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Evidence-based design, physiological efficacy testing and consumer analysis of an exemplar model of a fruit derived bioactive functional food for use in physical performance and exercise settings.

Thesis presented by

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for the degree of

Doctor of Philosophy

(Nutritional Sciences)

University College Cork

School of Food and Nutritional Sciences

Head of School: Prof. Alan Kelly

Supervisors: Dr Alice Lucey, Dr Lorna Doyle (SETU Waterford)

2023

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Abstract

The overall aim of this thesis is to examine the new product development practices used within the scientific design, development and testing of new nutritional products in the performance nutrition sector. Despite recent growth in the area there is a general lack of publicly available information to guide the incorporation of nutritional science into the processes underpinning the design and development of new performance nutritional products. Performance nutrition provides a promising area for the creation of value-add products using Irish agrifood outputs, a key priority in Irelands Food Vision 2030 strategy (Gov.ie - Food Vision 2030 – A World Leader in Sustainable Food Systems, 2021).

In chapter 1, frameworks that were created as part of this PhD thesis to inform and guide the incorporation of nutritional science in the development of new products are introduced. These frameworks are applied throughout the subsequent chapters in the design and development of an exemplar model of a functional food to support post exercise recovery and endurance exercise performance. This exemplar model took the form of a blackberry polyphenol enriched high protein milk.

Chapter 2 reviews the literature on new product development and discusses the role of polyphenols and protein in post-exercise recovery and supporting endurance exercise performance. The review confirms the efficacy of dairy protein, particularly in the form of bovine milk, for muscle repair and regeneration post-exercise. RCTs have consistently shown the benefits of consuming between 1.2-1.6g/kg/day of high-quality protein for those undergoing frequent exercise. While there's growing evidence to support the use of plant-derived polyphenols for exercise-induced muscle damage (EIMD) recovery and endurance exercise performance, gaps remain in understanding their mechanisms, dosing, and optimal dietary sources.

Chapter 3 presents an online survey that assesses the nutrition priorities, practices and preferences of athletes and active individuals. This survey was designed as part of this thesis following a series of focus group sessions with athletes of various levels and backgrounds. This survey provides key end-user insights into the nutritional wants and needs of athletes and active individuals. Key findings highlight muscle recovery as the primary nutritional priority among the athletic community and reveal a clear preference for food product forms over supplements. The data collected provides

invaluable guidance for new product development in sports nutrition and allows the meaningful incorporation of end-user insights into the process.

Chapter 4 bridges a crucial knowledge gap by conducting a systematic review and meta-analysis investigating the efficacy of flavonoid-rich polyphenols to support post-exercise recovery. The meta-analysis of 26 randomised controlled trials (RCTs) observed significant benefits in muscle strength and soreness recovery post intensive exercise. However, a notable limitation in the included studies was the inadequate characterisation of the polyphenol subclasses and dosages used, emphasising the need for more detailed reporting in future human studies.

Chapter 5 presents an RCT assessing the efficacy of the RubusElite beverage prototype to support post-exercise recovery. Results suggest that this beverage augments recovery of muscle strength and reduces muscle soreness in comparison to both high protein and low protein milks. In collaboration with RubusElite project partners, this RCT includes detailed characterisation of the beverage's polyphenol content and a verification of polyphenol stability within the treatment beverage for the study duration.

Chapter 6 further investigates the efficacy of the RubusElite prototype in relation to physical performance during a 15km cycling trial. In contrast to previous findings, no significant improvement in performance or exertion-related measures was observed. However, thorough polyphenol characterisation alongside these findings offers critical insights into the lack of observed benefits and provides direction for the reformulation of the prototype. This also provides considerable insights into future RCT design to reassess the efficacy of the reformulated prototype.

In summary, this thesis offers a comprehensive investigation into the development of performance nutrition products through the creation of an exemplar model using novel frameworks which allow for the incorporation of scientific best practice. It provides innovative insights into the role of polyphenols and protein in supporting exercise and is one of the first undertakings to assess their efficacy in combination. By combining rigorous scientific evaluation, consumer insights, and product prototyping, the research offers a comprehensive roadmap for future product development in the rapidly evolving domain of performance nutrition.

Acknowledgements

Starting with the cornerstones of this project, my sincere appreciation goes to my supervisors. Alice, your unwavering support and guidance throughout the past 5 years have been invaluable; every meeting left me enlightened and motivated. Lorna, thank you for your valuable input and for hosting me during my time at SETU.

A particular mention to Tessa, my counterpart in food science on the RubusElite project. Working alongside you has been an absolute pleasure, and I wish you all the success in your future endeavours.

Morgan, your help during the trial was nothing short of Phenomenal. The project would not have been possible without your input, and I hope we get the opportunity to work together again in the future… hopefully during a less successful period for Limerick!

Thank you to the staff at SETU Waterford, especially Adam and Bruce, for your assistance and for enduring many early mornings to facilitate the trial.

Also I would like to show my appreciation to all of the participants who volunteered their valuable time to take part in the research.

A significant nod to the wider RubusElite team, in particular Yvonne, Seamus, Shane, and Dilip, your expertise has been instrumental in bringing considerable depth to this thesis. Collaborating with you has been an enriching experience and one I have thoroughly enjoyed.

To my officemates in room 105. Elaine, Áine, Andrea and Siobhán, you made me feel so welcome when I first arrived to UCC and I am extremely grateful for the knowledge and experience you shared during the first years of my PhD. More recently, thanks to João, Aoife and Bernadette for being great company during the final run in.

To Finbarr and the lads who have come and gone through St Marys during my time in Cork, thank you all for providing a low stress, comfortable and entertaining home throughout the majority of my PhD.

I can’t not mention my (mostly) reliable Ford Fusion, which, against all odds, survived countless journeys up and down the country in the honourable pursuit of science. A noble steed indeed!

To my parents, Val and Seán, thank you being with me on every step of this PhD journey. Your continual willingness to lend an ear and offer sound advice made everything so much easier.

To my dog Rudi who never failed to greet me at the door with a ferocious enthusiasm. You always put a smile on my face!

And, of course, to my girlfriend Clara. Your support has been incredible, providing a second home, a sanctuary in the north from the hardships of research. Your belief in me and constant encouragement has made all the difference.

I would finally like to also thank the Department of Agriculture Food and the Marine who provided financial support for this research.

Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism and intellectual property.



Conor C. Carey

List of Publications

Scientific Publications

1. Nutritional priorities, practices and preferences of athletes and active individuals in the context of new product development in the sports nutrition sector.
   Conor C. Carey*, Lorna Doyle, Alice Lucey*. Front. Sports Act. Living 2023
   doi: 10.3389/fspor.2023.1088979
   Impact Factor 2.7. Citations – 2.

2. Flavonoid Containing Polyphenol Consumption and Recovery from Exercise-Induced Muscle Damage: A Systematic Review and Meta-Analysis.
   Conor C. Carey*, Alice Lucey, Lorna Doyle.* Sports Med. 2021
   doi:10.1007/s40279-021-01440-x.
   Impact Factor 12.6. Citations – 19
   Awarded Review of the Year at the UCC School of Food and Nutritional Sciences Publication of the Year Competition.

3. Effect of a blackberry polyphenol enriched high protein dairy beverage on recovery from exercise induced muscle damage and oxidative stress: a randomised controlled trial (In preparation for submission to the Journal of the International Society of Sports Nutrition – Impact Factor 5.1)
   Conor C. Carey*, Morgan Lewis, Dilip Rai, Tessa Van De Langerijt, Yvonne O'Callaghan, James A. O'Mahony, Shane V. Crowley, Lorna Doyle, Alice Lucey*

4. Effect of a blackberry polyphenol enriched high protein dairy beverage on performance in a 15km cycling time trial: a randomised controlled trial (In preparation for submission to the International Journal of Sports Nutrition and Exercise Metabolism – Impact Factor 4.6)
   Conor C. Carey*, Morgan Lewis, Dilip Rai, Tessa Van De Langerijt, Yvonne O'Callaghan, James A. O'Mahony, Shane V. Crowley, Alice Lucey, Lorna Doyle*

5. The bioaccessibility and apparent permeability of phenolic compounds in blackberry-dairy blends containing different leucine concentrations after in vitro digestion (Under Peer Review at Food Bioscience – Impact Factor 5.3)
   Tessa van de Langerijt; Yvonne C O'Callaghan; Katerina Tzima; ***Conor C Carey****; Alice Lucey; Nora M O'Brien; James A O'Mahony; Dilip K Rai*

Published Abstracts

6. Analysis of the Nutritional Priorities, Practices and Preferences of Athletes and Active Individuals to Inform New Food Product Development in Sports Nutrition.
   Conor C. Carey*, Lorna Doyle, Alice Lucey.* Proceedings of the Nutrition Society, doi:10.1017/S0029665121002536. Impact Factor 6.4.

Other Publication

RubusElite Industry Facing Report - Insights and Perspectives on the Performance Nutrition Sector (Appendix 1)

Training Undertaken During PhD

August 2023 – UCC Odyssey Programme

Graduate of the UCC Odyssey Programme, a course designed to enable researchers in UCC to adapt, integrate and expand on their existing expertise to prepare for the many diverse career choices ahead of them.

September 2022 – INRAE PhenolAcTwin PhD Summer School - Innovative approaches and tools to substantiate the link between dietary polyphenols and Health.

Hosted by the French Governmental Agriculture and Environmental Research Centre 'INRAE', this course covered approaches to develop research on polyphenols and health, including meta-analysis, microbiome analysis, nutrigenomics and in silico docking.

June 2022 – University of Copenhagen NEXS Grand Depart Symposium & PhD Training Course (Appendix 4)

Both practical and oral training sessions in state-of-the-art techniques related to exercise and performance focussed human clinical trials.

International Council for Harmonisation (ICH) – Good Clinical Practice (GCP) – Full Course (Appendix 5)

September 2020 – European Institute of Innovation food INNOFOOD 2020 (Appendix 6)

I was awarded a scholarship to take part in this pan-European competitive programme organised by EITfood, focussed on the food new product development process. As participants we spent one week designing and simulating an NPD strategy for a product which we then pitched to leading members of the food industry including Lidl and Strauss.

November 2020-April 2021 – ST6013 Statistics & Data Analysis for Postgraduate Research Students

Research training module to introduce the statistical methods relevant to data analysis and practical applications of these methods.

December 2020 – Epigeum Research Integrity Course – Natural and Physical Sciences (Appendix 7)

May 2019 – PG7016 Systematic Reviews for Health Sciences
UCC postgraduate research training module for the conduction of systematic reviews and meta-analyses

June 2019 – PG6015 An Introduction to Research Integrity, Ethics and Open Science
UCC postgraduate research training module on the appropriate conduction of scientific research, particularly considerations when working with human subjects and vulnerable populations, data protection, conflicts of interest and the ethical review process.

January 2019 – Nature Research Masterclasses
Completed a series of online masterclasses hosted by the world leading journal 'Nature'.

Abbreviations

ACSM	American College of Sports Medicine
ADA	American Dietetic Association
AIS	Australian Institute of Sport
BCAA	Branched Chain Amino Acid
bpm	beats per minute
CI	Confidence Interval
CK	Creatine Kinase
CMJ	Countermovement Jump
CONSORT	Consolidated Standards of Reporting Trials
CPET	Cardiopulmonary Exercise Test
DIAAS	Digestible Indispensable Amino Acid Score
DNA	Deoxyribonucleic acid
DOMS	Delayed Onset Muscle Soreness
DRV	Dietary Reference Value
EFSA	European Food Safety Authority
EGCG	Epigallocatechin gallate
EIMD	Exercise-induced muscle damage
ERE	Electrophile Response Element
ESPEN	European Society for Clinical Nutrition and Metabolism
EU	European Union
FAO	Food and Agriculture Organisation
FDBP	Freeze Dried Blackberry Powder
FRAP	Ferric Reducing Antioxidant Power
FSR	Fractional Synthetic Rate
GI	Gastrointestinal
HPLC	High Performance Liquid Chromatography
HPM	High Protein Milk
HR	Heart Rate
IMTP	Isometric Mid-Thigh Pull
IOC	International Olympic Committee
ISSN	International Society of Sports Nutrition
KEAP1	Kelch-like ECH-associated protein 1

LPM	Low Protein Milk
MD	Mean Difference
MDA	Malondialdehyde
MMRM	Linear mixed models repeated measures
MPS	Muscle Protein Synthesis
mTOR	Mammalian Target of Rapamycin
MVC	Maximum Voluntary Contraction
NCAA	National Collegiate Athletics Association
NO	Nitric Oxide
NPD	New Product Development
NRF2	Nuclear factor erythroid 2-related factor 2
PHPM	Polyphenol enriched high protein milk
PI	Prediction Interval
PPT	Pressure Pain Threshold
PUFA	Polyunsaturated Fatty Acid
RCT	Randomised controlled trial
ROS	Reactive Oxygen Species
RPE	Rating of Perceived Exertion
RSI	Reactive Strength Index
SD	Standard Deviation
SEM	Standard Error of Mean
SETU	South East Technological University
SREC	Social Research Ethics Committee
TPC	Total Phenolic Content
TT	Time trial
TTE	Time to exhaustion
UCC	University College Cork
UEFA	Union of European Football Associations
UN	United Nations
USD	United States Dollars
VAS	Visual Analogue Scale
WADA	World Anti-Doping Agency
WIT	Waterford Institute of Technology

Chapter 1 – General Introduction

1. Opening Statement

Amidst the environmental challenges confronting the Irish agri-food sector, there is an urgent need to identify sustainable and economically viable alternatives to traditional revenue streams. Ireland's renowned dairy and produce sectors offers an avenue for innovation, particularly through the development of novel value-add foods to support performance nutrition. However, the successful diversification into this sector demands an evidence-based approach to ensure product efficacy and end-user value.

In the evolving landscape of performance nutrition, athletes and practitioners alike are continually seeking products that offer an edge in performance. Yet, as the domain of performance nutrition products expands, a discernible divide persists between offerings founded on rigorous evidence-based research and those exclusively driven by commercial motivation. This dichotomy merits a wider discussion, amidst the burgeoning array of products available, what can be done to ensure the development of performance nutrition products are both scientifically robust and genuinely efficacious?

This PhD thesis focuses on establishing a framework for the evidence-based creation of nutrition products tailored for sport and exercise performance. Using this framework and guided by scientific evidence and end-user insights, we have designed a functional food, specifically a blackberry polyphenol enriched high protein milk, as part of the multi-disciplinary RubusElite project funded by the Irish Department of Agriculture Food and the Marines, Food Institutional Research Measure (FIRM). The systematic approach employed for this research project serves as an exemplar model for the principled design and development of future nutritional products and functional foods within the exercise and sporting landscape.

2. Problem Statement

The use of performance nutrition supplements is extremely prevalent at an estimated 60% in mixed athletic groups (Knapik *et al.*, 2016; Garthe and Maughan, 2018; Jovanov *et al.*, 2019). Although supplements play a leading role within the sports

nutrition sector, this has been extended to include the development of specific functional foods to support exercise performance(James, Naughton and Petróczi, 2010; Bigliardi and Galati, 2013; Reid, 2013; Arenas-Jal *et al.*, 2019). Considerable concerns have been expressed in relation to the use of nutritional supplements for sport and exercise by sporting bodies, practitioners and academics alike. There is a consensus that in many situations dietary supplementation may not provide the purported ergogenic benefits and may actually be detrimental to both performance and health when used inappropriately (Garthe and Maughan, 2018). This often occurs when supplements are used as substitutes rather than to support a balanced diet (Garthe and Maughan, 2018).

A further challenge is the poor regulation of the efficacy and health claims for performance nutrition products within Europe, with much of the regulation left to the individual member state, many of whom cannot feasibly evaluate every product that enters the market (Garthe and Maughan, 2018; Rodriguez-Lopez *et al.*, 2022). Limitations in governance and regulation has led to the development of many nutrition products that are marketed on the basis of spurious or greatly exaggerated efficacy claims (Kerksick *et al.*, 2018; Tiller, Sullivan and Ekkekakis, 2023). Additionally, poor oversight of manufacturing facilities and the sourcing of raw materials can also present a challenge with many products not containing the advertised dosages and some even shown to be contaminated with compounds banned by the World Anti-Doping Agency (WADA) resulting in anti-doping rule violations (Outram and Stewart, 2015; Duiven *et al.*, 2021; Lauritzen, 2022; Cohen *et al.*, 2023).

Consequently, many well regarded sporting bodies including the International Olympic Committee (IOC), Union of European Football Associations (UEFA) and the American College of Sports Medicine (ACSM) warn against the use of sports supplements where possible, advocating for a ‘food first approach’ to performance nutrition (ACSM and ADA, 2016; Maughan *et al.*, 2018; Collins *et al.*, 2021). Despite these recommendations, there is consistent research evidence supporting certain supplements and sports foods, where potent ergogenic properties have been demonstrated when used in the appropriate circumstances, exemplars include caffeine and creatine monohydrate (Peeling *et al.*, 2018). Therefore, the growing consensus supports a more balanced approach such as the recently proposed “food first but not

always food only" framework to deliver an optimal and safer performance (Close *et al.*, 2022).

While sports foods and supplements undeniably support athletic performance and possess commercial viability; this sector would greatly benefit from structured guidelines which integrate nutritional science and end-user engagement in the new product development process.

3. Incorporating Sports Nutrition Science into the New Product Development Process

Although the field of performance nutrition has highly specific requirements for product development, to date there is a dearth of peer-reviewed literature outlining the incorporation of nutritional science within the development and design of new products. The core aim of this thesis is to provide a blueprint to inform the incorporation of scientific methods into the new product development process in the sports nutrition sector. These frameworks were actively assessed through their application within an exemplar model of a sports nutrition product development. This model takes the form of a blackberry polyphenol enriched high protein milk.

The novel framework developed as part of this PhD thesis takes the form of a five-phase model where performance nutrition science and research can pragmatically inform new product development for sport supplements and foods (Figure 1). The overarching goal of this five-phase model was to provide a stepwise approach for the design and development of new products for the sports nutrition sector. The outcome is a sum of the parts, with each stage being instrumental for the NPD success; a brief overview of these five critical elements is provided below.

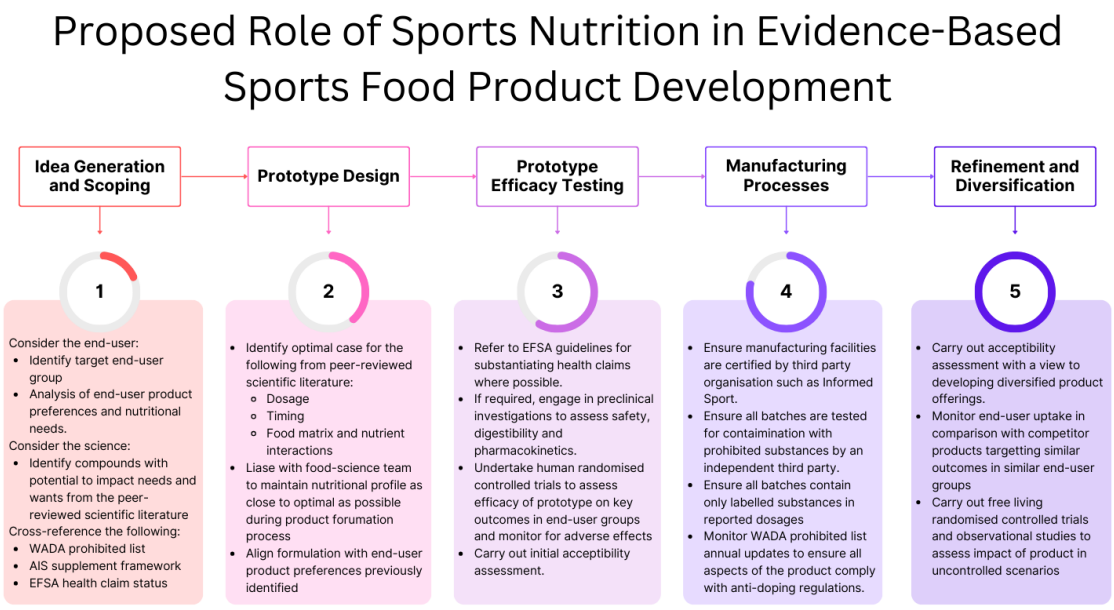


Figure 1. The five key phases model, developed as part of this PhD thesis, in which nutrition science can be meaningfully incorporated into the new product development process of performance nutrition products.

3.1 Idea Generation and Scoping

Consider the end-user: Key to the initial idea generation process is engagement with the end-user. Mixed methods research using both quantitative and qualitative tools such as surveys and focus groups can identify the key wants and needs of athletes. This will allow new products to align with the key goals of the end-user. Food choice in athletic groups has been shown to be multi-facetted with physiological demands and sporting culture adding complexity to the already intricate and complex field of food choice (Birkenhead and Slater, 2015). Validated research surveys which investigate food choice in targeted end-user athletic groups are also helpful to provide key information regarding how to better cater for specific populations within sport and exercise population groups (Pelly, Burkhart and Dunn, 2018; Thurecht and Pelly, 2019).

Consider the 'Science': Assessment of published scientific evidence underpinning product efficacy claims of active nutritional compounds is essential. Peer reviewed scientific evidence should be prioritised, paying particular attention to the scientific hierarchy of evidence which places more weight on evidence generated through randomised controlled trials and systematic reviews and meta-analyses. Lower levels

of evidence may also be of assistance when evaluating specific elements for example mechanisms of action, dose response and safety concerns. A comprehensive review of the literature supporting the efficacy of key competing products should also be undertaken to determine whether end-user needs are being sufficiently met. This stage also provides an important opportunity to highlight any knowledge gaps and areas of limited knowledge or concern.

Cross-Reference the guidance: At this early stage of the process, it is also essential that key guidelines and regulations are consulted to assess the initial viability of a product concept. Product concepts must not contain any compound which appears on the WADA prohibited substance list (The Prohibited List | World Anti-Doping Agency, 2023).

It should then be established whether the active compound in the proposed product has received European Food Safety Authority (EFSA) health claim status. When a compound or product receives a health claim approval from the EFSA, it gains the immediate advantage of being marketable based on its scientifically proven efficacy. In contrast, if a product has not received EFSA health claim status, the lack of approval pinpoints the areas of research that must be addressed in future stages of the new product development process.

The Australian Institute of Sport (AIS) has developed an evidence informed 'Sports Supplement Framework' which ranks supplements and sports foods on the basis of the scientific evidence underpinning their efficacy claims and can also be used to initially when profiling the viability of a product concept (Kim and Kim, 2019). The WADA prohibited list, EFSA health claim submissions and the AIS Sport Supplement Framework collectively should be consulted at this early stage in the process to ensure appropriate resource allocation.

3.2 Prototype Design

The development of an early product prototype is a key step in the development of new food and nutritional products. Generally, in food product design this prototype allows for initial sensory acceptability (Ruiz-Capillas and Herrero, 2021), however in the field of functional food and nutritional supplement development this also allows

for an evaluation of physiological efficacy (Granato *et al.*, 2020) (the process of which will be discussed in section 3.3 below).

During the design of this prototype, it is imperative to conduct a comprehensive review of the 'totality' of scientific literature published in this broader area; discerning the scientific evidence supporting the suggested dosage, the timing of ingestion, and interactions between the food matrix and nutrients is of critical importance for prototype design. Initially, each of these three aspects should be scrutinized to identify potential safety concerns and any risk of adverse effects, when the product takes a food format.

Subsequent to this, based on published evidence from higher order human studies (if available), the literature review should pinpoint the optimal characteristics for efficacy, which will then inform the creation of a "*paper prototype*". This paper prototype represents the ideal nutritional profile based on scientific evidence. Working collaboratively, it provides an explicit reference target for the food science and prototype formulation teams, setting clear nutritional benchmarks to achieve. Lastly, the prototype must align with the preferences of the target market. This alignment is determined by the insights gathered during the idea generation and scoping phase, which involves direct engagement with potential end-users.

3.3 Prototype Efficacy Testing

It is essential that performance nutrition supplements and food products are investigated for efficacy prior to their release especially if marketed on the basis of a functional claim. If certain elements underpinning prototype design were unclear following review of the scientific literature basic, preclinical investigations may be required to clarify pertinent elements which may lead to refinement of product prototypes, these elements may include safe dosage limits, pharmacokinetics, digestibility, bioavailability and bioaccessibility among others.

The efficacy of the product should then be assessed via a human randomised controlled clinical trial. The EFSA have issued key recommendations for the substantiation of health claims relating to muscle function and physical performance (Turck *et al.*, 2018). While these recommendations appear to be poorly engaged by the sports nutrition industry for the most part, these provide clear standards for the assessment

of health claims related to physical capacity, performance and muscle recovery among others (Turck *et al.*, 2018).

More general guidance for study design and assessment of health claims are publicly available and while not specific to sports nutrition, set scientific best practice standards for such efficacy testing and as such should be adhered to where possible (Lucey, Heneghan and Kiely, 2016; 'Scientific and technical guidance for the preparation and presentation of a health claim application (Revision 3)1', 2021). During these investigations it is also pertinent to conduct assessments of end-user acceptability. It is helpful at this stage to obtain sensory feedback relating to the prototype to quantitatively assess whether it aligns with end-user product preferences or requires further refinement (Ruiz-Capillas and Herrero, 2021).

3.4 Manufacturing Processes

It is pragmatic to consider nutritional science within the manufacturing process of a new performance nutrition related product. A key challenge facing food innovation within the field of performance nutrition is poor quality standards within manufacturing procedures and processes resulting in adverse consequences such as reduced ergogenic effects of a product due to products not containing the intended dosages of active compounds or product contamination with potentially banned or dangerous substances posing considerable risk to the health and reputation of athletes (Outram and Stewart, 2015; Duiven *et al.*, 2021; Lauritzen, 2022; Cohen *et al.*, 2023). In a bid to mitigate these risks, 'NSF Certified for Sport' and 'Informed Sport' are organisations which have been established which certify manufacturing facilities and provide third party batch testing of sports nutrition products for contaminants (Certified for Sport®; Manufacturing Certification | Informed Manufacturer; Sports Supplements Certification | Informed Sport). This independent third-party approach to quality control results in greater assurances for athletes and sporting organisations that the products are both efficacious and safe (Vento and Wardenaar, 2020). These procedures should be of paramount importance in the manufacture and release of performance nutrition products. The WADA prohibited list is also updated annually and should be monitored closely for changes which may impact product formulation even following release to market (The Prohibited List | World Anti-Doping Agency, 2023).

3.5 Product Refinement and Diversification

Following the launch of nutritional products larger scale end-user engagement can be undertaken to obtain valuable product feedback and where product refinement, diversification and development of alternative product offerings may represent further benefits for the athlete. Less controlled 'free living' randomised controlled trials can be conducted to investigate the impact of nutritional products and how they impact overall dietary intakes. Similarly, co-supplementation is common in this cohort, with 15% reporting using 4 or more supplements simultaneously (Jovanov *et al.*, 2019). It is important to consider the impact of poly-supplementation of sports nutrition products and supplements. It is entirely feasible that a product may need to be reformulated on the basis of these key factors.

3.6 Product Development Decision Tree

While there is a clear role for scientific evidence in performance nutrition to inform all of the above key phases of the new product development process, the above process only reflects the best-case scenario where products successfully pass through each phase of the process. In practice, this process is likely to act as a phase-gate or stage-gate model where products must pass through various stop-go decisions which determine whether the product is a viable candidate for the next phase of product design and development. These models have successfully been applied in fields such as medical device development (Pietzsch *et al.*, 2009). Based on the novel multi-disciplinary collaborative research in the RubusElite project, a decision framework to inform the screening of the potential new products from a nutritional science perspective has been created. This will act to inform the phase-gate model to determine whether a product should proceed from one phase to the next and ultimately, it's viability to be released in the marketplace (Figure 2.).

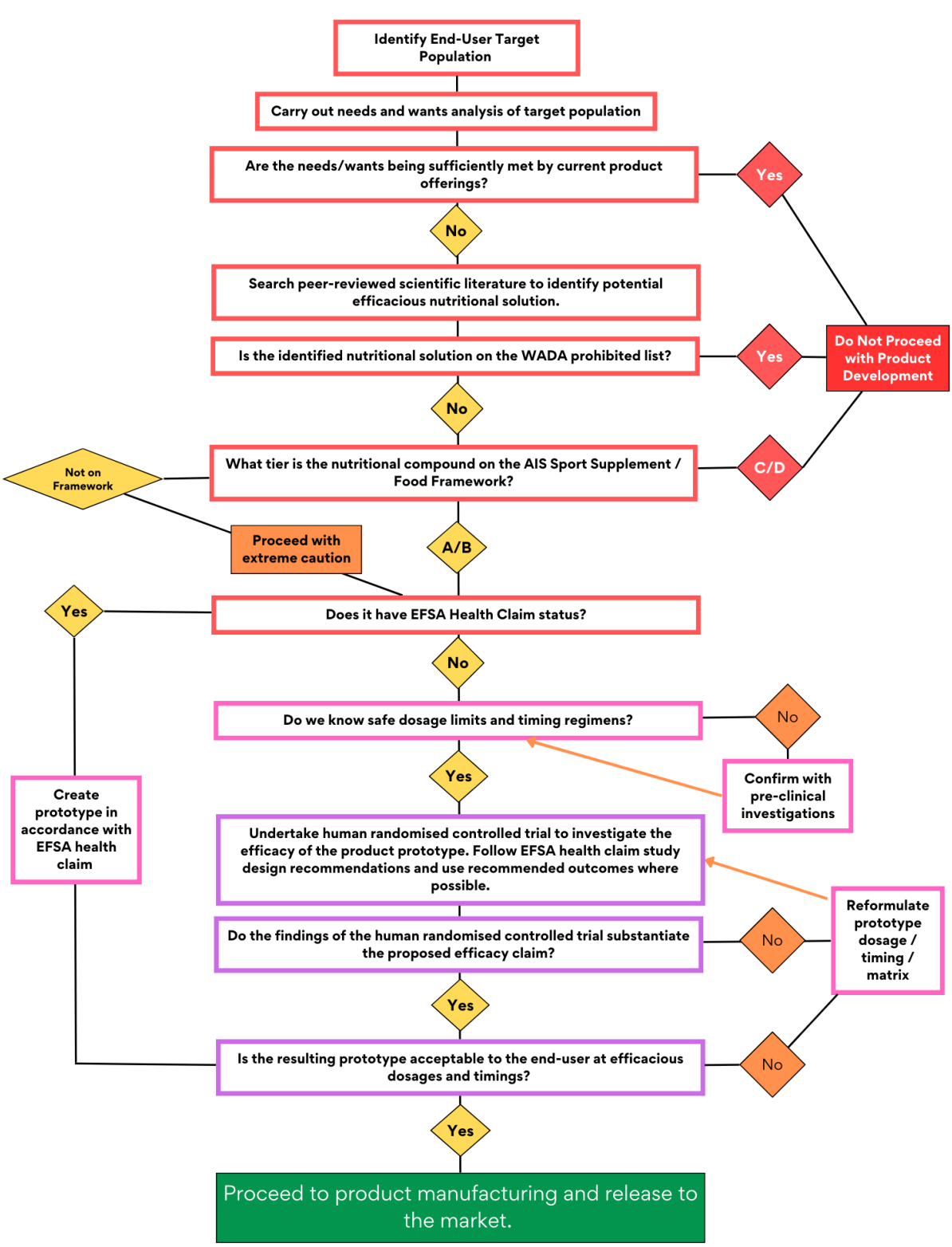


Figure 2. A proposed decision framework which incorporates key nutrition science actions and decisions within the new product development process of sports nutrition products.

4. Exemplar Model Product Concept

This thesis is anchored in the research objectives set out by the RubusElite project. This project seeks to systematically create an exemplar model of a new food product enriched with bioactives for the performance nutrition sector. A distinguishing feature of the RubusElite project is its multidisciplinary, multi-institution methodology, where different disciplines collaborate to develop and validate the product concept. This collective effort has resulted in the formulation of the blackberry polyphenol-enriched high protein milk, representing an integration of nutritional science and product development principles.

Berry polyphenols have been suggested to be efficacious to support exercise performance and recovery post exercise in previous randomised controlled trials, while dairy protein and milk has been shown to support muscle repair, recovery and hydration during exercise (Shirreffs, Watson and Maughan, 2007; Hulmi, Lockwood and Stout, 2010; Phillips, 2012; Desbrow *et al.*, 2014; Somerville, Bringans and Braakhuis, 2017; Bowtell and Kelly, 2019). This RubusElite Project is the first of its kind to incorporate blackberry polyphenols in a high protein milk matrix to support exercise performance and recovery.

For this exemplar model, dairy protein in the form of bovine milk was chosen to be the protein source. Milk protein is both a complete protein, containing all essential aminos acids, and is a particularly rich source of leucine (Vliet, Burd and van Loon, 2015; Gorissen *et al.*, 2018). Milk protein has also been shown to be one of the more digestible protein sources for humans with an average ileal digestibility of 95.3% (Dupont and Tomé, 2020). This suggests that milk protein is one of the most appropriate sources of protein to support muscle protein synthesis stimulation. The carbohydrate protein blend of bovine milk has also been suggested to augment not just muscle protein synthesis but also recovery from exercise-induced muscle damage (Cockburn *et al.*, 2008; Cockburn, Bell and Stevenson, 2013), when enhancement of recovery with protein supplements alone has been mixed (Pasiakos, Lieberman and McLellan, 2014; Pearson, Hind and Macnaughton, 2022). Carbohydrate is particularly important for endurance exercise as the major fuelling macronutrient (Impey *et al.*, 2018), and has been shown to enhance muscle protein synthetic response to exercise when combined with protein due to the role of insulin and protein synthesis (Figueiredo and Cameron-Smith, 2013). Protein is in group A of the AIS Sport

Supplement framework and has received health claim status from EFSA stating that there is an established cause and effect relationship between dietary protein and the growth and maintenance of muscle mass (Agostoni *et al.*, 2010).

Berry polyphenols have been the subject of numerous randomised controlled trials in recent years, many of which have shown considerable benefits to both endurance exercise performance and post exercise recovery (Cook et al., 2015, 2017; Craig Perkins et al., 2015; Godwin et al., 2017; Lim et al., 2012; McAnulty et al., 2004; McLeay et al., 2012; Murphy et al., 2017; Sadowska-Krępa et al., 2015; Strauss et al., 2018; Willems et al., 2016; Willems et al., 2015). A recent meta-analysis has shown that chronic polyphenol consumption improves endurance exercise performance (Somerville, Bringans and Braakhuis, 2017). Fruit polyphenols are currently in group B of the AIS sport supplement framework however have not received health claim status from EFSA.

Both polyphenols and high protein milk possess potential advantages for athletes and those engaging in active lifestyles. Yet, a gap exists in the current market for a polyphenol-enriched high protein milk product. While the potential synergy of polyphenols and dairy in enhancing exercise performance is evident, comprehensive research into their combination is lacking. The envisaged blackberry polyphenol-enriched high protein milk not only serves as an innovative exemplar model for functional foods but also holds promise for commercial viability. This project thus propels the evidence-based evolution of nutritional product design, setting a precedent for future offerings in the domain of sports and exercise nutrition.

5. Overarching Goals of the PhD Thesis

The overarching goal of this PhD thesis is to design a food product to support exercise performance and recovery using the proposed science informed frameworks. The research in this thesis focuses on the initial three phases of the product development process described in Figure 1, bringing the product to the point of a decision to manufacture and release to the marketplace. A full application of the decision-making framework presented in Figure 2 is also considered. The development of this product will act as an exemplar model to inform the future development of products to support performance in exercise and sporting settings.

Chapter 2 - Narrative Literature Review**:** Evidence-based considerations in performance nutrition new product development and the potential for dairy protein and polyphenols to support exercise performance and recovery.

Aims of Chapter 2

- To contribute to **phase 1**: '*Idea generation and scoping*', identifying key considerations within the new product development process for bioactive functional foods.
- To inform **phase 2**: '*Prototype design'* within the product development framework.
- To extensively review the literature to:
    - Evaluate and establish the evidence underpinning the proposed product concept, related to dairy protein and berry polyphenols.
    - Identify core research gaps relating to the role of polyphenols and dairy protein in exercise performance and recovery.
- To establish a foundational knowledge resource which will facilitate:
    - The food science team in their preliminary prototype design with which considered the required nutrition-led evidence-based product design attributes.
    - The nutritional biochemistry team by identifying areas requiring pre-clinical investigations such as digestibility and bio-accessibility.

Chapter 3 – Nutritional priorities, practices and preferences of athletes and active individuals in the context of new product development in the sports nutrition sector.

Aims of Chapter 3

- To develop a tool enabling the performance nutrition end-user to actively participate in **phase 1**: '*Idea generation and scoping'*, by conducting a needs assessment and analysing their perceived nutritional priorities.
- To gather data related to nutritional product preferences of athletes and active individuals to inform **phase 2** '*Prototype design'*.
- To identify hidden needs of athletes and active individuals by identifying where their practice does not align with evidence-based best practice. This information can also be used to inform **phase 2** '*Prototype design'*.

Chapter 4 – Flavonoid containing polyphenol consumption and recovery from exercise-induced muscle damage: A systematic review and meta-analysis.

Aims of Chapter 4

- To augment the evidence base for **phase 2**: '*Prototype design'*, specifically focusing on the role of polyphenols in exercise recovery in terms of polyphenol dosage, timing, composition and food matrix interactions.
- To critically assess the existing evidence in light of the known complexities associated with polyphenol interventions ensuring a strong scientific foundation for the recovery claims made for polyphenol use.
- To identify key protocol parameters from existing muscle recovery RCTs that are crucial for designing an effective RCT in **phase 3**: *'Prototype efficacy testing'*.
- To evaluate the commercial and scientific implications of incorporating polyphenols in sports nutrition products aimed at muscle recovery.

Chapter 5 - Effect of a blackberry polyphenol enriched high protein dairy beverage on recovery from exercise induced muscle damage and oxidative stress: a randomised controlled trial

Aims of Chapter 5

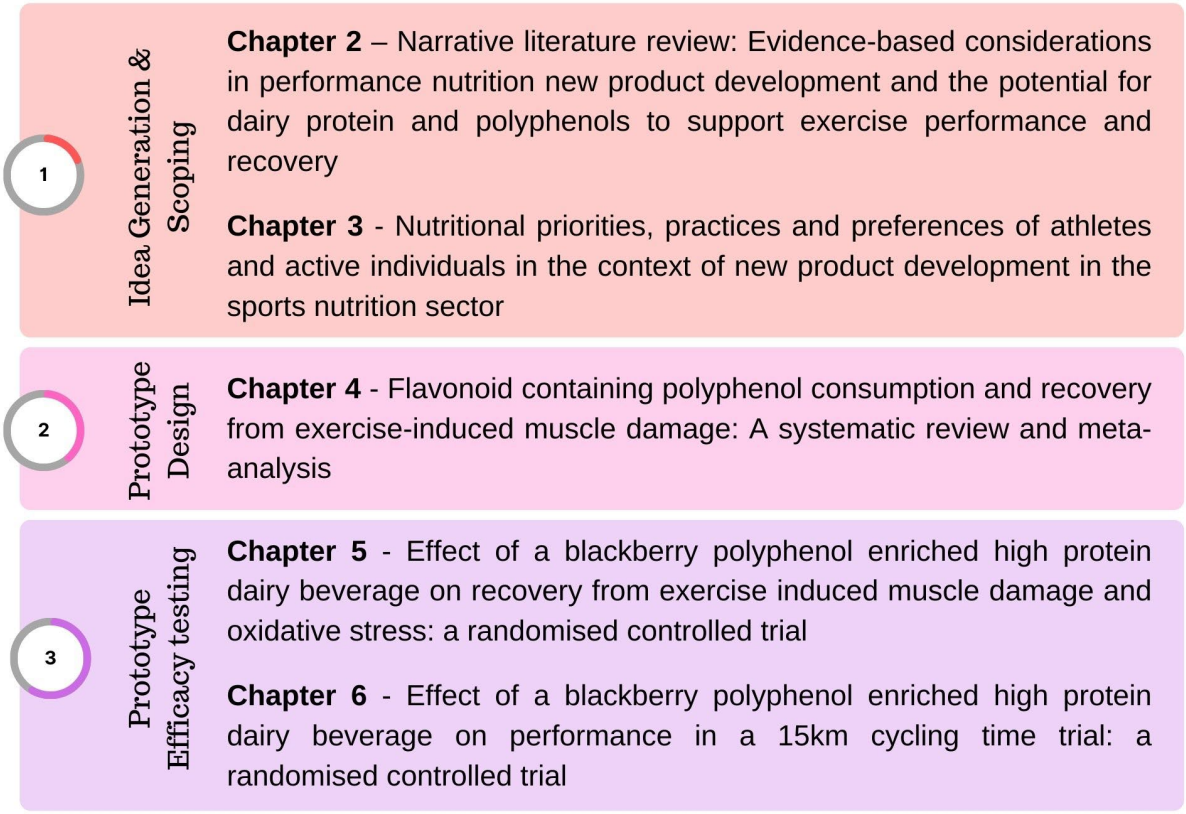
- As part of **phase 3:** '*Prototype efficacy testing'*, the objective was to investigate the efficacy of the RubusElite beverage product prototype in aiding recovery from exercise-induced muscle damage and oxidative stress.
- To conduct a single-blind, proof of concept RCT (Randomised Controlled Trial) which adheres to and utilizes EFSA (European Food Safety Authority) recommended outcomes for evaluating the product's impact on muscle function recovery after intensive exercise.
- To perform an initial acceptability assessment of the RubusElite beverage prototype as part of **phase 3** *'Prototype efficacy testing'*.
- To provide further empirical evidence to support the identification of optimal polyphenol dosage and subclass recommendations through extensive product characterisation.

Chapter 6 – Effect of a blackberry polyphenol enriched high protein dairy beverage on performance in a 15km cycling time trial: a randomised controlled trial.

Aims of Chapter 6

- As part of **phase 3:** '*Prototype efficacy testing'*, the objective of this chapter was to assess the efficacy of the RubusElite beverage product prototype concerning its secondary claim of enhancing endurance cycling exercise performance.
- To conduct an RCT (Randomised Controlled Trial) adhering to EFSA (European Food Safety Authority) recommended procedures for muscle performance claims.
- To conduct a single-blind, proof of concept RCT (Randomised Controlled Trial) which adheres to and utilizes EFSA (European Food Safety Authority) recommended outcomes for evaluating the product's impact on endurance exercise performance.

- To provide vital data to the existing evidence base underpinning the efficacy of polyphenols by combining data from recommended exercise performance outcomes with extensive polyphenol characterisation.



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Chapter 2 – Literature Review

Title: Designing a High Protein Dairy Beverage Enriched with Polyphenols: An Evidence-Based Approach to Sports Nutrition New Product Development

1. Introduction

There has been considerable interest and commercial growth within the sports nutrition sector in recent years. This has been suggested to be the result of the expansion of the sports nutrition product market into the general food sphere rather than just being of interest to those performing at a high athletic level (Arenas-Jal et al., 2019; Cui et al., 2022). The field of sport and exercise nutrition places a particular focus on the required macro and micronutrients to fuel performance, as well as fluid requirements needed to sustain such high-level performance (ACSM and ADA, 2009). While these aspects should be the primary focus of any performance nutrition strategy, even when these are accomplished, the desire to push the limits of human performance remains. One area in which further enhancement could potentially be achieved is the addition of "bioactive" functional foods to the athlete diet.

1.1. Bioactive Functional Foods in Performance Nutrition

Bioactive compounds have been defined in the scientific literature as '*extra-nutritional constituents that typically occur in small quantities in foods' and are being studied for their effects on health'* (Kris-Etherton *et al.*, 2002). Functional foods have been defined as '*novel foods that have been formulated so that they contain substances or live microorganisms that have a possible health-enhancing or disease-preventing value, and at a concentration that is both safe and sufficiently high to achieve the intended benefit*' (Temple, 2022).

Within the context of performance nutrition, bioactive functional foods contain compounds that possess the ability to improve an aspect of performance beyond the macro or micronutrient functionalities of the food. With an appropriate scientific process to evaluate the validity of sports nutrition product claims, combined with intelligent marketing structures, bioactive functional foods can provide an attractive avenue for sports nutrition products (James, Naughton and Petróczi, 2010; Bigliardi and Galati, 2013; Reid, 2013). In many cases however, there is a tendency to use substances without scientific substantiation in an attempt to gain a performance benefit, while other performance nutrition products are often used on the basis of misinformation and spurious product claims (Heuberger and Cohen, 2019). A further example is the use of exogenous ketones within elite endurance sport. This has been

widely reported in the media, with many pro cyclists and top world tour teams being reported to use them extensively (PezCycling News, 2020). Despite this, there is very limited scientific evidence to support their efficacy (Shaw *et al.*, 2020; Evans *et al.*, 2022). In contrast, when appropriate scientific procedures are carried out to substantiate efficacy claims, bioactive functional foods provide one of the most exciting avenues in the advancement of performance nutrition.

1.2. Objective of the literature review

The primary objective of this review is to offer direction for the creation of innovative bioactive functional foods designed to enhance athletic performance, using an exemplar model that combines berry polyphenols with a high-protein dairy beverage. During the first section of this literature review, evidence-informed considerations for the development of bioactive functional foods for sports nutrition will be discussed which will consider the latest research in the field (Figure 1). These considerations will endeavour to outline the crucial evidence-based processes necessary for the nutritional design and development of performance nutrition foods. This evidence-informed approach can serve as a valuable reference for future research and development of other bioactive functional foods.

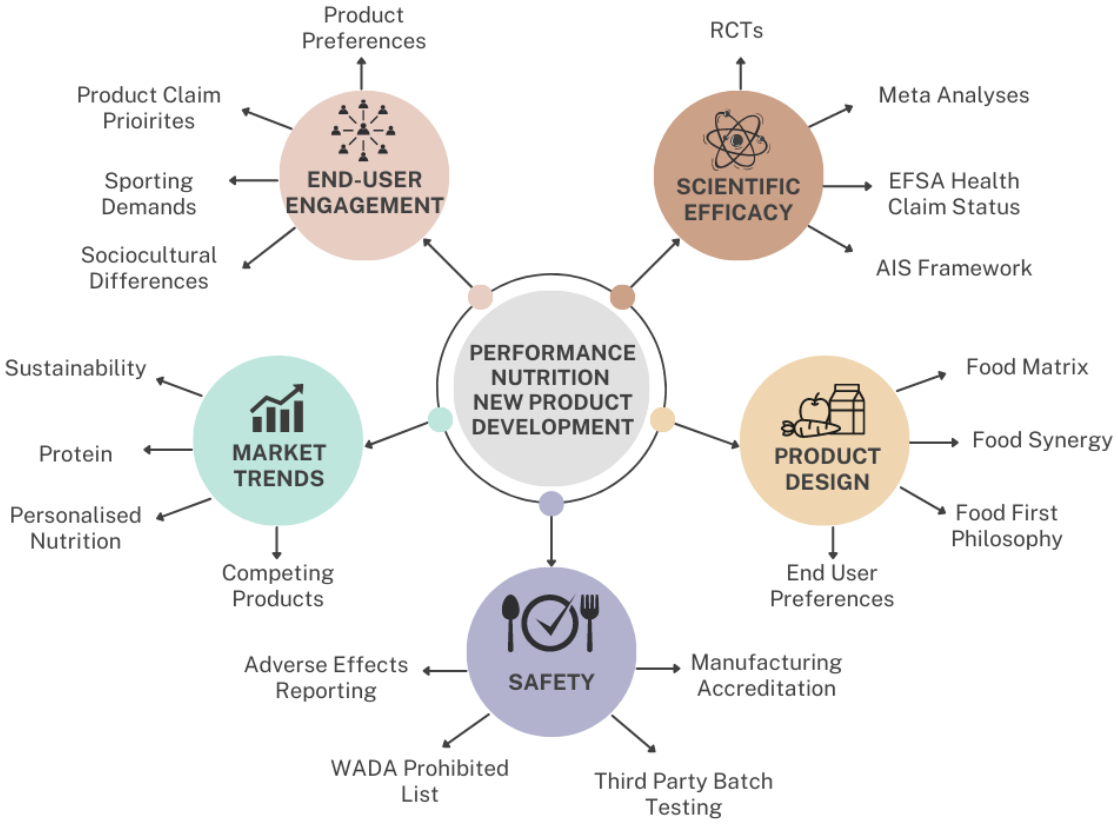


Figure 1. Considerations for New Product Development in Performance Nutrition

2. Evidence-Based Design and Efficacy Testing of Bioactive Functional Foods

In the design and development of evidence-based sports nutrition products, there are a number of key considerations which should be observed.

A) There must be sufficient scientific evidence to support the ultimate efficacy claim of the active ingredient or compound of the product (Peeling *et al.*, 2018).

B) Food-synergy and the interaction of the food matrix on the digestion and absorption of nutrients and bioactive compounds should be assessed as these may impact on the ergogenic benefit of the product (Capuano and Janssen, 2021).

C) Safety: The risk of inadvertent anti-doping violations arising from the use of a product should be assessed and independent third-party batch testing should

be included in the ultimate production plan if any risk is observed (Vento & Wardenaar, 2020).

D) Finally, the wants and needs of the end-user should be considered and wider trends within the industry should be observed in order to optimise the final product (Malsagova *et al.*, 2021).

Each of these key considerations have been discussed in depth in the following sections.

2.1 Six Key Considerations for Development of Bioactive Functional Foods for Sports Nutrition

Reported and anticipated growth in the sports nutrition industry necessitates a rigorous scientific approach to new product development processes to ensure that products are safe, effective, and meet the needs of athletes and active individuals. Within the scope of the present literature review, six key considerations for the methodical, science-based development of bioactive food products in performance nutrition are presented. This model, shown in Figure 2., underlines the essential factors for consideration during the design and development of research-backed bioactive foods for athletes and active individuals. By considering these factors, sports nutrition researchers and industry can develop innovative bioactive functional foods designed to enhance athletic performance while ensuring safety and efficacy.



Figure 2. Key considerations for food product development in performance nutrition identified during research and literature searches as part of this PhD thesis. Abbreviations: EFSA- European Food Safety Authority, AIS – Australian Institute of Sport

2.2 *Standards of Scientific Evidence to support Efficacy Claims in Sports Nutrition*

The importance of scientific evidence in sports nutrition research cannot be overstated. The quality of scientific evidence that substantiates the efficacy claims of sports nutrition products is essential for evaluating the safety and efficacy of these interventions, and for informing sports nutrition guidelines and recommendations (Peeling *et al.*, 2018).

One of the key principles of scientific evidence is the hierarchy of evidence, a framework that is used to evaluate the strength and quality of scientific evidence (outlined in Figure 3). Randomised controlled trials (RCTs) have been widely considered the best method to determine a causative relationship of an intervention and within the context of sport and exercise are widely considered as the methodology with the lowest risk of bias (Shah *et al.*, 2023).

While RCTs represent the lowest risk of bias within study structures, often within the field of performance nutrition, numerous RCTs are present on the same topic, the results of such can sometimes be contradictory. Systematic reviews and meta-analyses play an important role in this case as they act to quantitatively synthesise the results of multiple RCTs to gain an improved understanding of the consensus on a topic (Kelley and Kelley, 2019). In human nutrition, meta-analyses play a particularly vital role in the scientific process to derive scientific understanding (Lichtenstein *et al.*, 2021). While the resource intensive nature of RCTs and dietary interventions can result in underpowered and compromised RCT design (Lichtenstein *et al.*, 2021); the conduction of meta-analyses allow many of these compromises to be accounted for and an understanding of the overall trends to be quantitatively attained (Kelley and Kelley, 2019).

The validity of the efficacy claims of sports nutrition products should routinely be assessed with the hierarchy of scientific evidence in mind. Those products with positive results found in multiple well controlled RCT's and large meta-analyses should be prioritised for recommendations to athletes and active individuals (Kelley and Kelley, 2019; Lichtenstein *et al.*, 2021). This should form the basis of practitioners and end-users' decision making process on what products are worth considering including within a nutrition regimen.



Figure 3. Scientific hierarchy of evidence (Yetley *et al.*, 2017)

2.3 European Regulatory Governance of Efficacy Claims in Sports Nutrition

While international research is included in this review, the remit in regard to food regulation and health claims is described as per the jurisdiction of the EU. The regulation of health claims for bioactive functional foods in sports nutrition is an important aspect of consumer protection, transparency and public health, along with providing clarity and legal guidance on the level of efficacy claims which can be placed on a product (Domínguez Díaz, Fernández-Ruiz and Cámara, 2020). There are specific standards of scientific evidence set out for foods and nutritional products to achieve health claim status from the European Food Safety Authority (EFSA) (Lucey, Heneghan and Kiely, 2016). However, there are currently, no specific regulations governing sports nutrition products and performance nutrition claims (Varvaštian, 2015). EFSA have a set of regulations surrounding health claims regarding muscle function and physical performance that are particularly pertinent to this field (Turck *et al.*, 2018). This guidance suggests that prior to the application for a health claim for use related to enhanced muscle function such as improved recovery and muscular adaptation, or physical performance a minimum standard of scientific evidence must be which substantiated for the food product (Turck *et al.*, 2018). All applications for health claims are rigorously assessed by an expert panel at EFSA via the official health

claim process which culminates in the publication of a Scientific Opinion of the health claim (Turck *et al.*, 2018).

However European Directive 2000/13/EC legally only prohibits the use of health claims on the labels of foodstuffs without EFSA approval when the claim is conducive to pharmacological health benefits that may prevent / cure / treat an ailment (Visioli, 2022). As the majority of sports nutrition efficacy claims are not related to illness or ailment, they fall in a legal grey zone meaning that the process of application for an EFSA health claim status are often not engaged with sports nutrition products as evidenced by the reduced number of application submissions on this basis (Turck *et al.*, 2018). As such the ability to distinguish between legal and illegal claims in this area is incredibly difficult and often is left to the European Union member states to govern individual food labelling legislation on foodstuffs intended for sportspeople, specific guidelines are currently not available in Ireland (Bradley, 2015).

Regardless of the current lack of regulation for efficacy claims placed on sports nutrition products, there is a clear need for a minimum scientific standard to be met before a product can be promoted on the basis of physiological efficacy claims within the performance nutrition landscape. In the absence of robust legislation governing this field perhaps guidance initiatives such as the sports supplement framework put forward by the Australian Institute of Sport could be a basis for decisions as to whether products are sufficiently substantiated with scientific evidence (Kim and Kim, 2019). This Australian framework provides a 4-tier classification system using expert-led assessment of the current scientific evidence supporting performance claims of supplements and sports foods (Kim and Kim, 2019). Along with the framework comes a series of informative infographics for both athletes and sports nutrition practitioners enabling essential dissemination about the vast array of products on the market for the different key stakeholders (framework is exhibited in Figure 4).

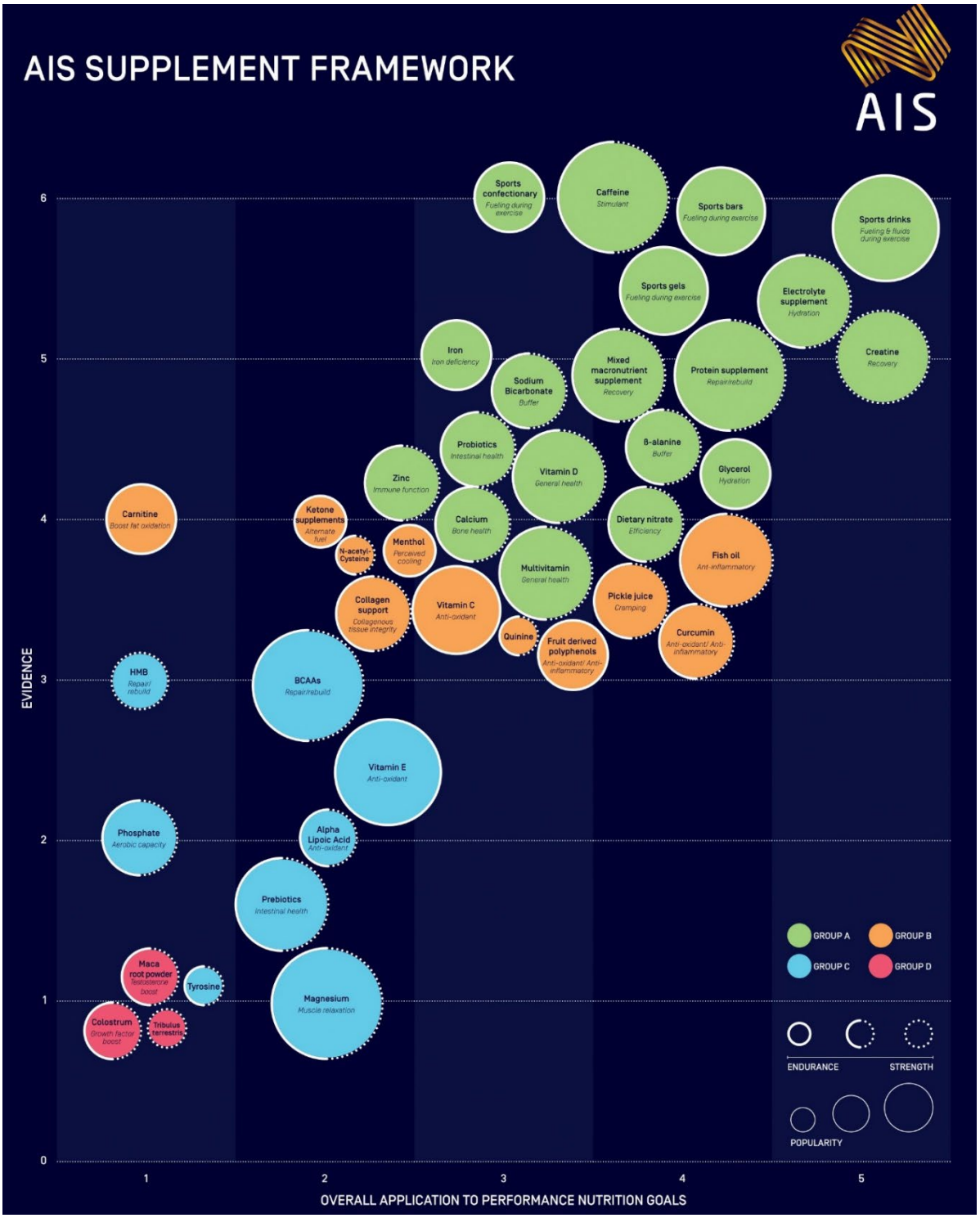


Figure 4. Australian Institute of Sport, Sport Supplement Framework

https://www.ais.gov.au/nutrition/supplements

2.4 Food first Approach to Performance Nutrition

The food first philosophy to sports nutrition has been consistently advocated for in position stands and consensus statements on sports nutrition by a number of international sporting organisations including the International Olympic Committee (IOC) (Maughan *et al.*, 2018), Union of European Football Associations (UEFA) (Collins *et al.*, 2021) and the American College of Sports Medicine (ACSM) (Thomas, Erdman and Burke, 2016). A food first approach to sports nutrition emphasizes the importance of obtaining nutrients from whole foods rather than relying on a supplement-based approach to reaching nutritional goals (Close *et al.*, 2022). This approach is centred on the idea that whole foods provide a wide array of essential nutrients and other beneficial compounds that are often lacking in supplements, and that the nutrients and bioactive compounds present in whole foods may be easier to digest and utilize by the body (Capuano and Janssen, 2021). Another key argument in favour of the food first approach is that whole foods are more nutrient-dense than supplements. For example, fruits and vegetables provide a wide range of vitamins, minerals, and macronutrients as well as fibre, which are essential for maintaining good health and supporting athletic performance. In contrast, many supplements, such as whey protein, provide only a single nutrient or compound, and are often lacking in other important nutrients vital for the overall health and performance of an individual (Garthe and Maughan, 2018). Additionally, the 'food first but not always food only' framework acknowledges that there may be situations where it is difficult or impossible to obtain all the necessary nutrients from food alone (Close *et al.*, 2022). In these cases, supplements may be necessary to bridge nutritional gaps and support optimal athletic performance (Close *et al.*, 2022). This is likely the most sensible approach to performance nutrition for the elite athlete who is trying to push the boundaries of performance, as there are compounds and nutrients which have been shown to be ergogenic when consumed in dosages which are wholly impractical if not impossible to achieve when exclusively adopting a food only approach (Peeling *et al.*, 2018). A key example of this is creatine monohydrate which has been shown to have considerable ergogenic benefit to athletes, but effective dosages cannot be reasonably obtained from whole food sources (Lanhers *et al.*, 2015).

2.5 Food Synergy and Food Matrix Effects in Performance Nutrition

Food synergy refers to the concept that the nutrients and other beneficial compounds in whole foods can have a greater overall impact on health and athletic performance when they are consumed together, rather than in isolation (Jacobs, Gross and Tapsell, 2009). This is because different foods and food components can interact with each other in ways that enhance their absorption and utilization by the body (Jacobs, Gross and Tapsell, 2009).

For example, the absorption of plant-based (non-haem) iron is enhanced by the presence of vitamin C, which is found in many fruits and vegetables. Consuming foods that are rich in both of these nutrients, such as citrus fruits and leafy green vegetables, can therefore provide a greater benefit than consuming non-haem iron sources alone (Ballot *et al.*, 1987).

Considering plant based bioactive foods such as dietary nitrate in beetroot and polyphenols in berries and teas are a present in substantial quantities in their natural food forms, it is entirely possible that the effect of the food matrix on digestion and nutrient absorption may alter the activity of bioactive compounds. Co-ingestion of polyphenols with a fat source has been shown to potentially enhance polyphenol absorption through enterohepatic recirculation of polyphenols associated with their interactions with bile secretions from the gall bladder (Manach *et al.*, 2004; Ortega *et al.*, 2009; Martínez-Huélamo *et al.*, 2016). However, the food matrix may also have a negative effect on bioavailability of bioactive compounds such as the effect of dietary fibre in certain fruit sources of polyphenols inhibiting the release of compounds during digestion (Palafox-Carlos, Ayala-Zavala and González-Aguilar, 2011). As such it is imperative to consider the interaction of the food matrix and co ingestion of nutrients on bioavailability and bioaccessibility of bioactive components when designing bioactive functional food products for performance nutrition.

2.6 Anti-Doping

Doping is a considerable issue within sport and exercise and is particularly pertinent within the field of sports nutrition. First and foremost, in the new product development landscape for sport and exercise, all proposed formulations should be cross referenced with the World Anti-Doping Agency prohibited list to ensure the prospective product

is approved for use by athletes (The Prohibited List | World Anti-Doping Agency, 2023). This list is updated annually and should be monitored each year. In particular sports supplements pose considerable risk to participants due to the risk of containing unlabelled substances and varying dosages of active ingredients (Judkins and Prock, 2012; Mathews, 2018). The World Anti-Doping Agency (WADA) have recently stated that "the risks of taking supplements should be weighed against the potential benefit that may be obtained, and athletes must appreciate the negative consequences of an anti-doping rule violation as a result of taking a contaminated supplement." A study indicates that of a selection of 114 products from Europe's leading supplement brands, 10% have been shown to be contaminated with substances prohibited for use in elite sport (Judkins and Prock, 2012). New product development ventures must create procedures to minimise if not eliminate this risk in future manufacturing plans.

The ability to alleviate this risk through the availability and use of food-based solutions is a massive advantage for a natural, food first approach to performance nutrition. An emerging process in the development and production of sports nutrition products is the introduction of third-party testing of each batch of the product. This independent, unbiased and publicly available process provides a certain level of trust that the product being purchased contains only the labelled substances and is free from contamination with any prohibited substances (Vento and Wardenaar, 2020). Organisations such as 'Informed Sport' have been set up to carry out this batch testing for contaminants in sports nutrition products and to provide certification to sports nutrition brands who undergo these processes (Sports Supplements Certification | Informed Sport). Informed Sport also provide a quality assurance programme to certify manufacturing facilities ensuring they meet minimum standards in their procedures to avoid supplement contamination (Manufacturing Certification | Informed Manufacturer). While most athletes in a recent survey were aware of the importance of third-party testing of nutritional supplements, only 57% of supplements purchased were actually third party tested (Vento & Wardenaar, 2020).

It is essential that new product development projects in the field of performance nutrition carry out a risk assessment for potential anti-doping violations by consulting the WADA prohibited list and ensuring this is consistently and thoroughly cross referenced on an annual basis throughout the NPD process as well as following the products release. Inclusion of provisions to ensure manufacturing facilities are

accredited by an organisation such as Informed Sport and third-party batch testing for product contamination should be built into the business plan from the outset.

2.7 Food Choice in Sport and Exercise – Optimisation of Product Prototypes

The food choices made by sporting and active populations can differ from the general population based on their unique motivations and nutritional needs. A review by Birkenhead and Slater (2015) comprehensively discusses the multi-facetted and complex dynamics which influence the food choice of athletes. Athletes may view food as fuel for performance and prioritize nutritional content over taste and enjoyment. Athletes from different sporting and sociocultural backgrounds can also have very different factors affecting food choice (Birkenhead and Slater, 2015). While this complex topic has received considerable research attention with the context of the general population (Wahl *et al.*, 2017; Chen and Antonelli, 2020), athletic population groups are largely under researched. Additionally, given the added complexity sporting variables such as differing body composition and the energy demands of various sports, it is very difficult to determine whether a sports nutrition product will be successfully adopted by the targeted end-user (Pelly, Burkhart and Dunn, 2018; Thurecht and Pelly, 2019).

A potential approach to support the future development of bioactive functional foods would be to undertake science-backed consumer analysis of new products which have been shown to be efficacious. Tart cherry juice and extracts represent one of the bioactive functional foods with considerable scientific evidence of its efficacy for improved post exercise recovery and performance benefits (Vitale, Hueglin and Broad, 2017; Ortega *et al.*, 2020). One recent study conducted on athletes' perceptions and experiences using tart cherry products observed that 68.8% of participants enjoyed the taste of tart cherry. In terms of product format preferences, opinion was largely divided with 35% requesting a concentrate product, 25% indicating they preferred a beverage format and 22% requesting a juice product. Interestingly, only 2% of athletes wanted powder products and a further 2% wanted a tablet product (Wangdi *et al.*, 2021). This further supports the statement that athletes are calling for more food-based product format over supplements to achieve their specific needs. Further end-user engaged, targeted research into specific products following their release may be a

pragmatic undertaking to better understand the factors affecting food choice in efficacious bioactive functional foods for sporting individuals.

2.8 New Product Development Trends in Sports Nutrition – Recent Case Study Examples

There are a number of wider trends and advancements in the sports nutrition landscape which have gained notable traction. Keeping abreast of market trends should be a vital part of the new product development strategy as they may provide added value for the end-user as well as desired product attributes. Examples of key trends in food product development within performance nutrition are discussed in detail below in the following section. These trends have been chosen on the basis of their relevance within the field of performance nutrition as well as global consumer trends within food industry as discussed in the comprehensive review by Arenas-Jal et al (2019). While these trends are of particular importance to sport and exercise, they do not represent a complete overview of consumer trends in performance nutrition and are presented merely to act as examples of some current consumer trends in this fast-moving space. The process of analysing current consumer trends should be an integral part of new product development in performance nutrition new product development. The trends to be discussed are:

A) Personalised Performance Nutrition

B) High Protein in Performance Nutrition

C) Sustainable Performance Nutrition

2.8.1 Personalised Performance Nutrition

Personalised nutrition uses information on individual characteristics to develop targeted nutritional advice, products or services (Jose Ordovas, 2018). The field of personalised nutrition is still largely at an early stage and most evidence is based on observational rather than interventional research designs (Jose Ordovas, 2018). There have been notable advancements in the field of analysis of phenotypic and genomic analysis to inform personalised nutritional practices (Cole, Florez and Hirschhorn, 2020). This has extended to field of bioactive functional foods within sports and

exercise nutrition with the identification of genes which influence the impact of differing dosages of caffeine on exercise performance and adverse effects (Pickering and Kiely, 2018). This review showed that by assessing the genotypic variation between athletes in the CYP1A2 and ADORA2A genes you can more effectively prescribe dosage and timing strategies for athletes (Pickering and Kiely, 2018). Evidence has been published concluding that the CYP1A2 gene affects the speed of caffeine metabolism (Womack *et al.*, 2012) and the ADORA2A gene affects nervous system excitability (Loy *et al.*, 2015). Those with the CYP1A2-C allele benefiting from greater caffeine dosages while those with the ADORA2A – TT allele at higher risk of anxiety so potentially benefitting from lower dosages. While there has been considerable research into personalised nutrition from a health perspective (Cole et al., 2020), this application in caffeine use for athletes may be the first example where genetic testing is able to provide specific personalised nutrition advice within the field of plant derived functional foods for sporting performance. While significant further research is required, this shows considerable rationale for further investment and research into the genetic variation in response to particular nutrition strategies to support sporting performance.

2.8.2 High Protein Performance Nutrition

A further popular trend in sports nutrition is the fortification of products with added protein. Protein is important for the growth and maintenance of muscle mass and function, particular for triggering the growth of new muscle and as a mediator of the adaptive response post exercise (Phillips, 2012). Protein products such as protein bars, powder, beverages and other products are the most common and lucrative sector of the sports nutrition sector (Arenas-Jal *et al.*, 2019). In Europe it was reported that 54% of all new sports nutrition products had the high protein/ source of protein positioning claim (*Sports Nutrition: European market, consumer trends, and innovation*, 2021). Considering the inclusion of a protein source within the formulation of a new product may provide added nutritional benefit to an athlete as well as a desired product attribute.

2.8.3 Sustainability in Performance Nutrition

There is a considerable movement towards greater sustainability of global food systems within nutrition, with climate action one of the primary goals outlines in the

UN Sustainable Development Goals (UN DESA, 2022), and global targets for climate action inherently linked with food production. There is notable scope for further development of efficacious sport nutrition products which use sustainable approaches. Plant-based proteins are one of the key growing areas of this sustainable development in sports nutrition with many plant proteins shown to have the capability of stimulating muscle protein synthesis to the same extent as animal proteins when certain strategies are used to augment the anabolic properties of the proteins such as combining various protein sources and fortifying certain amino acids (Vliet, Burd and van Loon, 2015). Performance nutrition also shows opportunity for contributing to a circular food economy and reducing food waste. One example of this is the extraction of polyphenols from food waste products such as peels, stems, seeds and pomace from the processing of other products such as juices and oils, given the promising efficacy of polyphenols to support sporting performance as outlined later in the review (Kumar *et al.*, 2017).

2.9 Concluding Notes on NPD Considerations in Performance Nutrition

In the field of new product development of bioactive functional foods to support sports nutrition strategies there are a vast array of factors to be considered.

The Scientific Evidence: A key priority is the evidence base underpinning the performance claim on which the ultimate product will be based. EFSA and AIS guidance should be complied with and the level of scientific evidence underpinning efficacy claims should be assessed with reference to the scientific hierarchy of evidence.

There is also a clear a trend towards adopting a food first approach as is supported by international sporting bodies such as UEFA, IOC and ACSM. This should be the first direction for NPD ventures although there is space for the inclusion of supplements where it is impossible or wholly impractical to achieve efficacy through a food product.

Safety for the End-user: High quality production standards should be maintained to minimise the risk of inadvertent doping violations and products should be third-party batch tested to ensure minimal risk.

Market Intelligence and Commercial Success: To ensure the success of any sports nutrition product in the market an end-user engaged approach should be undertaken. consumer needs and wants analysis should be completed due to the complex nature of food choice in athletes. Other trends such as sustainability and the growth of protein products should be considered as additional selling points especially when working with lesser-known bioactive products.

3. Competing bioactive functional foods in performance nutrition

3.1 Dominant Players within the Bioactive Functional Foods Market – Case Study Exemplars

In the initial design phase of new product development, it is well established that competitor analysis is a key cornerstone of a strategic new product development process (Hatzijordanou, Bohn and Terzidis, 2019). Within the scope of the design of the RubusElite high protein, polyphenol enriched dairy beverage, it was deemed pertinent to complete an analysis of alternative product offerings that may provide benefit to athletes and active individuals ulitising other, non-polyphenolic bioactive phytochemicals. This process aimed to identify such products on two fronts. Firstly, to identify established, highly researched products that have high quality scientific evidence underpinning their functional claims. Secondly, to identify products that are in the early stage of development and scientific research but present potential to be future competitors in the market. Two key bioactive functional foods were identified as being deep-rooted products on the market by virtue of having notable scientific evidence underpinning their performance claims as well as having numerous brands citing these benefits within their unique selling points. They are caffeine based functional foods such as coffees and teas, and dietary nitrate predominantly in the form of beetroot juice. Two emerging bioactive products requiring further research were ginseng and capsaicin.

3.1.1 Caffeine

Caffeine is the most widely used and thoroughly researched bioactive compound which is commonly used in functional foods to support sport and exercise (Doherty and Smith, 2005; Ganio *et al.*, 2009; Souza *et al.*, 2017). Found in many plant sources, such as cocoa and coffee beans and the leaves of tea plants, caffeine has become commonplace in everyday diets globally. A large volume of research has been undertaken to evaluate the effects of caffeine on exercise performance, in particular its effect on physical and cognitive performance (Doherty and Smith, 2005; Ganio *et al.*, 2009; Souza *et al.*, 2017).

The extent of research carried out has led to a considerable number of higher order scientific evidence in the form of systematic reviews and meta-analyses concentrating specifically on physical exercise performance and caffeine use. Three of these reviews

focussed on various aspects of endurance performance, one with a focus on muscular strength and one investigating a mix of both endurance and strength-based variables (Doherty and Smith, 2004, 2005; Ganio *et al.*, 2009; Warren *et al.*, 2010; Souza *et al.*, 2017). All five of these reviews concluded that there are statistically significant ergogenic effects of caffeine for exercise performance over a range of performance tasks such as endurance performance (Doherty and Smith, 2004), acute force generation in sport specific and maximum voluntary muscle contraction (MVC) tasks (Warren *et al.*, 2010; Souza *et al.*, 2017) and perception of exercise difficulty (Doherty and Smith, 2005) amongst others. Only one major facet of exercise performance in which no beneficial effect was observed was for sprint performance (Souza *et al.*, 2017). The extent of the benefit for these aspects of performance has, however been shown to be highly variable, which is often related to the dosage regimen of caffeine ingestion (Goldstein *et al.*, 2010). The International Society of Sports Nutrition (ISSN) provide loose guidelines of 3-9mg caffeine per kg bodyweight as an appropriate dosage to provide an ergogenic performance effect (Goldstein *et al.*, 2010). It has been shown that doses over 9mg/kg can in fact be detrimental and lead to side effects such as anxiety, nausea and insomnia (Burke, 2008). While caffeinated bioactive functional foods are well established to be ergogenic at appropriate dosages, an individualised approach to discerning the appropriate dosage should be undertaken to ensure adverse effects do not impact competition performance (Tyzw *et al.*, 1995; Sökmen *et al.*, 2008). Caffeine has been placed in 'Group A' in the Australian Institute of Sport Supplement and Sports Food Framework, stating that there is '*Strong scientific evidence for use in specific situations in sport using evidence-based protocols.'*

3.1.2 Dietary Nitrate

Nitrate is an anion found in the human body which has been known to impact on vascular function. It can be reduced into compounds of nitric oxide (NO) and nitrite. NO has been known to impact muscle contractility and a range of pulmonary and cardiovascular responses (Stamler and Meissner, 2001). Dietary sources of nitrate include green leafy vegetables and beetroot (Jones *et al.*, 2018).

To date a large number of RCT's have been undertaken to investigate the impact of consuming dietary nitrate on endurance performance in athletes and active individuals. Two meta-analyses have been undertaken which combine the results of these RCT trials. The first of these meta-analyses analysed the results of 17 RCT's investigating

the effect of dietary nitrate intake, mainly from beetroot and sodium nitrate on time trials, time to exhaustion trials and graded exercise tests (Hoon *et al.*, 2013). Results of this meta-analysis report that there was a significant moderate effect of nitrate intake during time to exhaustion (TTE) trials but there was no significant improvement in time trial (TT) protocols. However, while not reaching significance in TT protocols there was a small beneficial trend showed further RCTs were warranted. A qualitative systematic review was also carried out as part of this study which noted that performance benefits were more pronounced with prolonged (7+ days) intake of nitrate and in people in lower activity brackets.

An updated meta-analysis was undertaken in 2017 to advance knowledge on dietary nitrate supplementation following an influx of new RCTs completed in this area (McMahon, Leveritt and Pavey, 2017). In this analysis, 46 published papers containing 76 RCT's met their inclusion criteria. Even with this expanded body of evidence, the results observed were relatively similar to those of (Hoon *et al.*, 2013). The consumption of dietary nitrate resulted in significant improvements in TTE trials; however, the overall grouped analysis observed only a trivial trend towards improvement in performance as a whole.

The majority of these RCT trials in healthy individuals have focussed on the intake of nitrate from beetroot due to its naturally rich content of nitrate and its relatively palatable taste in concentrated forms. A placebo beetroot drink was also developed and made commercially available for research purposes to support the design of RCT's with dietary nitrate (Gilchrist *et al.*, 2014). Dietary nitrate has been placed in 'Group A' in the Australian Institute of Sport Supplement and Sports Food Framework, stating that there is '*Strong scientific evidence for use in specific situations in sport using evidence-based protocols.'*

3.2 Early stage emerging bioactive functional foods – Case study exemplars

Within the new product development process not only is it essential to assess the status of key high performing competitors in the commercial space but also those which are emerging into the space with potential for growth (Hatzijordanou, Bohn and Terzidis,

2019). Two of the key emerging bioactive functional foods with growing levels of scientific evidence underpinning their performance claims are ginseng and capsaicin.

3.2.1 Ginseng

Ginseng is a widely used herb originating from Asia. In traditional eastern culture it has been used for generations for a number of purposes, not least it's suggested ability to restore Qi or life-energy. Ginseng has also been proposed to contain physiological bioactivity with a number of RCT's investigating its effect on exercise performance. These actions include the ability to improve the utilisation of oxygen through increasing fat oxidation allowing greater preservation of glycogen stores (Bahrke and Morgan, 2000). The depletion of glycogen stores has been shown to be a major limiting factor when it comes to endurance exercise (Rapoport, 2010). The general evidence investigating the effect of ginseng is both widely varied and highly contradictory. RCT's have investigated a number of different variations of ginseng compounds which may provide varying physiological effects. The body of research has also investigated an assortment of exercise variables including endurance performance, strength performance and repeated sprint type activity. Eschbach et al., (2000) and Wasuntarawat et al., (2010) both failed to observe any effect of ginseng on endurance performance using TTE and TT protocols respectively. This is contrasted with the results of (Liang, Podolka and Chuang, 2005) who observed a ginseng variety, panax notoginseng, improved sub-maximal VO_2 and TTE performance in a sample of 29 untrained adults. However, with all three studies using different varieties of ginseng, considerably different dosing and timing regimens, a consensus on the efficacy of ginseng in TTE trials is impossible to ascertain. Lee and colleagues have also alluded to endurance performance enhancing effects of ginseng however their only measure which showed significant improvements was VO_2max following training with ginseng ingestion in 117 sedentary adults (Lee *et al.*, 2017). No improvement was observed in repeated wingate anaerobic sprint cycling tests with 8 weeks of supplementation in 38 healthy adults (Engels, Fahlman and Wirth, 2003). Two studies have investigated the effect of ginseng on muscular strength; however, the results of these trials are also contradictory with decreases in perceived exertion and muscle pain observed Caldwell et al., (2018), however no impact on muscular strength was observed (Lee *et al.*, 2017). The overall findings regarding the use of ginseng for exercise performance is largely inconsistent. The main limitation of these

studies is the use of different varieties of ginseng leading to poor coherence of findings. It is currently unclear as to whether a benefit can be obtained from the use of ginseng despite its longstanding use in eastern cultures. A more targeted and standardised approach must be undertaken in future RCTs with a focus on a specific genus of the plant and investigating a specific mode of exercise, potentially endurance exercise as this provides the most convincing theoretical mechanism for action. As such ginseng is a poor target for development of a functional food to support exercise performance at this point.

3.2.2 Capsaicin

Capsaicin is a chemical compound which has garnered some interest within the sporting community in recent years. It is the chemical in chilli peppers which contributes to their famed piquancy or spiciness. Mouse models have shown increases in the force generation capacity of skeletal muscle as well as reduction of energy cost at a given force production using capsaicin (Kazuya *et al.*, 2014). This is theorized to be a result of the binding of capsaicin and its analogues to TRPV1 receptors which allow greater release of Ca^{2+} from the sarcoplasmic reticulum into the cytoplasm of the muscle cell (Kazuya et al., 2014). Increased cytoplasmic Ca^{2+} concentrations have been shown to result in greater force generation capacity in rodent muscle (Smith *et al.*, 2013). This theory has been substantiated through a mouse model which observed significantly improved endurance swimming capacity with capsiate, yet these performance enhancements were attenuated when a TRPV1 antagonist was given (Haramizu *et al.*, 2006). While capsaicin's mechanisms have been clearly shown in animal models, there is a dearth of human exercise trials using capsaicin. A small RCT in 10 healthy trained young men showed acute capsaicin supplementation improves rating of perceived exertion and total weight lifted was higher with 12mg of purified capsaicin provided 45 minutes prior to exercise (de Freitas, Cholewa, Freire, *et al.*, 2017). Another small RCT in 10 physically active men showed significant improvements to 1500m performance with the same dosage and timing regimen (de Freitas, Cholewa, Gobbo, *et al.*, 2017). Yet despite these positive results a study investigating the effect of capsaicin on repeated sprint ability in 19 male experienced athletes showed no improvement to performance (Opheim and Rankin, 2012). Importantly, in this study there was also significant gastrointestinal distress

experienced by the participants which would be a serious risk for use of this compound during competition. However, the dose used in Opheim and Rankin's study was over double that of both of the studies by de Freitas suggesting this may be a contributing factor to this distress. Further human studies are required to substantiate that which has been shown in animal models. The establishment of safe dosage guidelines for use in humans in an overarching priority. One particularly promising avenue for food innovation is the development of non-pungent variations of capsaicin which have seen some success (Haramizu *et al.*, 2006). This may alleviate the risk of gastrointestinal distress while potentially maintaining the beneficial effects (Watanabe *et al.*, 1994). While a recent meta-analysis of RCTs investigating capsaicin on both endurance performance and strength endurance found no significant effect of capsaicin on endurance performance in time trials or time to exhaustion trials, a significant improvement to muscular strength endurance during resistance exercise was observed (Grgic *et al.*, 2022). Although there appears to be promising early results supporting the potential mechanism for action for capsaicin to support enhanced muscular endurance in particular, notable safety concerns are still prevalent behind the appropriate dosage and risk of GI upset. Although there is some promise from research conducted to date, further research is warranted using non-pungent variations before capsaicin can be a realistic target for new product development.

3.3 Concluding Remarks on Competing Bioactive Functional Foods for Performance Nutrition

In conclusion, it is evident that the performance nutrition landscape is highly dynamic with bioactive functional foods at various stages of research and market establishment. The evidence supporting the benefits of caffeine and dietary nitrate, which are dominant players in the functional foods market, is well-documented and robust (Pasman *et al.*, 1995; Doherty and Smith, 2004; Matthew S Ganio, Douglas Casa and Lawrence Armstrong, 2008; Hoon *et al.*, 2013; McMahon, Leveritt and Pavey, 2017). These bioactive compounds have demonstrated notable performance enhancements across a range of sport and exercise domains (Pasman *et al.*, 1995; Warren *et al.*, 2010; Lansley *et al.*, 2011; Thompson *et al.*, 2016; Jonvik *et al.*, 2017; Lowings *et al.*, 2017). Importantly, these findings have been translated into practical guidelines to ensure

appropriate dosing and consumption patterns for athletes, consequently paving the way for successful market penetration and commercial longevity.

Emerging bioactives, such as ginseng and capsaicin, exhibit promising physiological properties, however, they are yet to have a coherent and compelling body of evidence supporting their effectiveness in improving sport and exercise performance (Bahrke and Morgan, 2000; Eschbach *et al.*, 2000; de Freitas, Cholewa, Gobbo, *et al.*, 2017; de Freitas *et al.*, 2022). Furthermore, their current safety profiles and potential side effects warrant further investigation (Grgic *et al.*, 2022).

The analysis of these competitor products within the functional foods market is integral for new product development (Hatzijordanou, Bohn and Terzidis, 2019). This process enables a clear understanding of both the strengths and weaknesses of established and emerging bioactives, thus providing valuable insights to inform the development process of new functional foods. Having evaluated the current landscape of bioactive functional foods in performance nutrition, this review will now delve deeper into the science and application of two particularly promising components for functional food development in performance nutrition, dietary protein and polyphenols.

4. Role of Protein in Performance Nutrition

4.1 Overview of Proteins in Physiological Function

Proteins are crucial to almost every aspect of cellular and systemic physiological function in humans. Proteins are comprised of chains of amino acids, typically folded into three-dimensional configurations that determine their functional capabilities. They are synthesized in the cell through a process dictated by the genetic code embedded in the cell's DNA, known as translation (Scheper, Van Der Knaap and Proud, 2007). At the cellular level, proteins facilitate numerous important functions including structural support of the cell and intracellular transport (Paulson, 1992). Proteins also serve as enzymes in biochemical reactions in the body which are essential for homeostasis and energy production (Knowles, 1987). Additionally, proteins are integral to DNA replication and cell division, essential for the growth and repair of skeletal muscle tissue following strain or injury such as that experienced during exercise (Obinata, 1993; Tye, 2003). These proteins include those involved in myofibrillogenesis, which contributes to both the structure and contractile properties of skeletal muscle (Obinata, 1993).

In addition to their intracellular roles, proteins have extracellular purposes that influence overall physiological functioning. Many proteins, including collagen and keratin, are critical to the structural integrity of tissues and organs. Haemoglobin, a transport protein, plays a vital role in oxygen transportation (Winslow, 2007). Regulatory proteins, such as insulin and other hormones, are crucial for maintaining physiological balance and coordinating biological responses (Reaven, 1997). Immune proteins called antibodies, are essential to support the defence against pathogens and harmful substances (Forthal, 2014). These represent just a small number of the vital roles of proteins within physiological function.

From a nutritional perspective, the human body has capacity for the endogenous synthesis of eleven amino acids to support physiological function however there are nine essential amino acids which must be acquired through dietary sources. These essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Common sources of dietary protein include meats, dairy products, legumes, nuts and seeds.

Most current dietary recommendations for proteins recommend between 0.75 and 0.9g/kg/day (Phillips, 2007), with the EFSA dietary reference value (DRV) for protein sitting at 0.83g/kg/day (Agostoni et al., 2012). These dietary recommendations originate from a set of nitrogen balance studies which have shown that this range of protein consumption is sufficient to offset the loss of protein in the body resulting from an imbalance in the protein synthesis and protein breakdown processes favouring protein breakdown (Rand, Pellett and Young, 2003). However, there have been significant criticisms of the methods used with such nitrogen balance studies such as the difficulty in quantifying all sources of nitrogen intake and excretion along with the inability to detect subtle changes in protein redistribution (Bauer *et al.*, 2013). Given the short-term nature of these studies, they are also unlikely to reflect the long-term muscle protein turnover relevant in changes to muscle mass (Bauer *et al.*, 2013).

4.2 Dietary Protein and Exercise, an Overview

Considerable research has since shown that certain subsections of the population may benefit from increased protein ingestion beyond that of the DRV. In particular it has been put forward that higher protein intakes can be particularly beneficial to those engaging in regular exercise training such as resistance and endurance training as well as those at risk of age associated muscle loss conditions such as sarcopenia (Phillips, 2012, 2015; Bauer *et al.*, 2013). In fact, protein intakes of up to 2g/kg/day have been shown to be safe for healthy adults (Agostoni *et al.*, 2012), suggesting that the EFSA DRV may be insufficient to optimise physiological function in such population groups (Wu, 2016). As such the American College of Sports Medicine (ACSM) recommend 1.2 to 1.7g/kg/day for those undergoing resistance or endurance exercise training (ACSM Information On… Protein intake for optimal muscle maintenance. 2015). The International Society for Sports Nutrition have taken this even further, categorically stating in their position stand on protein and exercise that the recommended level of protein intake (0.8g/kg/day) is "*likely not sufficient to offset the oxidation of protein/amino acids during exercise nor is it sufficient to provide substrate for lean tissue accretion or for the repair of exercise induced muscle damage*" (Campbell *et al.*, 2007). As such they have set their protein recommendations for exercising healthy adults at 1.4 to 2.0g/kg/day (Campbell *et al.*, 2007). In an updated position stand they have maintained this stance and further added that there is emerging evidence that

even higher protein intakes (>2g/kg/day) may have further benefits for those trying to maintain lean mass in hypocaloric periods as well as those concerned with body composition in resistance trained individuals, a stark contrast to the EFSA DRVs (Jäger *et al.*, 2017). In ageing populations these higher protein recommendations have also been revised due to the benefits to muscle function and maintenance of muscle mass with the European Society for Clinical Nutrition and Metabolism (ESPEN) revising their protein recommendations for optimum muscle function in ageing to 1.0 to1.2g/kg/day for healthy older adults and 1.2-1.5g/kg/day for those at risk of muscle loss due to inactivity and/or malnutrition (Deutz *et al.*, 2014). In addition to this, isolated protein supplements have been placed in 'Group A' in the Australian Institute of Sport Supplement and Sports Food Framework, stating that there is '*Strong scientific evidence for use in specific situations in sport using evidence-based protocols.*

4.3 Dietary Protein and Muscle Mass & Function

The regulation of muscle mass is a result of the balance between muscle protein synthesis and muscle protein breakdown, with the difference referred to as the net muscle protein balance (Burd *et al.*, 2009). Protein balance is impacted by two main apparatuses, feeding and exercise (Witard, Bannock and Tipton, 2021). When exercise and protein feeding is utilised concurrently this results in a positive net muscle protein balance (Burd *et al.*, 2009). Muscle protein synthesis can be assessed through the measurement of the fractional synthetic rate which uses stable isotope labelling of amino acids administered by intravenous infusion to directly trace the incorporation of amino acids into newly synthesised muscle tissue (Witard, Bannock and Tipton, 2021). One pertinent randomised controlled trial showed 30g of protein following an endurance exercise bout resulted in muscle protein synthesis increases in the 360 minutes post exercise (Churchward-Venne *et al.*, 2020a). A number of studies indicate that protein boluses in this range of between 20 and 30g are sufficient to maximally stimulate muscle protein synthesis resulting in a net positive muscle protein balance (Tipton and Phillips, 2013; Churchward-Venne *et al.*, 2020b). While this may be good general guidance for healthy young adults undertaking exercise, the appropriate protein dosage is likely more individual and nuanced depending on factors such as dietary protein quality and timing as well as the physiological demands placed on the

individual (Moore, 2020). For example, in the case of ageing individuals and master athletes' factors such as protein sensitivity and anabolic resistance may result in a greater bolus of protein being required to achieve a positive net protein balance (Morton *et al.*, 2018).

The role of protein feeding and exercise on muscle protein balance in the longer term has also been investigated within RCTs. A recent systematic review of 74 RCTs investigating the role of protein feeding alongside longer-term exercise training has shown significant increases in muscle mass and function, highlighting the benefits of long-term positive muscle protein balance in healthy adults (Nunes *et al.*, 2022). This systematic review also noted that enhancement in lean body mass was significantly improved with protein ingestion of >1.6g/kg/day in younger subjects undergoing resistance exercise training (Nunes *et al.*, 2022).

Overall it is well established that dietary protein intake is positively associated with improved muscle protein balance, especially when combined with regular exercise. It appears that current DRVs such as that put forward by the EFSA may be insufficient to optimise physiological function in the context of both ageing and athletics.

4.4 Assessing Dietary Protein Quality

While total protein intake provides the predominant and most accurate metric by which to achieve sufficiently positive muscle protein balance post exercise to result in muscular adaptation, the composition of the protein source also plays a significant role. Traditionally the quality of a protein was assessed by Biological Value, a measure of the nitrogen absorbed following food ingestion expressed as a percentage of the amount of nitrogen present in said food (Hoffman and Falvo, 2004). There is a number of inherent flaws in this measure of protein quality, predominantly as it does not take into account the digestibility of the protein, and as such provides an overestimation of protein utilisation (Hoffman and Falvo, 2004). The quality of dietary protein comprises of three key factors, the essential amino acid content, the digestibility of the protein and in the context of skeletal muscle, the anabolic signalling capacity of the protein resulting from the leucine content. The current best practice in the assessment of protein quality in the context of performance nutrition is to use a mixed methods approach.

The first is to consider protein digestibility using the Digestible Indispensable Amino Acid Score (DIAAS), a method which assesses the amount of individual amino acids which are present in the digesta at the end of the small intestine (Mathai, Liu and Stein, 2017). This protocol was developed by the Food and Agriculture Organisation (FAO) of the United Nations (Leser, 2013). This accounts for both the digestibility of the protein itself along with the specific amino acid characteristics accounting for whether a protein may be limited in a specific amino acid, and thus limiting physiological function (Herreman *et al.*, 2020). Due to the need for surgical cannulation to access the digesta to be assessed this is typically undertaken in pig models due to the similarity in digestive systems to humans.

While the DIAAS allows for accurate assessment of the amino acid composition and digestibility it does not account for the amount of protein is incorporated into skeletal muscle, a factor which can be influenced by the anabolic signalling impact of an amino acid such as leucine (Holowaty *et al.*, 2023). This is key to performance nutrition given the primary goal of protein intake being the support of skeletal muscle mass gain and enhancement of skeletal muscle function as a result. As such the muscle protein synthetic (MPS) response through fractional synthetic rate protocol has been developed (Tang and Phillips, 2009). This protocol involves intravenous infusion of a traced amino acid, typically phenylalanine followed by feeding of the protein for assessment (Fiorotto, Sosa and Davis, 2012). Skeletal muscle biopsies are then taken at serial time points and samples assessed by mass spectrometry for amount of tracer incorporated into muscle tissue (Fiorotto, Sosa and Davis, 2012). This allows for direct assessment of the efficiency of a protein at stimulating and providing substrate for muscle protein synthesis (Fiorotto, Sosa and Davis, 2012).

4.4.1 Essential Amino Acid Content

The essential amino acid content of the protein source is a critical factor which significantly impacts the capacity for the muscle protein synthetic response (Vliet, Burd and van Loon, 2015). Protein sources containing greater essential amino acid to total protein ratios have been reported to enhance the muscle protein synthesis response (Church *et al.*, 2020). Other research studies indicate that incomplete protein sources struggle to increase muscle protein synthesis post feeding (Arentson-Lantz *et al.*, 2021). One study in particular showed that a meal containing >20g of an incomplete wheat-based protein which was low in the essential amino acids: lysine

and threonine; failed to significantly improve muscle protein synthesis compared with a low protein control meal and suggested that complete proteins are needed to achieve required responses to impact acute overall muscle protein balance (Arentson-Lantz *et al.*, 2021).

4.4.2 Protein Digestibility

Along with the amino acid content of the protein, it has been shown that the digestibility of the protein may also prove to be an important factor worth considering when it comes to influencing muscle protein synthesis through the use of dietary protein (Trommelen, Betz and van Loon, 2019). Animal based protein sources have been shown to be superior to most plant-based proteins in terms of the digestibility as measured by DIAAS scores (Berrazaga *et al.*, 2019; Herreman *et al.*, 2020). This has been attributed to the structures and indigestible cell walls present in plant versus animal-based protein sources (Berrazaga *et al.*, 2019) as well as greater splanchnic urea synthesis of plant-based proteins (Vliet, Burd and van Loon, 2015). A comprehensive report by Herreman and colleagues of the digestibility in terms of DIAAS scores and complete essential amino acid characterisation from multiple data sets is presented in Table 1.

Table 1. Amino Acid Composition and DIAAS of animal and plant proteins (Herreman *et al.*, 2020)

Protein source	Histidine	Isoleucine	Leucine	Lysine	Met + Cys	Phe + Tyr	Threonine	Tryptophan	Valine	DIAAS	Limiting AA[a]
Corn	110 ± 29.7	90 ± 14.6	162 ± 58.2	36 ± 14.9	126 ± 22.2	140 ± 42.8	86 ± 10.2	52 ± 35.4	90 ± 14.4	36	Lys
Rice	93 ± 7.0	89 ± 17.4	80 ± 12.4	47 ± 2.3	104 ± 11.0	119 ± 29.6	75 ± 4.1	114 ± 28.6	95 ± 18.0	47	Lys
Wheat	118 ± 21.7	91 ± 10.5	87 ± 11.1	48 ± 10.6	127 ± 19.4	109 ± 16.9	78 ± 7.1	127 ± 17.8	92 ± 9.8	48	Lys
Hemp[b]	124 ± NA	106 ± NA	85 ± NA	54 ± NA	121 ± NA	131 ± NA	87 ± NA	-	99 ± NA	54	Lys
Fava bean	108 ± 4.1	106 ± 2.2	95 ± 5.4	95 ± 4.3	55 ± 5.1	119 ± 3.4	91 ± 6.2	68 ± 7.8	83 ± 2.2	55	Met + Cys
Oat	91 ± 11.4	100 ± 4.2	94 ± 4.9	57 ± 5.8	151 ± 52.9	135 ± 9.2	85 ± 5.9	110 ± 17.2	102 ± 3.4	57	Lys
Rapeseed	107 ± 8.0	90 ± 4.9	78 ± 5.0	67 ± 10.3	125 ± 14.3	92 ± 12.3	97 ± 6.5	106 ± 9.4	92 ± 4.6	67	Lys
Lupin	121 ± 16.1	104 ± 27.2	89 ± 19.3	75 ± 12.3	68 ± 12.7	121 ± 35.6	97 ± 22.7	72 ± 22.5	78 ± 14.6	68	Met + Cys
Pea	99 ± 9.7	101 ± 13.1	87 ± 11.5	110 ± 10.8	70 ± 12.3	116 ± 16.3	94 ± 7.9	77 ± 7.1	83 ± 9.8	70	Met + Cys
Canola	105 ± 6.9	93 ± 9.9	79 ± 7.8	72 ± 9.2	121 ± 10.4	97 ± 6.1	97 ± 12.2	112 ± 19.5	87 ± 9.1	72	Lys
Soy	119 ± 9.4	124 ± 8.3	102 ± 6.1	96 ± 9.0	91 ± 11.5	147 ± 8.3	105 ± 6.0	132 ± 21.1	95 ± 7.3	91	Met + Cys
Potato	100 ± 7.3	156 ± 9.2	143 ± 11.2	122 ± 4.6	115 ± 6.0	210 ± 18.2	165 ± 12.0	128 ± 13.7	138 ± 5.1	100	NA
Gelatin	34 ± 9.5	34 ± 10.6	35 ± 8.7	60 ± 11.5	27 ± 10.3	36 ± 13.0	46 ± 4.9	2 ± 3.0	46 ± 8.6	2	Trp
Whey	85 ± 10.8	166 ± 23.2	138 ± 22.9	131 ± 25.2	132 ± 21.6	101 ± 14.0	174 ± 22.8	180 ± 47.0	116 ± 14.3	85	His
Egg	101 ± 11.7	129 ± 25.5	103 ± 16.2	133 ± 58.4	123 ± 53.2	144 ± 18.9	106 ± 14.1	129 ± 49.7	105 ± 32.3	101	NA
Casein	147 ± 9.4	153 ± 4.3	141 ± 6.6	134 ± 4.3	117 ± 5.0	201 ± 8.0	130 ± 4.3	159 ± 13.4	148 ± 2.7	117	NA
Pork	197 ± 13.6	153 ± 11.1	122 ± 9.2	157 ± 10.7	128 ± 10.7	148 ± 10.4	145 ± 10.1	144 ± 17.1	117 ± 9.0	117	NA

Note: Data expressed as mean of individual DIAA values ± standard deviation.

[a]Limiting AA when DIAAS <100, NA not applicable when DIAAS ≥100.

[b]Dataset does not include tryptophan measurements. This is however the only publication reporting the ileal digestibility of hemp in pig studies. These data were therefore included for comparison.

4.4.3 Leucine Content

The amino acid leucine has been shown to have a particularly influential role in the muscle protein synthetic response following both feeding and exercise (Pasiakos and Mcclung, 2011; Vliet, Burd and van Loon, 2015). Animal based proteins such as dairy and meats have been shown to be rich in leucine and contain greater proportions of leucine in comparison to many plant-based proteins (Muscaritoli *et al.*, 2021). In addition to its role as a substrate in the generation of new proteins like other essential amino acids, leucine has been shown to act as a signalling molecule for the initiation of muscle protein synthesis (Wilkinson *et al.*, 2013). The method of action of this signalling process has been shown to occur due to its interaction of the anabolic mTOR pathway (Holowaty *et al.*, 2023). Although leucine has been shown to play a key role in the muscle protein synthetic response, supplementing with leucine alone has been shown to be insufficient to promote sufficient muscle protein synthesis likely due to the need for greater substrate availability in the form of other essential amino acids to optimally stimulate muscle protein synthesis (De Andrade *et al.*, 2020). While supplementing with leucine may not be an effective strategy to support muscle growth, a randomised controlled trial has shown that fortifying a low dose of complete protein (6.25g whey) with an abundance of leucine (5g total leucine) resulted in similar stimulation of muscle protein synthesis, suggesting that leucine may be one of the key limiting factors of the muscle protein synthetic response after exercise (Churchward-Venne *et al.*, 2013). These findings indicate that fortifying low doses of complete proteins with leucine may be a viable strategy for those concerned with limiting total protein intake such as those trying to reduce caloric intake or those concerned with the satiating effect of protein. This may include those in weight category sports and in cases of sarcopenic obesity.

A number of studies have postulated that a “trigger point” occurs with the ingestion of leucine, with the concentration of blood leucine following protein ingestion critical for the muscle protein synthetic response as a result of anabolic signalling (Moore *et al.*, 2009; Tang *et al.*, 2009; Churchward-Venne *et al.*, 2013, 2020b). While a recent systematic review of the ‘leucine trigger point’ hypothesis has reported that this ‘physiological event’ is only particularly evident in older adults and it is still unclear whether this is a limiting factor in younger healthy adults and athletes where anabolic resistance in less of a factor (Zaromskyte *et al.*, 2021).

4.5 Dietary Protein Timing

The timing of protein intake has been suggested to moderate the immediate muscle protein synthetic response to exercise due to the limited rate of protein absorption (Stark *et al.*, 2012; Schoenfeld and Aragon, 2018). It has been shown that due to the timing of digestion of protein, that one can technically achieve their total daily protein goals (1.2-1.6g/kg/day) but if this intake comes predominantly from one major bolus per day, this may not be sufficient due to these limitations on the rate of absorption with excess protein being excreted in urine (Tome and Bos, 2000). Fast acting proteins such as whey protein have been shown to have absorption rates of roughly ten grams per hour (Bilsborough and Mann, 2006). With this in mind it has been suggested that ingesting protein evenly at three-to-four-hour intervals may be appropriate to achieve daily protein goals within the limitation of protein absorption rates (Jäger *et al.*, 2017). It has been frequently suggested that there may be a '*window'* post exercise where protein intake immediately post exercise results in optimal muscle protein synthesis response (Candow and Chilibeck, 2008). However more recent research has shown that there is little to no evidence of the presence of such an anabolic window enhancing muscle protein synthetic response (Aragon and Schoenfeld, 2013). One other consideration of protein timing which may present an opportunity for maximising the impact of dietary protein is pre-sleep protein (Trommelen and van Loon, 2016). Pre-sleep protein ingestion has also been shown to improve mitochondrial synthesis post endurance exercise as well as enhance muscle protein synthesis within protein matched studies (Trommelen *et al.*, 2023). Although it must be considered that large dosages of protein may be needed to achieve these benefits, potentially of up to 40g of protein (Trommelen and van Loon, 2016).

4.6 Effects of protein on post-exercise recovery from exercise induced muscle damage (EIMD)

Along with the effect of protein on muscular protein synthesis, protein has been suggested to improve recovery from EIMD (Pearson, Hind and Macnaughton, 2022). EIMD is the transient period of post exercise decrements to performance, increased inflammation and oxidative stress and periods of stiffness and soreness which follows intense exercise (Clarkson and Tremblay, 1988; Owens *et al.*, 2018). EIMD is caused

by exposure to unaccustomed high intensity muscle contraction and, particularly eccentric muscle contractions (Clarkson and Sayers, 1999). It is commonly assessed through the use of muscle force measurements such as maximal voluntary contractions, muscle pain measurements and blood biomarkers of inflammation, oxidative stress and muscle damage (Warren, Lowe and Armstrong, 1999; Clarkson and Hubal, 2001).

While the role of protein on muscular adaptation through muscle protein synthesis is clear (Phillips and van Loon, 2011), the role on enhancing post exercise recovery from muscle damage is less clear (Owens *et al.*, 2018). A systematic review and meta-analysis of 27 RCTs investigating protein supplementation and its role in recovery from EIMD reported no measurable reductions in muscle soreness, muscle damage and no measurable enhancement to maintenance of muscle function (Pasiakos, Lieberman and McLellan, 2014). While a more recent systematic review and meta-analysis of 29 RCTs reports enhancements in maintenance of muscle function and reductions in creatine kinase but no effect on muscle soreness (Pearson, Hind and Macnaughton, 2022).

4.7 Bovine Milk Protein

Bovine milk represents an attractive food-based source of protein for athletes and active individuals. Bovine milk contains approximately 3.25% protein (Roy, 2008). The protein comprises of a ratio of roughly 80% casein and 20% whey (Kanda et al., 2016). Both whey and casein have complete amino acid profiles containing all nine essential amino acids (Herreman *et al.*, 2020). Whey in particular contains one of the highest proportions of branched chain amino acids, leucine in particular, of commonly consumed protein sources meaning it offers strong potential for shifting muscle protein balance in the favour of synthesis (Herreman *et al.*, 2020). Both whey and casein offer a highly digestible protein source with reported DIAAS scores of 85 for whey and 117 for casein in a recent extensive analysis of the digestibility of various protein sources (Herreman *et al.*, 2020). As a result of the essential amino acid content, the high leucine content in particular and the high digestibility of bovine milk protein, it has been shown to be an effective and efficient source of protein to promote muscle protein synthesis following exercise (Kanda et al., 2016). While the evidence of the role for isolated protein supplements to increase the rate recovery from EIMD is

mixed, protein rich foods may represent a beneficial alternative (Roy, 2008; Burd *et al.*, 2019). The protein, carbohydrate blend present in bovine milk has shown particular promise in this regard with promising results being observed recovery of muscle function from EIMD (Cockburn *et al.*, 2008; Cockburn, Bell and Stevenson, 2013; Rankin, Stevenson and Cockburn, 2015).

4.8 *Concluding Notes for the Role of Protein in Sports Nutrition*

The feeding of dietary protein is a key factor in ensuring a positive muscle protein balance resulting in important muscular adaptation to exercise (Phillips, 2012). However current DRVs for protein are outdated and inappropriate for those undergoing frequent exercise, especially resistance exercise (Phillips, 2007; Jäger *et al.*, 2017). Athletes should aim to achieve a minimum of 1.2g/kg/day plus of protein ingestion to achieve a positive muscle protein balance (Phillips and van Loon, 2011; Jäger *et al.*, 2017). This protein intake should be evenly spaced across the day into 3-4 meals (Phillips and van Loon, 2011). Animal based proteins offer advantages in terms of leucine content and digestibility over most plant-based proteins, and while it is possible to achieve a similar muscle protein synthesis response through plant-based proteins, a greater total protein intake may be required (Vliet, Burd and van Loon, 2015). Appropriate protein intakes can be achieved through dietary sources which may provide added benefit as a food matrix, for example the particular advantage being observed with bovine milk ingestion and the natural carbohydrate and protein blend present to support recovery as well as strong protein characteristics to support muscle protein synthesis (Cockburn, Bell and Stevenson, 2013; Rankin, Stevenson and Cockburn, 2015; Burd *et al.*, 2019).

5. Role of Polyphenols in Post Exercise Recovery and Endurance Exercise Performance

5.1 Polyphenols Overview

Polyphenols are a class of chemical compounds which contain multiple phenolic ring structures in their chemical make-up (Scalbert, Johnson and Saltmarsh, 2005). Polyphenols are non-nutritive phytochemicals which are found abundantly from a variety of food sources including various fruits, berries, teas among many others (Manach *et al.*, 2004). They exhibit several hydroxyl groups attached to two or more phenolic ring structures and as such there are various differing structures which fall under the 'polyphenol' umbrella term (Manach *et al.*, 2004). In fact there have been several thousand molecules discovered which exhibit a polyphenolic structure leading to large numbers of subclasses (Manach *et al.*, 2004) A table of common polyphenol food sources and subclasses is presented in Table 2 (Castro-Barquero *et al.*, 2020).

Polyphenols have been shown to exhibit antioxidant radical scavenging abilities, especially in-vitro, in a similar manner to that of antioxidant vitamins (Leopoldini, Russo and Toscano, 2011), however polyphenols may also provide further mechanisms for enhanced antioxidant action (Zhou *et al.*, 2019). Polyphenols are well known for their cardiovascular health benefits (Cory *et al.*, 2018), many of which have been shown to occur in dosages which are readily acquired from whole food sources (Heneghan *et al.*, 2018). Fruit derived polyphenols have been classed in "Group B" of the AIS sport supplement framework, stating that there is "Emerging scientific support, deserving of further research."

5.2 Polyphenols and Post Exercise Recovery

5.2.1 Potential Mechanism of Action

Polyphenols are one of the most widely researched plant-based bioactive groups to boast antioxidant effects (Scalbert, Johnson and Saltmarsh, 2005; Sureda *et al.*, 2014). Their ability to scavenge free radicals is thought to be beneficial for exercise recovery by reducing the secondary oxidative damage to healthy cell membranes surrounding EIMD. However, the capacity of polyphenols to accumulate in concentrations in vivo, in sufficient concentrations to produce a notable antioxidant effect through this

mechanism has been questioned (Schaffer and Halliwell, 2012; Forman, Davies and Ursini, 2014). Despite this, potent reductions in oxidative stress have been repeatedly shown with consistent polyphenol intake (Serafini *et al.*, 2000; O'Byrne *et al.*, 2002; García-Alonso *et al.*, 2006; Charles *et al.*, 2013).

More recent research has attempted to shed light on potential mechanistic evidence to support, highlighting the impact of polyphenols on the body's endogenous antioxidant systems through upregulation of the NRF2 – electrophile response element (also known as the antioxidant response element) pathway, through interactions with the NRF2 inhibitor KEAP1 (Birringer, 2011; Kanner, 2020; Zhou et al., 2019). This pathway provides a key regulatory function to phase II antioxidant enzymes and results in the production of greater levels of endogenous enzymes which regulate antioxidant activity such as superoxide dismutase and peroxiredoxin (Forman, Davies and Ursini, 2014; Huang *et al.*, 2015). Further human studies are required to confirm this as the primary mechanism of polyphenol antioxidant activity, especially post-EIMD. Yet the present evidence does appear to suggest this as the most likely cause of the in-vivo antioxidant effects which have been repeatedly shown.

Although the bioavailability of polyphenols is limited in humans, an emerging area of polyphenol research involves investigations into gut microbiome interactions (Sorrenti *et al.*, 2020) Large concentrations of polyphenols have been shown to reach the lower sections of the digestive system such as the colon through which polyphenols have been shown to impact positively on the composition of the gut microbiome (Singh *et al.*, 2019). The role of polyphenols on epigenetic transcription factors controlling key physiological functions such as NRF2 on antioxidant function has been attributed to the metabolism occurring in the gut microbiome which produces bioactive molecules which act directly on such transcription factors (Sorrenti *et al.*, 2020). To further elucidate the precise processes involved in this mechanism, further research employing omics technologies and bioinformatics approaches are necessary (Sorrenti *et al.*, 2020).

Table 2. Polyphenol subclasses and dietary sources (Castro-Barquero *et al.*, 2020)

Polyphenol Subclasses	Contribution, Mean (mg/d) ± SD, (%)	Polyphenol Contribution as Aglycones, Mean (mg/d) ± SD, (%)	Food Sources (% of Contribution)
Total polyphenols	846 ± 318	620.9 ± 273.5	
Flavonoids	491 ± 253, (58.0)	406.3 ± 237.2 (65.44)	
▪ Anthocyanins	43.5 ± 37.8, (5.14)	24.7 ± 21.7 (3.98)	Cherries (42.2), red wine (24.1), olives (10.5), strawberries (10.1), grape (9.30), other foods (3.8)
▪ Chalcones	0.009 ± 0.18, (<0.01)	0.006 ± 0.01 (<0.01)	Beer (100)
▪ Dihydrochalcones	1.72 ± 1.59, (0.20)	0.98 ± 0.91 (0.16)	Apples (93.2), fruit juices from concentrate (6.77)
▪ Dihydroflavonols	2.62 ± 4.92, (0.31)	1.81 ± 3.43 (0.29)	Red wine (97.6), white wine (1.80), rosé wine (0.59)
▪ Catechines	28.1 ± 22.4, (3.32)	27.1 ± 20.7 (4.36)	Tea (23.0), red wine (19.2), apples (18.6), chocolate (11.6), peaches (6.0), cocoa powder (3.18), fruit juices from concentrate (2.83), other foods (15.6)
▪ Proanthocyanidins	204± 185, (24.1)	200.7 ± 189.4 (32.32)	Chocolate (42.7), apples (20.4), plums (9.53), red wine (7.09), cocoa powder (5.68), strawberries (4.20), other foods (10.4)
▪ Theaflavin	0.70 ± 1.81, (0.08)	0.57 ± 1.46 (0.09)	Tea (100)
▪ Flavanones	83.2 ± 76.6, (9.83)	58.1 ± 55.0 (9.35)	Oranges (71.3), natural orange juice (23.0), fruit juices from concentrate (3.22), other foods (2.09)
▪ Flavones	73.2 ± 47.4, (8.65)	54.7 ± 32.9 (8.81)	Whole-grain bread (30.0), bread (23.6), oranges (21.6), natural orange juice (8.53), artichoke (3.80), other foods (12.5).
▪ Flavonols	54.0 ± 22.3, (6.40)	35.6 ± 15.3 (5.73)	Onions (27.8), spinach (26.7), lettuce (11.9), red wine (6.02), olives (5.10), asparagus (4.93), other foods (17.55)
▪ Isoflavonoids	0.002 ± 0.004, (<0.01)	0.002 ± 0.003 (<0.01)	Beer (100)
Phenolic acids	280 ± 131, (33.1)	164.2 ± 70.8 (26.44)	
▪ Hydroxybenzoic acids	15.5 ± 10.3, (1.83)	20.5 ± 12.4 (3.30)	Red wine (21.2), olives (19.9), walnuts (18.1), tea (9.46), swiss chard leaves (6.15), white wine (1.34), other foods (23.8)
▪ Hydroxycinnamic acids	264 ± 129, (30.9)	141.6 ± 66.8 (22.80)	Decaffeinated coffee (37.7), coffee (26.1), plums (5.66), potatoes (5.50), olives (4.21), red wine (1.79), other foods (19.0)
▪ Hydroxyphenylacetic acids	0.90 ± 1.04, (0.10)	1.16 ± 1.40 (0.19)	Olives (87.2), red wine (6.57), beer (3.86), extra virgin olive oil (1.52), white wine (0.65)
▪ Hydroxyphenylpropanoic acids	0.48 ± 0.65, (0.06)	0.91 ± 1.23 (0.14)	Olives (100)
Stilbenes	2.13 ± 3.92, (0.25)	1.78 ± 3.19 (0.29)	Red wine (91.9), white wine (3.94), grapes (1.60), rosé wine (1.21), other foods (0.07)
Lignans	1.53 ± 0.56, (0.18)	1.33 ± 0.55 (0.21)	Extra virgin olive oil (16.7), seeds (9.84), oranges (9.73), green bean (5.42), pepper (5.32), peaches (4.97), broccoli (4.71), bread (4.48), red wine (4.16), cabbage (2.77), other foods (31.9)
Other polyphenols	70.8 ± 41.5, (8.37)	45.6 ± 27.8 (7.34)	
▪ Alkylmethoxyphenols	0.93 ± 0.87, (0.11)	0.93 ± 0.87 (0.15)	Decaffeinated coffee (74.1), coffee (16.2), beers (9.77)
▪ Alkylphenols	13.7 ± 17.8, (1.62)	13.8 ± 18.5 (2.23)	Whole-grain bread (69.1), whole-grain pastries (14.8), breakfast cereals (8.40), pasta (3.29), other foods (4.41)
▪ Furanocoumarins	0.37 ± 0.38, (0.04)	0.37 ± 0.39 (0.06)	Celery stalks (98.3), grapefruit juice (1.7)
▪ Hydroxybenzaldehydes	0.42 ± 0.65, (0.05)	0.42 ± 0.66 (<0.01)	Red wine (78.9), walnuts (14.5), beer (2.61), white wine (1.95), other foods (2.04)
▪ Hydroxybenzoketones	0.002 ± 0.004, (<0.01)	0.002 ± 0.003 (<0.01)	Beer (100)
▪ Hydroxycoumarins	0.10 ± 0.19, (0.01)	0.09 ±0.18 (<0.01)	Beer (73.6), white wine (26.3), cocoa powder (0.10)
▪ Methoxyphenols	0.13 ± 0.12, (0.01)	0.11 ± 0.12 (0.01)	Decaffeinated coffee (81.3), coffee (18.7)
▪ Naphtoquinones	0.82 ± 1.12, (0.09)	0.84 ± 1.14 (0.14)	Walnuts (100)
▪ Tyrosols	52.4 ± 37.8, (6.19)	30.0 ± 21.2 (4.83)	Olives (50.0), extra virgin olive oil (34.8), refined olive oil (5.17), red wine (3.29), other foods (6.74)
▪ Other	1.96 ± 2.30, (0.23)	0.66 ± 0.54 (0.11)	Orange juice (45.4), pears (18.2), coffee (16.0), other fruit juices (9.98), olives (5.86), other foods (4.56)

5.2.1 Polyphenols and Maintenance of Post- Exercise Muscle Function and Reduced Soreness

A considerable number of RCTs of varying quality have been conducted to investigate the impact of polyphenol rich foods, concentrates, supplements, extracts and whole foods on recovery from exercise induced muscle damage. In terms of health claims on food products/supplements, the EFSA have published guidance on the measurement of muscle recovery claims stating that to measure recovery, tests of muscle performance parameters should be performed in a test-retest manner before and after a strenuous bout of exercise (Turck *et al.*, 2018). Measures of subjective perceived muscle soreness and fatigue can also be used to as supportive evidence (Turck *et al.*, 2018). Many RCTs have been conducted using these outcomes to assess the impact of polyphenols on post exercise recovery. Numerous RCTs have investigated the impact of acute polyphenol consumption on EIMD with most showing no impact on outcomes on post exercise recovery such as muscle strength and pain post exercise (Jówko *et al.*, 2012; Kim and So, 2019; Rojano-Ortega, 2021). Further RCTs have investigated the ability of longer-term polyphenol ingestion on recovery from EIMD, arising to the publication of a number of key meta-analyses (Doma, Gahreman and Connor, 2020a; Carey, Lucey and Doyle, 2021; Rickards *et al.*, 2021). A meta-analysis carried out as part of this thesis showed improvements in maintenance of muscle strength and reduced muscle soreness post exercise associated with 6 or more days of polyphenol ingestion and will be presented in full in a later chapter of this thesis (Carey, Lucey and Doyle, 2021). Another meta-analysis of 25 RCTs reports improvements in recovery of muscle from polyphenol supplementation which reported up to 13% improvement in muscle function (Rickards *et al.*, 2021). This review also notably reported considerably improved reduction of muscle soreness post exercise of up to 29% reduction occurring at 72 hours post exercise (Rickards *et al.*, 2021). This review included short (2-3 days) as well as longer term supplementation within their included studies (Rickards *et al.*, 2021). Doma and colleagues in a systematic review and meta-analysis of 25 RCTs also report significantly improved recovery of muscle strength associated with prolonged ingestion of polyphenol rich fruit supplements in a pooled analysis which included both acute and chronic polyphenol ingestion RCTs (Doma et al., 2020). This review also showed significant reductions in the concentrations of several biomarkers of muscle damage, inflammation and oxidative stress which was associated with improved post exercise recovery (Doma et al., 2020).

Furthermore, a systematic review and meta-analysis of anthocyanin rich whole fruit consumption which included 22 RCTs reported benefits for anthocyanin rich whole fruit reducing post exercise inflammation and oxidative stress, indicating that the benefits can been obtained through whole food solutions (Bloedon *et al.*, 2019). They recommended that an effective dosage of >100mg of anthocyanins be consumed 2 to 3 times daily to induce positive effects on oxidative stress and inflammation (Bloedon *et al.*, 2019).

5.2.2 Polyphenols and Recovery, Remaining Knowledge Gaps

While the efficacy of polyphenol products for recovery from exercise induced muscle damage is certainly promising given the strength of results from these systematic reviews and meta-analyses, there are still notable knowledge gaps. It is clear that a greater understanding of the precise mechanisms of action and their interaction with the gut microbiome is required to enable targeted products which influence correct transcription factors for the desired function (Sorrenti *et al.*, 2020). In RCT reporting there is often a very poor characterisation of polyphenol composition and dosage of study treatments employed which has severely hampered the elucidation of optimal dosing and polyphenol subclasses (Carey, Lucey and Doyle, 2021). A key example is that many important RCTs have only reported the dosage of the extract or food source which is providing the polyphenols but have not measured the precise polyphenol concentration present in the intervention (Arent *et al.*, 2010; Trombold *et al.*, 2010, 2011; Bell *et al.*, 2015; Jajtner *et al.*, 2016). Bowtell and Kelly in their comprehensive narrative review have postulated that the total polyphenol dosage required for recovery benefit is >1000mg per day for 3 or more days prior to and following exercise, and while this is likely the most appropriate guidance to follow at this moment the empirical data to support this statement is lacking (Bowtell and Kelly, 2019). Bloedon et al., (2019) have proposed a dosage strategy of >100mg anthocyanins two to three times per day for exercise induced oxidative stress and inflammation. While two individual studies have reported early evidence for a dose response relationship further dose response RCTs and ultimately meta-regressions are of critical importance once appropriate characterisation is also included within RCT reporting (Bloedon et al., 2019; Cook et al., 2017; Herrlinger et al., 2015).

5.3 Polyphenols and Training Adaptation

In recent years there has been considerable concern in relation to the use of high dose antioxidant supplementation alongside exercise training due to the potential for a blunting of the training effect (Merry and Ristow, 2016). Reactive oxygen species released during exercise do pose a risk of secondary oxidative muscle damage resulting in exacerbated post exercise reductions to muscle function (Peake *et al.*, 2017). However reactive oxygen species also play an important role in cell signalling in a hormetic manner, resulting in key processes integral to the adaptive response to exercise (Merry and Ristow, 2016). These concerns arise from studies investigating the role of antioxidant vitamins C and E and their role alongside exercise training (Paulsen, Cumming, Hamarsland, *et al.*, 2014; Paulsen, Cumming, Holden, *et al.*, 2014). Due to the contrasting mechanisms of these antioxidant effects exhibited by polyphenols compared to that of vitamins C and E, it has been suggested that polyphenols may not attenuate training adaptation in the same manner as these vitamin antioxidants (Bowtell and Kelly, 2019). Polyphenols augment endogenous antioxidant systems rather than directly scavenge radicals in the manner of antioxidant vitamin supplementation, and as a result may preserve this adaptive signalling response (Bowtell and Kelly, 2019). However empirical data supporting this theory is lacking. Two key randomised controlled trials in this area have shown early promising results with no attenuation of training adaptation being observed with longer term polyphenol supplementation (Kuo *et al.*, 2015; Beyer *et al.*, 2017), however further research is required. Until further evidence categorically rules out this theory of blunted exercise adaptation, athletes must be cautious with the long-term use of polyphenol supplements, however in periods where recovery is critical, but adaptation is not, polyphenols may be an attractive proposition.

5.4 Polyphenols for Exercise Performance

While polyphenols have been shown to play a role in the recovery from strenuous exercise, there is also an emerging body of evidence suggesting that polyphenols may also play a role in improving acute endurance exercise performance (Bowtell and Kelly, 2019). It is suggested that the intake of polyphenols may increase mitochondrial biogenesis through increasing expression of the NRF2 gene, a similar mechanism as has been suggested to cause the antioxidant effect (Dos Santos *et al.*, 2018). This gene

has been strongly linked with endurance performance with greater expression observed in elite endurance athletes compared with sprinters and control groups (Eynon *et al.*, 2009). Polyphenols have also been purported to play a role in the production of nitric oxide (NO) (Ghosh and Scheepens, 2009). Nitric oxide (NO) has been shown to be a potent vasodilator and may enhance muscle contractility (Stamler and Meissner, 2001). As such, NO plays a role in sports where blood flow and cardiac output impact on the delivery of oxygen to working muscle cells, namely endurance events (Stamler and Meissner, 2001).

To investigate the impact of polyphenols on endurance performance, a number of RCTs have been undertaken. Many of these studies however have used small sample sizes and as such it is difficult to draw conclusion from any one particular RCT. However, a recent meta-analysis of these smaller trials has synthesised these results and reports that supplementation with polyphenols for a minimum period of 7 days, provides a moderate benefit on performance in endurance tasks (Somerville, Bringans and Braakhuis, 2017). This meta-analysis includes 14 RCT's with a total of 348 participants who consumed an average of 688 ± 478mg of polyphenols per day. A subgroup analysis of 7 RCT's using the polyphenols subclass quercetin found a further improvement in performance when compared with the use of other polyphenols. However, 5 of these 7 RCT's used a quercetin dosage of 1000mg/day, considerably higher than the pooled average of the 14 RCTs included within the meta-analysis. As a result, it remains unclear as to whether these findings are a result of a dose response relationship, which has been shown elsewhere in polyphenol research (Herrlinger, Chirouzes and Ceddia, 2015; Cook *et al.*, 2017), or a result of specific properties exclusive to quercetin itself. Further research should be carried out to clarify the benefits of specific flavonoids and their potential to impact on various aspects of endurance performance.

While this meta-analysis focused primarily on the impact of flavonoid polyphenols, some research has also been undertaken investigating the effects of phenolic acids (You *et al.*, 2010). The phenolic acid, ferulic acid has been added to various products of late for its performance enhancing effects. It can be found commonly in the leaves of certain variations of the bamboo plant (You *et al.*, 2010). The scientific evidence supporting its use however is confined to animal models. Ferulic acid supplementation has shown positive effects on TTE trials and during repeated fatiguing exercise in mice

(You et al., 2009; You et al., 2010). The application of these results is tentative without specific RCT's carried out in human populations and there is a dearth of more recent evidence progressing knowledge in this area.

5.5 Concluding Note on Polyphenols in Performance Nutrition

Polyphenols are non-nutritive bioactive phytochemicals, commonly found in fruits, teas and beans among other plant-based sources, they are well known for their antioxidant effects (Scalbert, Johnson and Saltmarsh, 2005; Sureda *et al.*, 2014). Their bioactive properties, once attributed to radical scavenging are now thought to arise through epigenetic interactions with the NRF2 gene, potentially as a result of interactions in the gut microbiome (Zhou *et al.*, 2019). These interactions have led to RCTs showing improvements in post exercise recovery from EIMD and enhanced endurance performance (Bowtell & Kelly, 2019; Doma et al., 2020; Rickards et al., 2021; Somerville et al., 2017). While precise dosages and subclass characterisation require further research there is considerable potential for their use in sporting activities and for future new product development ventures. Particularly since their bioactive efficacy can be easily achievable in dosages present in whole food sources.

6 Rationale for a High Protein Dairy Beverage Enriched with Blackberry Polyphenols

6.1 Synergistic Effects of Polyphenols and Protein?

It is clear from the research presented in this review that both polyphenols and dietary protein can play a considerable role in an athletes' performance nutrition strategy. There is considerable scientific evidence behind the consumption of dietary protein to support muscle repair and regeneration along with exercise training (Phillips, 2012). While polyphenols can help athletes in reducing the time and magnitude of performance decrements following intensive training bouts (Doma et al., 2020; Rickards et al., 2021); a combination of polyphenols and protein represents a formidable combination in theory given the potential benefits to reducing the time spent in a performance decrement while also enhancing the muscular adaptive response.

Despite this combination representing a promising nutrient combination, few studies have investigated the combination of proteins and polyphenols in the context of sport and exercise performance and recovery. One RCT used water soluble polyphenols from blueberry and green tea extract combined with a soy protein complex over a 17-day period which included a 3-day running regimen (Nieman *et al.*, 2013). This study showed no significant impact of the polyphenol protein blend on exercise induced oxidative stress or inflammation but did observe enhanced gut-derived phenolic signature (Nieman *et al.*, 2013). Using a metabolomics approach this RCT observed that metabolites characteristic of gut bacteria metabolism of polyphenols increased with the intake of the polyphenol soy protein complex (PSPC) and intensive exercise compared to the placebo powder. Specifically, there was a notable increase in hippurate, 4-hydroxyhippurate, and 4-methylcatechol sulfate. Additionally, exercise induced fatty acid oxidation and ketogenesis occurred in both groups, but PSPC supplementation led to a greater increase in ketone bodies (3-hydroxybutyrate) post-exercise. This suggests that while PSPC did not significantly alter traditional markers of inflammation and oxidative stress, it was associated with enhanced gut-derived phenolic metabolism and ketogenesis during recovery from heavy exercise (Nieman *et al.*, 2013). This adds further weight to the theory that for polyphenols, the mechanism of action does not arise from the traditional radical scavenging mechanisms commonly observed with vitamin antioxidants. The authors also

theorised that by providing this polyphenol stimulus via a protein complex, the stability and bioavailability of polyphenols was improved (Nieman *et al.*, 2013).

Another RCT using the same polyphenol protein blend observed reduced exercise induced susceptibility to viral infection (Ahmed *et al.*, 2014). Only one RCT has combined polyphenols with dairy to date in the context of performance nutrition (Kung *et al.*, 2022). This RCT investigated the impact of flavonoid polyphenols combined with milk to support physiological response to exercise induced intestinal injury arising from submaximal cycling in 12 cyclists (Kung *et al.*, 2022). They investigated a high (490mg) versus a low (5mg) dosage of polyphenols in a milk beverage ingested for 15 days surrounding a 45-minute submaximal cycling bout followed by a 15-minute maximal effort time trial. This study showed no significant change to intestinal permeability or injury post exercise associated with polyphenol and milk ingestion and no difference in distance covered in the 15-minute maximal effort (Kung *et al.*, 2022).

It is clear that there is a notable knowledge gap surrounding the impact of co-ingestion of polyphenols and dairy protein in the context of sport and exercise. There appears to be theoretical support for the use of a polyphenol enriched protein product to support muscle regeneration and repair post exercise, arising from RCTs investigating polyphenols and protein in isolation however further research is required to establish whether the theory of their co-ingestion bear's fruit.

6.2 Food Matrix Effects on bioavailability

As well as the clear theoretical synergistic effects of polyphenols and protein for sports nutrition, there is also potential for the enhancement of polyphenol absorption through their delivery in a protein rich food source (Sun, Sarteshnizi and Udenigwe, 2022). Polyphenol protein conjugates and complexes result in alterations to their structural and physiochemical properties causing a protective effect on polyphenols during digestion (Zhou *et al.*, 2020).

The fat content of milk may also play a role in the bioavailability of polyphenols (Zhong *et al.*, 2017). It has been shown that the pharmacokinetic profile of polyphenols often occurs in a biphasic manner owing to subsequent fat ingestion (Martínez-Huélamo *et al.*, 2016). It has been shown that blueberry polyphenols exhibit

a second peak in plasma polyphenol metabolites correlating with subsequent fat ingestion suggesting enterohepatic recirculation through storage in the gall bladder and re-secretion through bile production in response to fat (Zhong *et al.*, 2017). An increase in tomato polyphenols was also observed when co-ingested with a fat source (Martínez-Huélamo *et al.*, 2016).

Overall, it appears that the protein, carbohydrate and the fat content of milk represent a favourable vehicle for delivery of polyphenols to the system along with the synergistic effects of both polyphenols and protein for muscle.

6.3 Use of Blackberry Polyphenols

Although there is a dearth of previous research investigating the effects of blackberry polyphenols at enhancing endurance exercise performance or recovery following intensive exercise, blackberries present a promising theoretical source of polyphenols for use by athletes and active individuals. Blackberry polyphenols have been shown to have an extensive polyphenolic composition predominantly containing anthocyanins cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside, but uniquely also containing significant concentrations of ellagitannin and catechin polyphenols (Tzima et al., 2023). Dietary anthocyanins in the form of New Zealand blackcurrant have been shown to enhance endurance performance through vasoactive properties including the increasing the bioavailability of NO resulting in vasodilation (Murphy et al., 2017, Cook & Willems, 2018). The anthocyanins in Montmorency tart cherries have also been shown to consistently improve post exercise recovery from following strenuous exercise (Hill et al., 2021). Anthocyanin rich whole fruit consumption has also consistently shown benefits to exercise induced oxidative stress and inflammation (Bloedon et al., 2019). Ellagitannins have also been attributed to enhancing post exercise recovery in RCTs investigating pomegranate ellagitannins (Trombold et al., 2010, 2011), while green tea catechins have been shown to improve exercise induced oxidative stress and inflammation (Sugita et al., 2016). Blackberries represent an encouraging and comprehensive source of polyphenols for use by athletes and active individuals, however, require considerable further research to substantiate their theoretical ergogenic effects.

7 Conclusion

This comprehensive literature review has elucidated the roles of both polyphenols and dairy protein in relation to exercise performance and recovery, offering significant insights with broader implications not only for academia but also for the sports nutrition industry and new product development ventures.

Polyphenols, a vast group of phytochemicals, have potent antioxidant properties which could aid in diminishing exercise-induced muscle damage and inflammation (Bowtell & Kelly, 2019; Doma et al., 2020). In addition, they are hypothesized to enhance endurance performance and exercise adaptation via involvement in mitochondrial biogenesis and nitric oxide production (Somerville, Bringans and Braakhuis, 2017). Yet, it must be emphasized that this body of research remains in its early stages. The future direction necessitates more rigorous investigations to affirm these findings including improved characterisation of study treatments and a more nuanced understanding of indicative dosage strategies underpinned by quantifiable evidence.

Dairy protein, specifically derived from cow's milk, is noted for being a complete source of essential amino acids, including leucine, and being highly digestible (Cockburn *et al.*, 2008; Roy, 2008). Consuming it post intense physical activity has been shown to stimulate muscle repair and regeneration, thereby facilitating quicker recovery and potentially enhanced performance (Cockburn *et al.*, 2008; Roy, 2008; Cockburn, Bell and Stevenson, 2013; Rankin, Stevenson and Cockburn, 2015; Kanda *et al.*, 2016).

Drawing upon these insights, the possibility of developing functional foods, particularly combining polyphenols and dairy protein, offers a promising avenue for sports nutrition. This proposed synergy might provide athletes with a comprehensive nutritional strategy, promoting quicker recovery and advanced muscular adaptation. However, the existing evidence largely pertains to the separate effects of polyphenols and proteins, thereby leaving a considerable knowledge gap regarding the concurrent effects of these nutrients.

This endeavour to develop an evidence-based bioactive functional food could have profound effects on the wider performance nutrition industry. It could set the precedent for future product development efforts, emphasizing the importance of an evidence-based approach to formulating new functional foods.

In conclusion, the co-ingestion of polyphenols and dairy protein for sports and exercise nutrition is an area ripe for exploration. The potential advantages of such a combination, despite being under-investigated, are promising and warrant detailed research. This task represents an exhilarating interdisciplinary opportunity, where academia and industry can collaborate to advance performance nutrition science and its application, ultimately benefitting athletes and consumers. The project thus sets a vital precedent for evidence-based practice in new product development, fostering innovation rooted in rigorous research.

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Chapter 3 – Nutritional Priorities, Practices and Preferences of Athletes and Active Individuals in the Context of New Product Development in the Sports Nutrition Sector

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Abstract

Introduction: Sports nutrition is a rapidly growing sector with increasing demand for evidence-based nutritional products to support competitive and healthy lifestyles. The product development process for novel foods should rely heavily on end-user engagement to facilitate future success, however there is a dearth of published information available. An understanding of the practices and self-reported nutritional priorities of athletes and active individuals is required for the development of new food products, facilitating evidence-based product formulation.

Methods: Participants were at least 18 years of age, actively participating in competitive sport or structured physical activity on at least two occasions per week. Participants were asked to undertake a comprehensive online survey assessing their nutritional practice, perceived nutritional priorities and preferences for product characteristics. Questions were developed on the basis of critical evaluation of the current scientific literature and the hosting of two scoping focus group sessions with prospective end-users.

Results: 405 individuals (29±9 years) completed this questionnaire. 295 participants reported active participation in competitive sport while the remaining 110 participants undertook structured physical activity exclusively. When asked to rank their top three most sought-after product claims in sports nutrition, 'enhanced muscular recovery' was the most prioritised receiving 101 first choice preferences (25%) and 295 top 3 preferences. Fifty-eight percent of participants reported taking nutritional supplements. Caffeine containing functional foods (excluding caffeine supplements) were the most commonly used functional food group. A very low incidence of functional food usage was reported otherwise. When asked to rank the importance of various food product attributes, 'nutritional profile' was ranked as the most important with rating of 3.37±0.7 out of 4 followed by 'taste' and 'accessibility'. Whole food nutritional products received the most first preference selections and most top 3 selections when presented with a number of popular performance and recovery products on the market.

Conclusions: The transition towards a food first approach in sports nutrition is vital for athletes and active individuals to achieve their goals; with the development of evidence-based functional foods, particularly with a focus on muscle recovery, endurance, and strength enhancement at the forefront for new food product design and innovation.

1. Introduction

Sport and exercise nutrition is a rapidly growing sector, with the global market valued at $40 billion USD in 2021 and projected to grow annually by 8.5% between 2022 and 2030 (Sports Nutrition Market Size Worth $82.3 Billion By 2030). This increasing global demand has been matched with additional availability of evidence-backed nutrition solutions, some of which have been proven to assist athletes with performance, enhance post exercise recovery, and augment body composition (Kreider *et al.*, 2018) The global increase in life expectancy which parallels concomitant increases in the prevalence of chronic disease, has resulted in growing demand for evidence-backed solutions to support population health (Crimmins, 2015). Given that physical activity (Rhodes *et al.*, 2017) and diet and nutrition (Roberts and Barnard, 2005) are two of the most effective and widely used prophylactic approaches for chronic disease prevention, the pragmatic development of food-based solutions not just for athletes, but to support healthy and active lifestyles across the lifespan is imperative. (Kerksick *et al.*, 2018). The development of an evidence base to enable and underpin new product development strategy is essential to support the forthcoming growth in this sector and to tackle the challenge of credibility of product claims within this sector.

Advances driven by both the scientific community and food industry have underpinned a clear transition towards personalised (Jose Ordovas, 2018; Pickering and Kiely, 2018) and periodised (Stellingwerff, Morton and Burke, 2019) nutritional practices, where a number of products have been shown to be efficacious in randomised controlled trials, when best practice protocols are applied to appropriate population groups (van de Walle and Vukovich, 2018; Nielsen, Lambert and Jeppesen, 2020; Gomez-Bruton *et al.*, 2021; Wu *et al.*, 2022). However, converting these positive findings from highly controlled scientific trials to free living situations is a considerable challenge for this industry. Furthermore, the availability of information about nutritional strategies for these population groups has increased dramatically in recent years related to the rise of internet and social media use, with Bourke et al. (2019) reporting 65% of athletes using social media for nutrition purposes in the previous 12 months (Bourke, Baker and Braakhuis, 2019a). While the internet and social media provides end-users with rapid and cost-effective access to nutritional information, the conditions and caveats that arise from personalised and periodised approaches are often difficult to fully comprehend through the communication limitations of social media. This poses notable difficulties for athletes and active individuals with some athletic groups in

recent years even exhibiting poorer nutrition knowledge than their community counterparts (Spendlove *et al.*, 2012) while others have reported that while general nutrition messages were well understood, notable gaps in nutrition knowledge were present (Brooke L. Devlin and Belski, 2015). To date much of the literature assessing the perceived nutritional priorities of this population has focused on assessing nutrition knowledge in athletes and their support staff, with a number of studies suggesting a critical need for greater food and nutrition literacy (Lamarche *et al.*, 2014; Brooke Lea Devlin and Belski, 2015; Andrews *et al.*, 2016). It is clear that those with access to qualified nutritional professionals such as sports dietitians are at a considerable advantage on this front (Wardenaar and Hoogervorst, 2022). Potential reasons for this disparity may be poor access to qualified nutrition professionals among different sporting groups, particularly for those not at elite level and those of lower socioeconomic backgrounds (Torres-McGehee *et al.*, 2012; Burke, Meyer and Pearce, 2013; Beck *et al.*, 2015). It is clear that improving nutrition and health literacy in the athletic population represents a significant opportunity to improve both performance, health and wellbeing (Tam *et al.*, 2019; Foo *et al.*, 2021). Gaining a quantifiable understanding of the current practices is critical to shape future food innovations in the sports nutrition sector, allowing for greater understanding of where gaps in knowledge exist and whether current perceived priorities of this population are misevaluated.

Factors influencing food choice in athletes include culture, physiological demands, and socioeconomic factors amongst others, highlighting the complexity of food choice in sporting populations (Birkenhead and Slater, 2015). However, one study showed that within NCAA division I and III athletes, there was no influence of demographic or athletic characteristics on supplement usage (Hamilton *et al.*, 2022). Wesana et al. (2020) analysed the brand equity and preference for sports nutrition brands concluding that sporting factors such as competition level and sport type as well as more generic socio-demographic determinants including age, gender and education play a considerable role in brand perception and food choice among consumers (Wesana *et al.*, 2020). The characteristics of the food product itself also have an impact on whether a product will be adopted by the target population in free-living scenarios. It is well established that sensory factors such as taste, aroma, texture and appearance play a crucial role in food choice within the general population (Clark, 2022). While these may not be quite as important in elite athletic populations (Birkenhead and Slater, 2015), they may prove to be the deciding factor when

two competing sports nutrition products have physiologically equivalent outcomes. A key example of this product optimisation has been the emergence of low volume, high nitrate concentrated beetroot juice shots in place of larger volumes of standard beetroot juice. Despite this however, there is a complete dearth of scientific knowledge investigating athletes and active individuals' preference for sensory characteristics of sports nutrition food products despite their importance for food choice and decision to purchase.

Taking a broad overview of the sports nutrition sector, it is unclear as to the appropriate focus when aiming to design and produce products to bring to the sport and exercise market. Due to the multitude of factors affecting food choice (Birkenhead and Slater, 2015), it is largely unclear which prototype may develop into a successful product in the market. Combine this with the reported key nutritional knowledge gaps reported in these populations (Spendlove *et al.*, 2012; Brooke L. Devlin and Belski, 2015), it is exceptionally difficult for the sports nutrition sector to effectively identify the correct avenue for future new product development ventures in order to develop a scientifically proven efficacious product that will be adopted by the end-user. This novel cross-sectional research was conducted with an overarching objective to inform key priorities and design elements for future new product development through assessment of current practices, perceived nutritional priorities and product preferences of athletes and active individuals.

2. Methods

2.1 Research Approach

The research tool (a comprehensive online survey questionnaire) underpinning the data presented in this study was developed using a combination of critical review of current scientific evidence, in conjunction with end-user engagement through the conduction of two scoping focus group sessions with athletes and active individuals of mixed backgrounds and abilities (Appendices 10 & 11). The findings of these focus groups enabled the design of the key questions utilised in the online questionnaire allowing the targeted identification of particular disconnects between current product offerings and the needs of end-users, both known and hidden. Data from these focus group sessions were analysed and did not reach idea saturation while further focus groups sessions were cancelled due to the onset of the covid-19 pandemic. Through critical review of the available literature investigating nutritional knowledge, practices and food choice within sport and exercise, knowledge gaps were identified which are of high value for informing future product development and the creation of this holistic research tool (Zinn, Schofield and Wall, 2005; Sobal and Bisogni, 2009; Trakman *et al.*, 2017, 2018; Corallo *et al.*, 2019; Thurecht and Pelly, 2019). Notably, these included the lack of published publicly available data to underpin design elements of new sports nutrition products such as product claim prioritization, the relative importance of nutritional and product characteristics to the end user and consumer preferences for various product forms currently available on the market.

2.2 Participants

Participants in this study were at least 18 years of age. The targeted population group for this study was athletes and active individuals which was defined as anyone undertaking competitive sport or actively participating in a form of physical activity program on at least two occasions per week. This was to ensure that participants were at least at a level where diet and nutritional practices would be potentially influential to their exercise or sporting performance and represented the predominant end-user group of sports nutrition products. Participants were recruited through social media advertisement, internal university email list and word of mouth. Clubs, organizational bodies and sporting facilities were also approached to distribute this survey to their athletes and members. A convenience sampling method was

used to achieve this sample and the sample size is in line with that of similar survey-based research in nutrition, food choice and sports nutrition (Ares and Gámbaro, 2007; Torres-McGehee *et al.*, 2012; Bourke, Baker and Braakhuis, 2019b; Corallo *et al.*, 2019).

2.3 Procedures

This questionnaire was granted ethical approval from the Social Research Ethics Committee of University College Cork (log 2020-046) (Appendix 9.). All responses were provided anonymously, and no identifying data was collected. Prior to participation, participants were presented with the purpose of the questionnaire, their role within the research and were given contact details of the researchers should they have any questions regarding the research project. Following this, participants were required to provide their informed consent to participate.

This questionnaire was carried out using the online platform Qualtrics (Qualtrics, Utah, USA) between April and June 2021. The questionnaire utilised a series of thirty-four questions including multiple-choice questions, rating scale questions, rank order questions and matrix scale questions (Appendix 12). Questions were designed to assess the current priorities, practices and preferences of athletes and active individuals regarding their nutrition and food choices. Questions related to preferences for product characteristics and food types were based of common product offerings in the sports nutrition market following review of various a wide array of sports nutrition product offerings available in Ireland and online.

2.4 Data Analysis

Data was extracted from Qualtrics for further statistical analysis. All data was analysed statistically using IBM SPSS (IBM SPSS Statistics version 26.0, IBM Corp, NY, USA). Data is described as mean ± standard deviation (±SD), frequencies and percentages where appropriate. Certain questions contained open text fields labelled “other” which were grouped for analysis when possible and analysed quantitatively. All data was initially analysed as an entire dataset, following this, various questions were analysed by subgroup using Mann Whitney U tests. Subgroups assessed were gender, competition level and total activity time per week. Statistical significance was set at $P < 0.05$ for all statistical tests.

3. Results

3.1 Demographics

Four-hundred and five (164 female, 241 male) athletes and active individuals successfully completed this questionnaire and were eligible for inclusion in analysis. The mean age of respondents was 29±9 years. A total of 295 participants (73%) reported active participation in competitive sport while the remaining 110 participants (27%) undertook a non-competitive structured physical activity programme exclusively. Participants reported undertaking 10.3±6.6 hours per week of either sporting or structured physical activity with those involved in competitive sport reporting 8.4±4.9 hours of sporting activity or physical preparation for competition per week alongside an additional 4.5±4.0 hours of structured physical activity separate from their sporting activity.

Table 1. Demographics of questionnaire respondents (n = 405)

	Mean ± SD (range)	**Frequency (%)**
Gender		
Males		241 (59.5%)
Females		164 (40.5%)
Age (yrs.)	29 ± 9 (18 to 64)	
Total Activity Time Hours per week	10.3 ± 6.6 (1 to 51)	
Competitive Sport Participation		
Yes		295 (72.8%)
No		110 (27.2%)
Sport Category		
R.I.D.S		192 (58.9%)
Endurance		37 (11.3%)
Combat		27 (8.3%)
Aquatic		23 (7.1%)
Racket		22 (6.7%)
Resistance/power		10 (3.1%)
Dance		5 (1.5%)
Equestrian		3 (0.9%)
Other		7 (2.1%)
Level of Competition		
International		31 (10.5%)
National		56 (19.0%)
Regional		73 (24.8%)
Local		133 (45.2%)

RIDS – Random intermittent dynamic type sports e.g. Soccer, Rugby, Basketball

3.2 Food and Nutritional Priorities of Athletes and Active individuals

3.2.1 Rank your top 3 product claims when choosing whether to use a performance food or product

When asked to rank their top three most sought-after claims of sports nutrition products, '*enhanced muscular recovery'* received the most first choice preferences at 25% (n=101); while 73% (n=295) participants ranked enhanced muscular recovery in their top 3 priorities overall (Fig. 1.). '*Improving endurance*' and '*improving strength and power*' were the next most sought-after nutritional product claim with 58% (236) and 57% (234) of participants rating in their top 3 priorities respectively. However, '*improving endurance'* received considerably more top priority selections (24%, 96) compared to *'improving strength and power' (16%, 66)*.

3.2.2 Does your engagement in physical activity frequently cause a stiffness and/or pain in your muscles in the hours and/or days following exercise? If so, how frequently do you experience this stiffness or pain?

Seventy percent of participants reported frequently experiencing muscular pain and stiffness post exercise, with 56% (228) reporting pain or stiffness at least once per week.

Table 3. Importance of product features and scientific proof on nutrition product choice using a scale of 1 (not important) to 4 (crucial). (n=405)

On A Scale of 1-4 how important would the following features be in your decision to purchase a food for performance / recovery:					
	1 *(not important)*	**2**	**3**	**4** *(crucial)*	**Mean Score ± SD**
Nutritional Profile	<1%	7%	46%	46%	3.37±0.65
Taste	6%	9%	51%	35%	3.15±0.80
Accessibility	2%	16%	53%	29%	3.09±0.72
Ease of Preparation	4%	20%	47%	29%	3.02±0.80
Price	4%	15%	57%	24%	3.01±0.74
Sustainability	16%	37%	34%	14%	2.45±0.92

On a scale of 1-4, how important is it that the effects of a sports nutrition product have been scientifically proven?					
	1 *(not important)*	**2**	**3**	**4** *(crucial)*	**Mean Score± SD**
Scientific Proof	<1%	13%	41%	45%	3.31±0.72

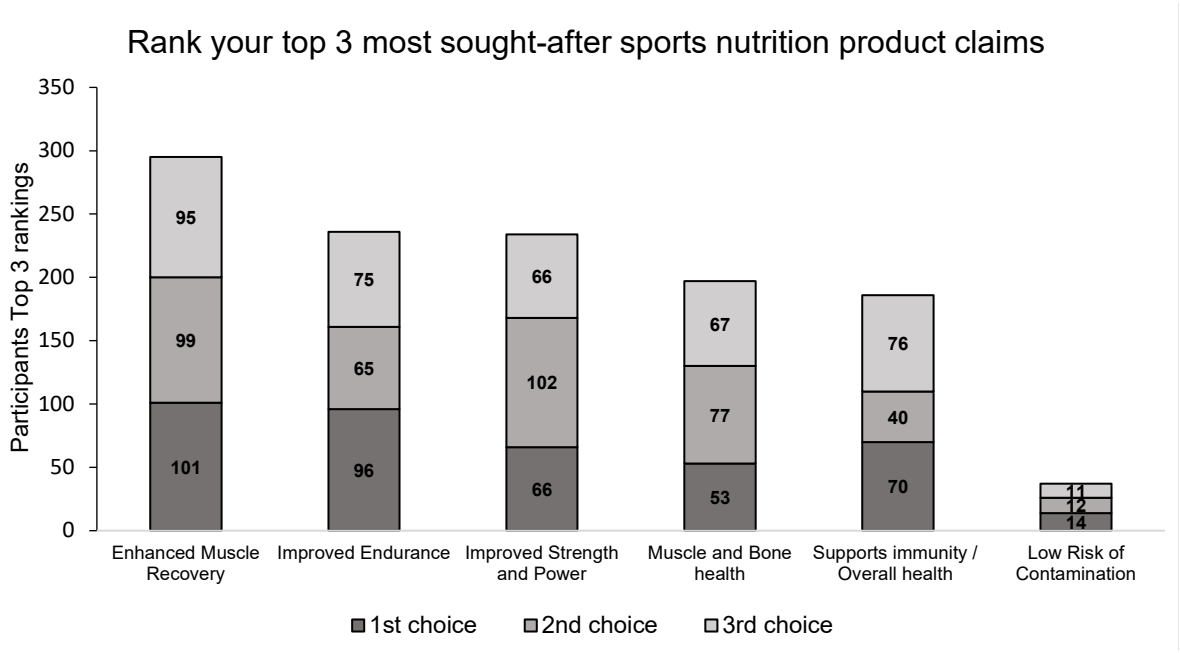


Figure 1. The most sought-after sports nutrition product claims of athletes and active individuals

3.3 Current Practices of Athletes and Active Individuals

3.3.1 Do you currently take nutritional supplements or bioactive functional food products?

Fifty eight percent of participants reported currently taking nutritional supplements, with 52% (n=210) reporting supplement use for health and wellbeing purposes and 35% (n=141) reporting supplement use to support their exercise performance or recovery. Multivitamins were the most commonly reported supplement used for health and wellbeing while protein supplements were the most commonly used exercise-related supplements (Table 2.). Forty three percent (n=173) of participants reported using bioactive functional foods as part of their nutritional routine to improve exercise performance or recovery. Caffeine-containing functional foods were the most commonly used group of functional foods with 135 participants reporting ingesting coffee or other caffeine containing food products to support their exercise performance (Table 2.).

3.3.2 Do you alter your routine after training/exercise/competition to improve recovery?

Seventy percent of participants reported altering their routine surrounding exercise with the intention to improve their recovery. Of the 285 participants who alter their routine, the most popular recovery routines were stretching (n=230) and foam rolling (n=166) (Figure 2.).

Table 2. Prevalence of supplement and functional food usage in athletes and active individuals (n=405)

What nutritional supplements are you currently taking for health and wellbeing purposes?		**What nutritional supplements do you currently take for exercise performance or recovery?**		**Do you use any 'functional food products', specifically for exercise performance or recovery?**	
Multivitamin	114 (28%)	Protein	125 (31%)	Caffeine	135 (33%)
Vitamin C	92 (23%)	Creatine	56 (14%)	Polyphenols	24 (6%)
Vitamin D	79 (20%)	Caffeine	46 (11%)	Dietary nitrate	14 (4%)
Omega-3 PUFA	77 (19%)	BCAA's	28 (7%)		
Iron	50 (12%)	Carbohydrate	8 (2%)		
B Vitamins	50 (12%)	Beta Alanine	4 (1%)		
Calcium	29 (7%)	Glutamine	3 (1%)		
Magnesium	13 (3%)	Citrulline	1 (<1%)		
Folic Acid	10 (2%)	L Carnitine	1 (<1%)		
Zinc	4 (1%)	Arginine	1 (<1%)		
Melatonin	2 (<1%)				
Glucosamine	2 (<1%)				

3.4 Food and Nutritional Preferences of Athletes and Active Individuals

3.4.1 On a scale of 1-4 how important would the following features be in your decision to purchase a food for exercise performance or recovery?

When asked to rank the importance of various features of a product for exercise performance or recovery, nutritional profile was ranked as the most important with an average rating of 3.37±0.65 out of 4, followed by taste (3.15±0.80) and ease of access (3.09±0.72) (Table 3.). Mann Whitney U analysis indicates that females rated 'taste' (U=14311, Z=-5.031, $P < 0.001$), 'price' (U=17311, Z=-2.021, $P = 0.043$), 'nutritional profile' (U=15537, Z=-3.710, $P < 0.001$), 'ease of access' (U=17086, Z=-1.989, $P = 0.047$) and 'sustainability' (U=13607, z=-5.220, $P < 0.001$) as more important than did their male counterparts, while gender had no impact on the 'ease of preparation'.

Athletes who reported competing at local or regional level rated the 'ease of preparation' of a product as significantly more important than did those competing at national or

international level (U=7467, Z=-2.209, *P* = 0.027), however 'level of competition' had no impact on the importance of 'taste', 'product pricing', 'nutritional profile', 'ease of access' or 'sustainability' (P>0.05). Participants reporting less than 10 hours per week of total sport or structured physical activity rated the 'price' of a sports nutrition product more important than those undertaking greater than 10 hours per week (U=17004, Z-2.129, *P* = 0.033). Hours of activity per week had no significant impact on importance of 'taste', 'nutritional profile', 'ease of preparation', 'ease of access' or 'sustainability'.

3.4.2 Rank in order, your top 3 preferred types of recovery product from the following list of common recovery products & rank in order, your top 3 preferred types of performance enhancing nutrition product from the following list of performance products.

Whole food type nutritional products received the most first preference selections and most top 3 selections when presented with a number of popular performance and recovery products on the market (Table 4). Whole foods received 186 and 180 first preferences for recovery and performance products, respectively. Food bars received the second most top 3 preferences in both recovery and performance categories with 68% (n=274) and 65% (n=262) participants ranking food bar products in their top 3 preferences respectively. Powder-based products received the second most number one preferences in recovery and performance categories with 56 top preference selections in the recovery category and 58 top preference selections in the performance category. When presented with a list of common food products which often carry performance or recovery claims, a smoothie or juice option received 202 top three rankings, however a hot food option received the most top rankings with 101 participants responding that it was their favourite of the food options presented.

Table 4. Descriptive data outlining the Sports nutrition food product formats preferences of athletes and active individuals (n=405)

Rank in order, your top 3 preferred types of recovery enhancing nutrition product.

	1st Choice			2nd Choice			3rd Choice			Total Top 3 selections		
	Males	Females	Total	Males	Females	Total	Males	Females	Total	Males	Females	Total
Whole Food	114	72	186	49	38	87	31	20	51	194	130	324
Bar	24	26	50	44	50	94	81	49	130	149	125	274
Dairy	23	31	54	52	33	85	46	40	86	121	104	225
Powder	46	10	56	61	17	78	34	20	54	141	47	188
Pre-made Beverage	27	14	41	24	14	38	27	17	44	78	45	123
Pill / Capsule	3	7	10	8	7	15	14	9	23	25	23	48

Rank in order, your top 3 preferred types of performance enhancing nutrition product.

	1st Choice			2nd Choice			3rd Choice			Total Top 3 selections		
	Males	Females	Total	Males	Females	Total	Males	Females	Total	Males	Females	Total
Whole Food	96	84	180	32	13	45	35	24	59	163	121	284
Bar	28	22	50	53	62	115	61	36	97	142	120	262
Pre-made Beverage	31	17	48	37	26	63	41	30	71	109	73	182
Powder	42	16	58	50	17	67	21	25	46	113	58	171
Gel	32	8	40	39	17	56	36	17	53	107	42	149
Pill / Capsule	5	8	13	14	10	24	17	7	24	36	25	61
Gum	2	2	4	5	4	9	11	9	20	18	15	33

Rank in order, your top 3 preferred form of sports nutrition food product.

	1st Choice			2nd Choice			3rd Choice			Total Top 3 selections		
	Males	Females	Total	Males	Females	Total	Males	Females	Total	Males	Females	Total
Smoothie / Juice	36	38	74	52	28	80	41	25	66	129	91	220
Shake	80	19	99	38	11	49	29	18	47	147	48	195
Bar / Flapjack	19	21	40	37	33	70	46	28	74	102	82	184
Hot Food	70	31	101	24	9	33	27	12	39	121	52	173
Yoghurt	5	12	17	14	16	30	24	15	39	43	43	86
Pancake / Brownie	5	9	14	17	13	30	19	18	37	41	40	81
Spreadable	4	18	22	12	25	37	6	12	18	22	55	77
Cereal	10	1	11	23	11	34	10	9	19	43	21	64
Biscuit / Cookie	3	3	6	6	5	11	15	7	22	24	15	39
Hot beverage	2	1	3	8	0	8	10	6	16	20	7	27
Shot	3	4	7	4	3	7	7	4	11	14	11	25

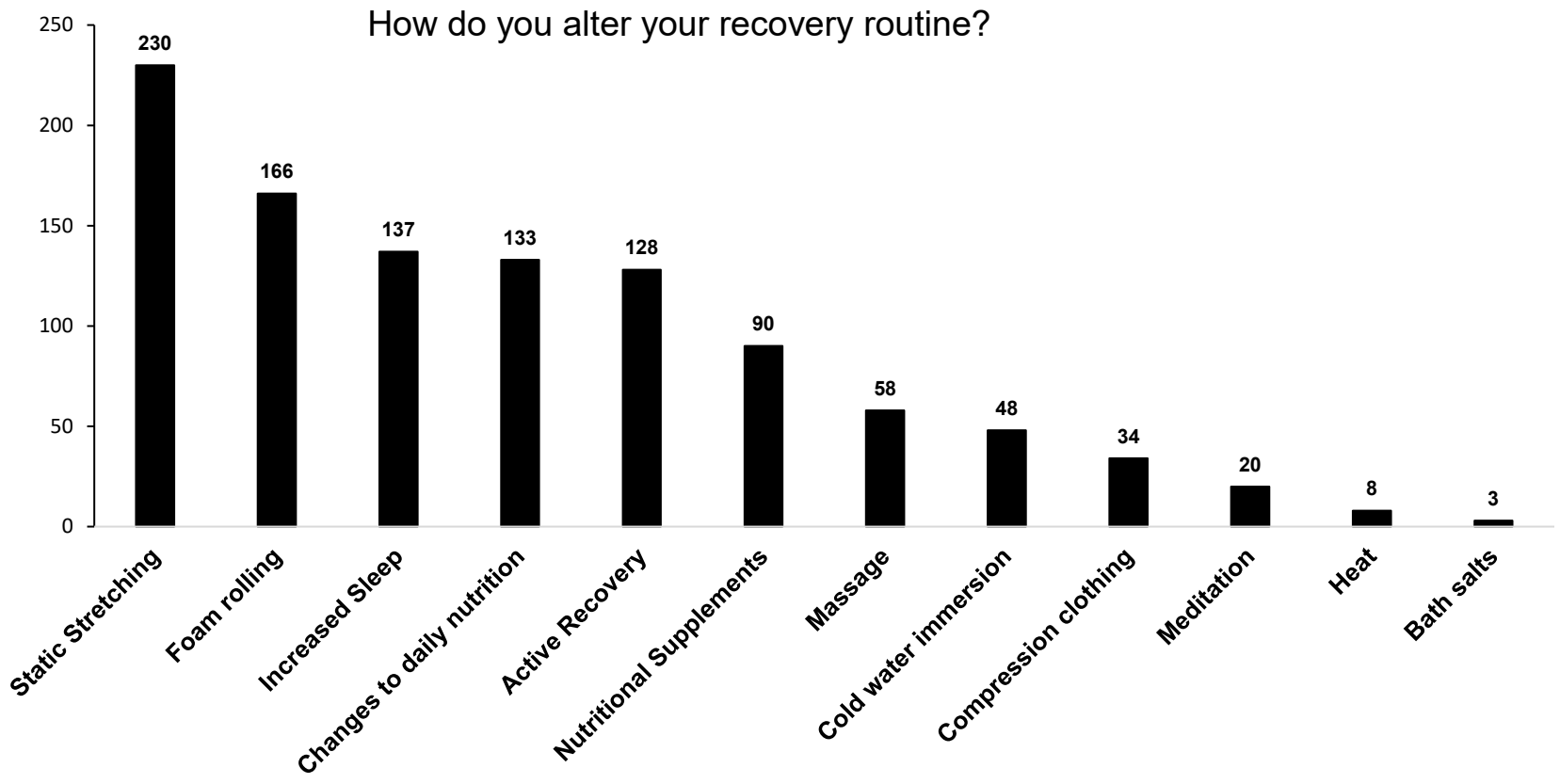


Figure 2. Recovery practices of athletes and active individuals (n=285)

Table 5. Descriptive data outlining preferences for place of purchase of a sports nutrition product

Rank your top 3 in order of likelihood, if you were to purchase a sports nutrition product, from where would you be most likely to purchase it?

	1st Choice			2nd Choice			3rd Choice			Total Top 3 selections		
	Males	Females	Total	Males	Females	Total	Males	Females	Total	Males	Females	Total
Supermarket	95	68	163	35	33	68	61	33	94	191	134	325
Health Food Shop	38	48	86	80	41	121	48	47	95	166	136	302
Online	87	29	116	49	26	75	44	25	69	180	80	260
Pharmacy	11	16	27	45	48	93	56	34	90	112	98	210
Café / Restaurant	1	0	1	19	10	29	12	15	27	32	25	57
Sport Shop	2	1	3	3	0	3	1	1	2	6	2	8
Gym	1	0	1	0	1	1	1	1	2	2	2	4

4. Discussion

4.1 Overview of study findings

While there has been substantial research advancement in efficacy testing of potential product prototypes (Arenas-Jal *et al.*, 2019); there is often considerable difficulty when it comes to translation of theoretically efficacious product prototypes to successful adoption amongst consumers (Menrad, 2003). In order to achieve this, an evidence-based needs analysis of target market must be evaluated to inform the conversion from food prototype to successful product.

The data gathered from this study has identified a clear disconnect between certain aspects of current practice of this population and advised evidence-based best-practice. Key findings from this study clearly demonstrate the demand for effective sports nutrition solutions to provide support for post exercise recovery, with 25% of participants highlighting this as their number one priority when it comes to their nutritional practice. While effective sports nutrition solutions to provide support to post exercise recovery are deemed of critical importance to these end-users; we detected that there is a clear disconnect between the current practice of end-users and the recommended scientific best-practice within the topic. There is also undoubtedly a desire for more food-based solutions to be developed, yet it is clear from current practice that supplementation still represents a considerable majority of the sports nutrition products produced and consumed (Knapik *et al.*, 2016). Future ventures should prioritise the development of fortified and functional food alternatives as evidenced in findings of this research study.

4.2 Priorities of Athletes and Active Individuals When Choosing a Sports Nutrition Product

It is critical to gain a quantifiable understanding of what aspects of physiological function this population prioritise as being important to support both their health and athletic performance. To the best of the authors knowledge this is the first study to analyse the specific product claims and attributes which athletes and active individuals prioritise when it comes to selecting sports nutrition products.

4.2.1 Post-Exercise Recovery: This study demonstrates for the first time that post-exercise recovery is the most sought-after sport and exercise nutrition product claim with almost 3 in 4 participants ranking muscular recovery as one of their top three priorities, and one quarter ranking it as their top priority. Exercise-induced muscle damage (EIMD) is wide ranging in its prevalence with 7 in 10 participants reporting frequently experiencing muscle stiffness or pain post exercise. EIMD is caused by unaccustomed strenuous exercise particularly when such exercise is at high intensity or contains high eccentric loading (Clarkson and Tremblay, 1988; Clarkson and Sayers, 1999). Over half of participants, reported experiencing stiffness or pain caused by EIMD at least once per week, highlighting the magnitude of this issue and the urgent need for an evidence-based food solution. Using appropriate methods to recover from EIMD allows athletes and active individuals to achieve the greatest possible adaptation to strenuous exercise through allowing for increased training frequency and also reduced the time spent in a state of compromised muscle function (Owens *et al.*, 2018). To enhance recovery after exercise, evidence suggests that protein supports muscle adaptation, and polyphenol-rich foods like tart cherry juice can be effective nutritional strategies to improve recovery from muscle soreness and damage (Owens *et al.*, 2018; Harty *et al.*, 2019).

4.2.2 Improving Strength and Endurance: Improving both endurance and strength through the use of nutritional products followed closely as important product claims prioritised by this population ranking them second and third respectively in terms of product claim importance The emergence of food forms such as isotonic sports drinks, gels and shots have attempted been developed to provide in-competition carbohydrate fuelling options, further innovation is duly warranted in this area. While protein ingestion coupled with resistance training improves strength and power adaptation in the long term (Andersen *et al.*, 2005; Hulmi, Lockwood and Stout, 2010); improving acute strength and power performance through nutritional means is a decidedly more difficult challenge. Caffeine shows considerable efficacy in this regard (Grgic *et al.*, 2018), and also in improving acute endurance performance (Matthew S Ganio, Douglas Casa and Lawrence Armstrong, 2008), however issues relating to dosages, habituation and genetic variance in response (Womack *et al.*, 2012) mean that achieving an optimal ergogenic effect may prove challenging for the athlete. Creatine monohydrate supplementation also provides potential for an ergogenic effect, increasing short term strength and power performance (Lanhers *et al.*, 2015, 2017),

however doses, particularly during loading phases appear achievable via supplementation and not by dietary means (Lanhers *et al.*, 2015).

4.2.3 Health and Immunity: Despite this research being carried out during the covid-19 pandemic which likely placed greater emphasis on maintaining health and immunity, this product claim was less prioritised in comparison with muscle recovery, enhancing strength and enhancing endurance. This strongly suggests that when it comes to nutrition and dietary interventions this population of athletes and active individuals prioritise seeking products that can have a direct impact on sporting performance rather than products that help maintain health and wellbeing exclusively. Given the considerable risk of absence from training and competition associated with illness and infection surrounding major sporting competition, this poses a considerable under prioritisation within this population (Dvorak et al., 2011; Engebretsen et al., 2013).

4.2.4 Scientific Efficacy: Due to the lack of regulation of the sports nutrition market, along with the recent growth in the industry, a considerable level of scepticism over the use of sports nutrition products has emerged in recent years (Lorraine, 2018; Luna, 2018). Particularly, the prevalence of mislabelling and contamination of sports supplements has led to a notable movement away from advocating for the use of sports supplements and towards a food first approach to sports nutrition (Close *et al.*, 2022). Within the wider food industry there has been considerable improvement within regulation of health claims of food products in recent years with the European Food Safety Authority and the European Commission introducing regulations on Nutrition and Health claims in 2007 (Regulation (EC) No. 1924/2006]. Establishing a minimum standard of scientific evidence underpinning these claims through regulation is a priority (Lucey, Heneghan and Kiely, 2016). This is now extending into the sports nutrition sector with the development of the Australian Institute of Sport ABCD classification of sports foods and supplements (Australian Institute of Sport 2018) and the International Olympic Council releasing their consensus statement on dietary supplements and their claims (Maughan *et al.*, 2018). In the current research, 87% of all participants rated the importance of having scientific proof to support a product claim at least a 3 out of 4 in terms of importance. Furthermore, 84% of participants stated they would be

willing to pay extra for a sports nutrition product with a product claim that is scientifically proven. It has been shown previously that scientific evidence backed health claims influence overall perception, food choice and willingness to pay (Hwang, Lee and Lin, 2016) This finding highlights the importance of a rigorous scientific process in new product development practice for sports nutrition food products, and also emphasises the importance of regulating sports nutrition efficacy claims to protect the consumer from spurious or fraudulent efficacy statements. Even when sports nutrition products have well established science-backed efficacy claims, risk of inadvertent product contamination is another particular issue within the sports nutrition industry. Paired with regulation of product claims, recommendation of third-party testing of sports nutrition products and their batches to ensure products contain the stated ingredients only and in the stated dosages is essential for the safety of end-users. Along with this education of end-users around the risks of supplement contamination and the importance of third-party testing is essential for the future of product regulation (Vento and Wardenaar, 2020)

4.3 Current Practices of Athletes and Active individuals

4.3.1 Supplementation: Supplementation type products represent a majority of the market share for sports nutrition products (Sports Nutrition Market Size Worth $82.3 Billion By 2030, Grand View Research). In our sample ~60% of participants reported using nutritional supplements, with protein supplements being the most common exercise-related supplement and multivitamins being the most used health-related supplement. These findings closely align with those reported in the meta-analysis of Knapik et al. (2016), who estimated prevalence of the use of any dietary supplement in combined athletic groups at 58% (Knapik *et al.*, 2016). Supplement usage in the general adult population in Ireland, as reported by the National Adult Nutrition Survey, is considerably lower at an estimated 28% highlighting that athletic groups are highly motivated and concerned with adapting their nutritional practices for either health or performance purposes (Browne *et al.*, 2011). One notable distinction between the results of this study and those presented in Knapik et al. (2016), is the greater prevalence of vitamin D supplementation with 20% of the sample in the current study reporting current supplementation compared to a supplementation prevalence estimate of 7% in the meta-analysis (Knapik *et al.*, 2016). This data was collected in Ireland which has an increased prevalence of vitamin D insufficiency and deficiency (Cashman *et al.*, 2009).

This study was conducted during the covid-19 pandemic during which increased focus was placed on vitamin D supplementation to support immune health (Martineau and Forouhi, 2020). The prevalence of vitamin D supplementation in Irish athletes and active individuals was higher (20%) than that reported in the general Irish population, at 17.5% (Cashman *et al.*, 2016). Given the growing knowledge surrounding the importance of avoiding vitamin D deficiency in athletes this is likely a positive development (Owens, Allison and Close, 2018).

4.3.2 Recovery: When participants reported the methods employed to improve recovery post-exercise the most commonly used methods were static stretching and foam rolling. Research into the effects of static stretching on recovery from EIMD have shown little to no effect on recovery of muscle strength or muscle soreness (Torres *et al.*, 2012), while foam rolling has been shown to have little effect, other than recovery of range of motion (Wiewelhove *et al.*, 2019; Skinner, Moss and Hammond, 2020). Despite the relative importance to the individual, the management of recovery post exercise appears to be largely misunderstood. Promotion of good sleep hygiene (Vitale *et al.*, 2019) and the incorporation of appropriate changes to daily nutrition practices (Owens *et al.*, 2018) during periods of intense exercise should be prioritised for the improvement of recovery over practices such as static stretching and foam rolling.

Our research indicates that protein supplementation was the most employed supplementation strategy relating to sporting performance or recovery in this population. While increased protein intake has been shown to positively impact muscle protein turnover and as a result augment the regeneration of muscle tissue post exercise and promote optimal muscle and strength gains particularly during resistance training (Phillips, 2007, 2012; Morton *et al.*, 2018), it remains unclear as to whether protein supplementation improves the time course of skeletal muscle recovery. A systematic review and meta-analysis showed little effect of protein supplements on recovery from symptoms of EIMD including muscle strength and muscle soreness (Pasiakos, Lieberman and McLellan, 2014). Another meta-analysis showed that whey protein supplementation had a small to medium temporal ergogenic effect on recovery of muscle function post resistance exercise training, however less than half of the included studies reported a beneficial overall effect (Davies, Carson and Jakeman, 2018). Although increasing protein intake will undoubtedly enhance adaptation to resistance training for most individuals, given this evidence, it cannot be relied upon as a primary

method to curb the issue of post exercise discomfort and reductions in performance capability in the aftermath of intense exercise. Alternative solutions should be sought to enhance recovery from EIMD such as those discussed in the key review of this topic by (Harty *et al.*, 2019).

4.3.4 Bioactive functional foods: A particularly underutilised avenue in the sports nutrition sector appears to be that of bioactive functional foods which provide physiological benefit beyond that of their macro or micronutrient content. Although there is a strong uptake in the use of caffeine-based functional foods, particularly coffee, the majority of participants in this study reported not using such functional foods at all. Only 24 participants reported using polyphenol-based functional food products such as tart cherry products, green tea and dark chocolate, and 14 participants reported the use of dietary nitrate based functional foods such as beetroot juice. While underutilised, the use of polyphenols for sporting performance (Somerville, Bringans and Braakhuis, 2017), recovery of muscle soreness and muscle strength (Carey, Lucey and Doyle, 2021) as well as providing health benefits (Amiot, Riva and Vinet, 2016; Cory *et al.*, 2018) has been the focus of recent research with much of the research showing performance, recovery and health benefits, although effects may be small and precise dosage required requires further investigation. Given the myriad of food sources naturally rich in polyphenols (Manach *et al.*, 2018), and their demonstratable capacity to address key priorities of athletes and active individuals, there appears significant scope for development of polyphenol rich functional foods for the sports nutrition market. The efficacy for the use of dietary nitrate functional food products, particularly beetroot juice is also well established with meta-analyses showing clear benefits in endurance capacity (Hoon *et al.*, 2013; McMahon, Leveritt and Pavey, 2017). In this survey, while almost 1-in-4 participants reported that improving endurance performance is their most sought-after attribute of a sports nutrition product and over half of the participants ranked it in their top 3, but only 14 (4%) participants reported using a dietary nitrate product. The emergence of functional foods has been a notable trend in the wider food industry in recent years and it is clear there is strong potential for this to extend to the sport and exercise nutrition sector, however challenges translating research to engaging strategies to support consumer uptake must be addressed (Bigliardi and Galati, 2013).

4.4 Product Preferences of Athletes and Active individuals

4.4.1 Factors affecting food choice: Nutritional profile was voted as the most important factor affecting a purchasing decision of a sports nutrition product, followed closely by taste. Although there is a fast-growing interest in sustainability in the wider food systems, the sustainability of a sports nutrition product received the lowest mean rating of features presented in this question. This suggests that athletes and active individuals are unlikely to be willing to compromise on other factors in favour of having an improved environmental impact, especially with regards nutritional profile and taste. However, product sustainability still has some importance to this population and may be a viable selling point of a product once other key factors are intact (Arenas-Jal *et al.*, 2019).

Gender appears to be a particularly important demographic influencer in this population with gender having a significant influence on the rank importance of taste, price, nutritional profile, ease of access and sustainability, which have been previously shown to influence the adoption of functional foods in the diet (Ares and Gámbaro, 2007). Outside of gender, competition level and time spent undertaking sport or structured physical activity may also be a factor in the food preferences of participants, particularly in the aspects of taste, ease of preparation and price (Ares and Gámbaro, 2007; Birkenhead and Slater, 2015; Clark, 2022).

4.4.2 *Towards a whole food approach:* Food choice in athletes is heavily influenced by the demands of the sport or exercise they are participating in, as well as the timing surrounding the exercise event (Birkenhead and Slater, 2015). As a result of this, separate questions were asked as to the types of sports nutrition product they would prefer for either performance or recovery. The most desirable food product type highlighted in both questions was “whole food” type nutrition products. A food first approach has been widely advocated for by sporting bodies as well as in three notable expert consensus statements on sports nutrition(ACSM and ADA, 2009; Maughan *et al.*, 2018; Collins *et al.*, 2021). There is also clearly considerable demand for sports nutrition food products given the results of the product preference section of this study (Table 4). This food-first approach is widely promoted by nutrition professionals over supplementation-orientated approaches owing to the importance of ‘food synergy’ and the interdependence of food constituents to derive the

greatest benefit to human physiology, especially with regards to nutrient digestion and absorption (Jacobs, Gross and Tapsell, 2009). This approach has been shown to be particularly beneficial when it comes to protein intake and muscle protein synthesis and the resulting remodelling of muscle tissue as a result of exercise (Burd *et al.*, 2019). While protein supplements have shown significant benefits for athletes and healthy ageing populations (Phillips, 2007; Morton *et al.*, 2018; Oikawa *et al.*, 2021), and research using protein supplements has been integral to the development of protein intake guidelines for these populations, whole protein foods have been suggested to have greater beneficial impact than that of their constituent amino acid content alone (Burd *et al.*, 2019).

Despite consumer demand and the scientific support for food-first approaches to sport and exercise nutrition, market insights note that 83.6% of the market share of this sector in 2019 was held by sports supplements (Sports Nutrition Market Size Worth $82.3 Billion By 2030). There is evidently major potential for a significant market swing towards foods for sport and exercise in the coming years. Although a food-first approach should be the first option for nutritional practice in sport and exercise, there is potential to include supplementation to augment this practice, particularly for nutrients which are difficult to consume in sufficient quantities from dietary sources to gain an ergogenic benefit. As such, the recently proposed '*food first but not always food only'* framework may represent the most impactful approach (Close *et al.*, 2022). This approach posits that athletes should adopt a food-first approach unless faced with one of six pre-defined scenarios which suggest supplementation may provide additional benefits (Close *et al.*, 2022). Future innovations in the sports nutrition market should reflect this and prioritise whole food products where possible, reserving supplementation approaches predominantly for nutrients in which it is impossible or wholly impractical to achieve exclusively from diet.

4.4.3 Opportunities for new product design: To date there is no previously published research to the authors knowledge that addresses end-user desires for particular product forms. As previously discussed, general food preference factors such as taste are of great importance to this population and as such creating products which meet the desired specifications are crucial for success in the sports nutrition market (Table 3.) (Birkenhead

and Slater, 2015). In this online survey participants were asked to rank their most preferable food products, when provided with a list of food product types found commonly on the sports nutrition market. Products such as smoothie/juice received the greatest number of top 3 selections, however hot food received the greatest number of first preference choices followed closely by a shake type beverage. This aligns considerably with the move towards a food first approach to sports nutrition as discussed above (Burd *et al.*, 2019; Close *et al.*, 2022) Given the fruit and vegetable derived nature of underutilised bioactive compounds such as polyphenols and dietary nitrates, smoothies and juices represent a particularly interesting direction for future development with juices such as beetroot juice and tart cherry juice showing particular scientific evidence (Connolly, McHugh and Padilla-Zakour, 2006; Wylie *et al.*, 2016). The creation of convenient and accessible hot meal solutions such as recipes and meal preparation methods for hot food, which meet the macronutrient nutritional demands of this population also appear to be in particular demand. Bar/flapjack type products also performed quite well receiving the third highest number of top three preferences. Participants were also asked to rank their most likely place of purchase for a sports nutrition product with supermarkets being the ranked the most likely place of purchase for such products. Developing food products which combine appealing sensory factors with favourable nutritional profile could revolutionise the sports nutrition sector from a supplement focussed one, to that of a food industry.

4.5 Limitations

It is worth noting that this study was undertaken in Ireland so the results may not be fully generalisable to that of the wider athletic population. The sporting activities of this sample, contains a considerable proportion of participants reporting engagement in random intermittent dynamic type sports such as soccer, rugby, Gaelic games and basketball which may not be representative of the sporting populations in certain areas of the world. As a result of the convenience sampling nature of this sample it may not be fully representative of views on a population level, and it is impossible to assess whether there would be a notable difference between responders and non-responders to the survey. Due to the nature of the format of the rank order questions, it was not possible to statistically compare answers against different population groups such as across gender and competition level, further

research should be considered to elucidate trends of these topics across population sectors and among specific sporting sectors.

5. Conclusion

There has been both significant growth in the sports nutrition sector as well as significant progression in the scientific knowledge surrounding nutritional practices to support sport and exercise in recent years. However, at this pivotal juncture for the sector it appears that by listening to the end user, greater efficiency and efficacy can be gained in the new product development process. In fields such as skeletal muscle recovery there are clear disparities between the current practice of athletes and active individuals and the scientific evidence of best practice. A transition towards a food first approach in sports nutrition is vital for athletes and active individuals to achieve their goals, with the development of functional foods, particularly with the focus of muscle recovery, endurance, and strength enhancement at the forefront. This population has also shown considerable support for the scientific process in developing such products and testing their respective efficacy. There appears to be particular enthusiasm towards beverages such as smoothies, juices and shakes as well as food products in bar or hot food format. This research merits consideration and priority in future new product developments in the sport and exercise nutrition sector.

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Chapter 4 – Flavonoid Containing Polyphenol Consumption and Recovery from Exercise-Induced Muscle Damage: A Systematic Review and Meta-Analysis

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Abstract

Background: Flavonoid polyphenols are bioactive phytochemicals found in fruits and teas among other sources. It has been postulated that foods and supplements containing flavonoid polyphenols may enhance recovery from exercise-induced muscle damage (EIMD) through upregulation of cell signalling stress response pathways, particularly the nuclear factor erythroid 2–related factor 2 (NRF2) pathway.

Objectives: This study aims to investigate the ability of polyphenol treatments containing flavonoids to enhance recovery of skeletal muscle strength, soreness and creatine kinase post EIMD.

Methods: Medline (Pubmed), Embase and SPORTdiscus were searched from inception to August 2020 for randomised placebo-controlled trials which assessed the impact of 6 or more days of flavonoid containing polyphenol ingestion on skeletal muscle recovery in the 96-hour period following a single bout of EIMD. A total of 2983 studies were screened in duplicate resulting in 26 studies included for analysis. All meta-analyses were undertaken using a random effects model.

Results: The pooled results of these meta-analyses show flavonoid polyphenols treatments can enhance recovery of muscle strength by 7.14%, (95% CI [5.50 to 8.78], P<0.001) and reduce muscle soreness by 4.12% (95% CI [-5.82 to -2.41] P<0.001), no change in recovery of creatine kinase concentrations was observed.

Conclusion: These results indicate that ingestion of polyphenol treatments which contain flavonoids has significant potential to improve recovery of muscular strength and reduce muscle soreness in the 4-day period post EIMD. However, characterisation of polyphenol dosage and composition of study treatments should be prioritised in future research to facilitate the development of specific guidelines for inclusion of flavonoid-rich foods in the diet of athletes and active individuals.

1. Introduction

Recovery from exercise-induced muscle damage (EIMD) is a critical issue facing athletes across most sports. EIMD occurs following a bout of unaccustomed exercise which contains either high intensity or eccentric muscle contractions (Clarkson and Hubal, 2001). Mechanical damage from muscle contractions during exercise results in muscle fibre degradation (Owens *et al.*, 2018), Z-line streaming (Proske and Allen, 2005) and damage to T-tubules (Proske and Morgan, 2001). Following this structural damage, there is a notable reduction in control of sarcoplasmic reticulum calcium ion (Ca^{2+}) release arising from this initial damage, which results in further damage and apoptosis to the muscle fibre (Gissel and Clausen, 2001). This apoptosis causes an inflammatory cascade which acts to clear necrotic cells resulting in the formation of reactive oxygen species (ROS) (Powers *et al.*, 2011), which may further damage proteins when produced in excess (Kozakowska *et al.*, 2015). This leads to substantial interference with subsequent muscular excitation contraction coupling causing a decreased performance capability of the muscle. This generally manifests as short-term loss of strength, reduced range of motion and delayed onset muscle soreness (DOMS) (Clarkson and Hubal, 2001). While this remains the most widely recognised mechanism for EIMD, it is suggested that team sport athletes may not undergo such a notable insult to muscle following match-play and that detriments may in part be attributed to neuromuscular mechanisms (Thomas *et al.*, 2017). Regardless, even in team sports it has been repeatedly shown to be detriments in indices of EIMD post such exercise including reductions of muscle strength, notable muscle soreness and elevated concentrations of biomarkers of oxidative stress, inflammation and muscle damage (Howatson and Milak, 2009; Twist and Sykes, 2011; Souglis *et al.*, 2015; Thomas *et al.*, 2017). It is clear that developing evidence-based strategies to combat EIMD is a priority for exercise science (Harty *et al.*, 2019).

Numerous novel recovery methods have been developed to reduce the impact of EIMD on subsequent training and performance, including cold water immersion (Leeder *et al.*, 2012), contrast water therapy (Bieuzen, Bleakley and Costello, 2013), compression garments (Hill *et al.*, 2014) and massage (Smith *et al.*, 1994), all of which have reported small beneficial impacts. However, it remains apparent that nutritional interventions provide prodigious potential to improve recovery from EIMD (Harty *et al.*, 2019). In performance nutrition, supplement use is prevalent, with muscle growth

and repair reported as one of the most common objectives for supplement intake in athletic populations (Maughan, Depiesse and Geyer, 2007). Commonly used supplements in athletic diets which have demonstrated potential to enhance recovery from EIMD include protein (Pasiakos, Lieberman and McLellan, 2014), branched chain amino acids (Howatson *et al.*, 2012), creatine monohydrate (Deminice *et al.*, 2013), omega-3 polyunsaturated fatty acids (Smith *et al.*, 2011) and vitamin D (Barker *et al.*, 2013). In addition to these well-established supplementation strategies bioactive compounds have begun to emerge as attractive additions in the field of sports nutrition. Bioactive compounds are defined compounds present in food that exerts reproducible biological effects at dietary levels (Frank *et al.*, 2020). Plant-based bioactive compounds such as polyphenols and dietary nitrate have gains significant traction in the sports nutrition sector.

Polyphenols are non-nutritive plant-derived bioactive compounds which have been extensively investigated for their potential to support recovery from EIMD (Bowtell & Kelly, 2019; Myburgh, 2014). They have been defined as compounds containing 2 or more phenolic rings in their chemical structure and are found in high concentrations in fruits, vegetables and teas among other sources (Frank *et al.*, 2020). Flavonoids are the largest subclass of polyphenols with over 6000 compounds identified to date (Panche, Diwan and Chandra, 2016). Flavonoids have been the primary focus of research relating to polyphenols in both sporting performance and recovery, with most prevalently investigated foods which contain flavonoids including tart cherry (Bell et al., 2015, 2016; Bowtell et al., 2011; Brown et al., 2019; Connolly et al., 2006; Howatson et al., 2010; Lamb et al., 2019; Levers et al., 2016), green tea (Jówko *et al.*, 2011, 2015; Kuo *et al.*, 2015; da Silva *et al.*, 2018) and pomegranate (Trombold *et al.*, 2010, 2011; Ammar *et al.*, 2018). The most widely researched flavonoid subclasses include anthocyanins, catechins and quercetin, among others. A review of randomised controlled trials (RCTs) investigating the use of fruit polyphenols as a recovery aid from EIMD suggested that chronic supplementation of >1000mg of polyphenols for 3 days prior to and following strenuous exercise may enhance recovery from EIMD (Bowtell & Kelly, 2019). While this review provides a valuable narrative analysis of the current research on this topic, many of the RCTs discussed in this review utilised small sample sizes and often reported conflicting results. A systematic review and meta-analysis are required to elucidate the potential efficacy and magnitude of any

benefits which may be accrued from flavonoid containing polyphenol ingestion in relation to EIMD.

A systematic review and meta-analysis of anthocyanin rich whole fruit consumption has shown small but significant reductions in both exercise induced inflammation and oxidative stress in healthy adults (Bloedon *et al.*, 2019). While oxidative stress and inflammation are inherently linked to recovery and muscle damage, there has yet to be a systematic review and meta-analysis to quantitatively synthesise data from RCTs assessing the direct impact of polyphenols containing flavonoids on indices of recovery from EIMD. A Cochrane review has previously assessed the impact of antioxidant supplementation on muscle soreness after exercise. While some polyphenol-based treatments were included in this review they were pooled with vitamin antioxidants and other antioxidant treatments such as N-Acetylcysteine (Ranchordas *et al.*, 2018). Given that this review made no attempt to subgroup by antioxidant type, and also that there is considerable difference between the mechanism of antioxidant action between polyphenols and such antioxidants (Bowtell & Kelly, 2019), a further review specifically investigating the impact of polyphenols containing flavonoids on post-exercise muscle damage is required.

The aim of this study is to synthesise the data collected in this body of research to objectively answer the question as to whether consumption of these supplements and extracts can improve recovery from EIMD, key outcomes prioritised were functional strength recovery; the time-course of skeletal muscle soreness and blood creatine kinase concentrations within the 96-hour period following intense exercise.

2. Methods

2.1 Search Strategy

This systematic review was undertaken in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). The following online databases were searched from inception to August 2020 to identify eligible RCTs: Medline (Pubmed), Embase and SPORTdiscus (fig.1). The following phrases were used alongside Boolean operators 'AND' and 'OR' to develop the search strategy: *Polyphenol, Flavonoid, Berry, Fruit, Tea, Anthocyanin, Quercetin, Catechin, Muscle, Recovery, Inflammation, DOMS, Soreness, EIMD, Creatine Kinase, Muscle Damage and Muscle Injury. (Polyphenol OR Flavonoid OR Berry OR Fruit OR Tea OR Anthocyanin OR Quercetin OR Catechin) AND (Muscle OR Recovery OR Inflammation OR DOMS OR Soreness OR Exercise Induced Muscle Damage OR Creatine Kinase OR Muscle Damage OR Muscle Injury).* Filters were used in the relevant databases to limit the results of such searches in terms of article type, language and to human participants. Reference lists of all eligible studies were screened to identify any further studies suitable for inclusion.

2.2 Study Inclusion and Exclusion Criteria

Criteria for inclusion: studies must be randomised placebo-controlled trials involving healthy human participants aged between 18 and 60 years, published in a peer reviewed academic journal in English language. Participants must be randomised to at least one of polyphenol treatment which contains flavonoids or placebo treatment for a minimum of 6-days in duration using either a crossover or parallel study design. Participants must have undergone a single bout of either high intensity exercise or eccentric muscle contractions causing EIMD. Primary outcomes extracted for this study were: muscle strength measured by either isometric or isokinetic maximal voluntary contraction (MVC), DOMS measured by visual analogue scale (VAS) and blood biomarker of muscle damage, creatine kinase (CK). Protocols must have taken follow-up measurements of these outcomes from at least one of the following timepoints: 24 hours, 48 hours, 72 hours or 96 hours post EIMD. Studies were excluded if the participants had any current, or a history of, any chronic illness which may have impacted on the outcomes measured. Studies were also excluded if the

intervention reported contained a non-polyphenol substance suspected to support or hinder muscular recovery that was not controlled for within the placebo treatment.

2.3 Study Selection

Search data was imported to a published software which allows for blinded duplicate screening of studies by two independent reviewers (CC, LD) (Ouzzani *et al.*, 2016). Where a trial could not be excluded based on information given in title and abstract, it was selected to be included for full text screening. Using a standardized form, full texts were assessed as to whether they met each of the above inclusion and exclusion criteria. Conflicts during either title and abstract screening or full text screening were resolved by consensus.

2.4 Data Extraction

Data extracted for meta-analysis was conducted by two authors in duplicate and included: sample size, means and standard deviation (SD) of each of the three primary outcomes when measured at any of the following timepoints: ≤2, 24-, 48-, 72- or 96-hours post EIMD. MVC data was extracted as a percentage of baseline strength values. When data was presented in graphical form only, data was extrapolated from graphs using WebPlotDigitizer (Drevon, Fursa and Malcolm, 2017). When standard error of mean (SEM) was the reported measure of variance, SD was calculated using RevMan v5.3 software (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Where multiple treatment arms were compared with a single placebo control group, the highest dose treatment arm was extracted only. When required data was presented in a form which did not facilitate inclusion in meta-analysis, corresponding authors were contacted to gain access to the necessary data.

2.5 Data synthesis & Meta-Analyses

Due to the inherent variability in study design across included RCTs, a random effects model was used for all meta-analyses conducted in this review (Borenstein *et al.*, 2009, 2010). As multiple different visual analogue scales were used for DOMS measurement, all VAS data was adjusted to a 100-point scale to facilitate comparison and quantification of the effect using the mean difference (MD). As all MVC data was

included as a percentage change from baseline and all CK measurements were in U/L, the MD was also used on extracted data. All meta-analyses were carried out in RevMan v5.3. Statistical significance for all meta-analyses in this review was set at $P<0.05$.

2.6 Heterogeneity and risk of bias

Heterogeneity was quantified by measurement of I^2, with values of low, moderate and high heterogeneity being assigned to values of ~25%, ~50% and ~75% respectively (Higgins *et al.*, 2003). Subgroups were assessed to evaluate subgroup differences, covariate distribution, plausibility and importance of interaction and possibility of confounding as described by (Richardson, Garner and Donegan, 2019). RCT quality was measured on all included studies using the Physiotherapy Evidence Database scale (PEDro) which has been repeatedly shown to be a valid and reliable measure of methodological rigour and risk of bias and has been widely used in the assessment of exercise trial quality (de Morton, 2009; Maher et al., 2003). The PEDro scale, based on the Delphi list (Verhagen *et al.*, 1998), contains eleven criteria aimed at assessing the internal validity and the sufficiency of detail to allow results of RCTs to be accurately interpreted.

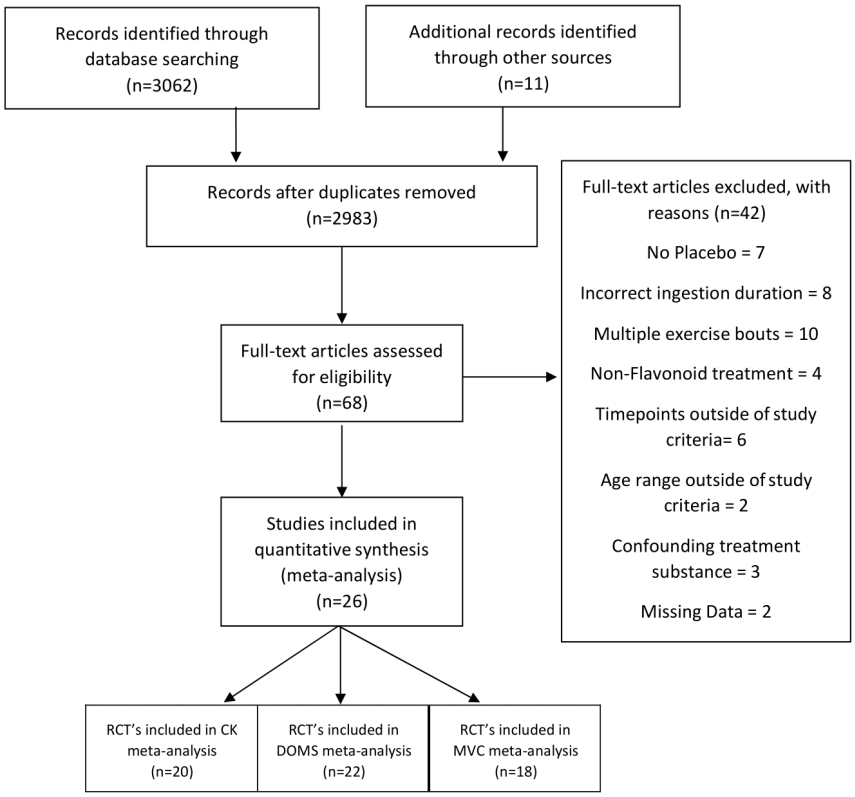


Fig. 1 Schematic representation of human study selection protocol from initial search to final inclusion.

3. Results

3.1 Study Selection

A total of 3062 published studies were identified through systematic searching. A further 11 studies were identified through screening of reference lists and other sources. Following duplicate removal 2983 distinct studies were screened by title and abstract by two authors (CC, LD). Of this sample, 2915 articles were excluded based on information provided in the title or abstract. The full text articles of the remaining 68 studies were assessed in duplicate for eligibility. Twenty-eight studies were deemed eligible for inclusion in this systematic review and meta-analysis by both screening authors (fig. 1). In the case of two studies, no appropriate data was obtained despite attempts to contact corresponding authors and, as such, these studies were excluded based on missing data (Kuo *et al.*, 2015; Lynn *et al.*, 2018). One study contained two discrete trials; an upper body muscle damage trial, and a lower body muscle damage trial, these trials were included in this analysis as independent RCTs (Trombold *et al.*, 2011). Two studies contained two differing treatment arms in comparison to a single placebo group, in these studies data from the highest dose treatment arm was extracted and included in the analysis (Herrlinger, Chirouzes and Ceddia, 2015; Lamb *et al.*, 2019).

3.2 Study Characteristics

Twenty-seven independent RCTs from 26 studies were included in at least one meta-analysis. MVC muscle strength analysis comprised of 18 RCTs, DOMS analysis comprised of 22 RCTs and creatine kinase analysis comprised of 20 RCTs. The total participant sample of included studies was 568 (MVC n=350, DOMS n=406, CK n=469). Of this sample 468 were male and 100 were female. Participants ranged in training status from untrained (Beyer *et al.*, 2017; da Silva *et al.*, 2018; Lamb *et al.*, 2019) to semi-professional athletes (Bell *et al.*, 2016). Eight studies implemented a crossover study design while the remaining 18 studies utilised a parallel design. Polyphenol sources included tart cherry (n=9) (Beals et al., 2017; Bell et al., 2015, 2016; Bowtell et al., 2011; Brown et al., 2019; Connolly et al., 2006; Howatson et al., 2010; Quinlan & Hill, 2020), various tea extracts (n=7) (Arent *et al.*, 2010; Jówko *et al.*, 2011, 2015; Herrlinger, Chirouzes and Ceddia, 2015; Jajtner *et al.*, 2016; Beyer *et al.*, 2017; da Silva *et al.*, 2018), pomegranate (n=3) (Trombold *et al.*, 2010, 2011;

Lamb *et al.*, 2019), quercetin (n=2) (O'Fallon *et al.*, 2012; Bazzucchi *et al.*, 2019), lemon verbena (n=1) (Buchwald-Werner *et al.*, 2018), black currant (n=2) (Hutchison *et al.*, 2016; Costello *et al.*, 2020), cacao (n=1) (Morgan *et al.*, 2018) and mixed fruit anthocyanin juice (n=1) (Lima *et al.*, 2019). Further study characteristics can be seen in Tables 1 and 2. Characteristics are reported as described in original publications.

3.3 Flavonoid Containing Polyphenols and Muscle Strength

A meta-analysis of the effects of flavonoids on recovery of muscle strength is presented in Figure 2. An overall significant pooled greater recovery in muscle strength over the 4-day period following EIMD was observed in comparison to placebo (MD=7.14, 95% CI [5.50 to 8.78], 95% PI (-1.25 to 15.53), P<0.00001). Significantly greater muscle strength is seen at 24h (MD= 6.65, 95% CI [3.35 to 9.95], 95% PI (-32.71 to 46.01), P<0.0001), 48h (MD=9.49, 95% CI [6.15 to 12.84], 95% PI (-1.17 to 20.15) P<0.00001), 72h (MD=9.12, 95% CI [3.54 to 14.70], 95% PI (-7.79 to 25.53) P=0.001) and 96h (MD=6.60, 95% CI [1.98 to 11.22], 95% PI (-5.62 to 18.82), P=0.005) post EIMD. Tests for subgroup differences indicated significantly greater recovery of muscle strength using tart cherry flavonoids compared with pomegranate interventions at both 24h and 72h post EIMD (P=0.007 and P=0.02 respectively), improved recovery of muscle strength at 24h post EIMD for interventions utilising beverage as the form of ingestion over capsules (P=0.03), and RCTs using trained participants showed grater recovery of muscle strength at 24h post EIMD compared to RCTs using untrained participants (P=0.01). No significant difference between subgroups was observed at any timepoint when stratified by trial design (parallel or crossover), duration of ingestion, or whether the ingestion protocol utilised a minimum of 3 days prior to and post EIMD as recommended by Bowtell, Kelly (Bowtell & Kelly, 2019).

3.4 Flavonoid Containing Polyphenols and Delayed Onset Muscle Soreness

Meta-analysis of the effects of flavonoids on DOMS is presented in Figure 3. There was a significant pooled reduction in DOMS over the four-days following EIMD when compared to placebo (MD= -4.12, 95% CI [-5.82 to -2.41], 95% PI (-11.77 to 3.53), P=0.00001). Significantly lower muscle soreness was observed at 24h post EIMD

(MD=-4.41, 95% CI [-7.82 to -1.00], 95% PI (-12.86 to 4.04), P=0.01), 48h post EIMD (MD=-5.26, 95% CI [-9.89 to -0.63], 95% PI (-20.94 to 10.42) P=0.03) and 72h post EIMD (MD=-6.21, 95% CI [-9.25 to -3.16], 95% PI (-9.68 to -2.74), P<0.0001). Tests for subgroup differences showed significantly better recovery of muscle soreness in RCTs using trained participants compared to RCTs using untrained participants at 48h post EIMD. No significant difference in muscle soreness was observed between subgroups at any timepoint when stratified by trial design, manner of ingestion, polyphenol treatment source, duration of ingestion, or whether the ingestion protocol utilised a minimum of 3 days prior to and post EIMD.

3.5 Flavonoid Containing Polyphenols and Creatine Kinase

The meta-analysis of the effects of flavonoid containing polyphenols on the CK response to EIMD is presented in Figure 4. There was no significant impact of flavonoid polyphenol ingestion on CK at any time-point over the 4-day period following EIMD or in the pooled analysis for the overall time period.

3.6 Risk of Bias

Risk of bias values from the PEDro scale are reported in Table 1. Risk of bias is low in this selection of RCTs, reporting a mean PEDro score of 9.57±1.15 out of a maximum 11 (no bias).

Table 1. RCT design characteristics for all studies included in this review.

Authors	Age (mean years ± SD)	Participant cohort (training status, sex, sample size)	Study Design (parallel / crossover)	EIMD inducing exercise	PP intervention	Reported Dosage	Duration of ingestion (pre and post EIMD)	Outcome variable and time of outcome measurement post exercise (h)	PEDro score
(Arent *et al.*, 2010)	21±0.4	Weight Trained males, (18)	Crossover (5-day washout)	Wingate anaerobic test, then 8x10s cycling sprints	Theaflavin enriched black tea extract	1760mg/day black tea extract	9 days (7 pre 2 post)	DOMS (24*, 48*)	8
(Bazzucchi *et al.*, 2019)	26±3	Moderately active males, (12)	Crossover (3-week washout)	10x10 isokinetic eccentric contractions at 45◦/sec	Quercetin	1000mg/day quercetin aglycone	14 days (14 pre)	MVC (0*) DOMS (0) CK (0, 24, 48*, 72*)	9
(Beals *et al.*, 2017)	Tart Cherry - 26±9 Placebo - 25±3	Recreationally active, (19, 10f)	Parallel	Maximal eccentric isokinetic extension until fatigue (3x less than 50% pktq)	Tart Cherry	733mg/day phenolic compounds	12 days (4 pre, day of, 7 post)	MVC (24, 48, 96) DOMS (24, 48, 96)	10
(Bell *et al.*, 2015)	30±8	Trained male cyclists, (16)	Parallel	High intensity stochastic cycling	Montmorency Tart Cherry	2x30ml of 90-110 Montmorency tart cherries, 9.2mg/ml anthocyanin	8 days (4 pre, day of, 3 post)	MVC (24, 48, 72) DOMS (24, 48, 72) CK (0, 24, 48, 72)	10
(Bell et al. 2016)	25±4	Semi-pro male soccer players, (16)	Parallel	12x20m sprints, then LIST	Montmorency Tart Cherry	73.5mg/L cyanidin-3-glucoside, 178.8 gallic acid equivalent	7 days (4 pre, 3 post)	MVC (24*, 48*, 72*) DOMS (24*, 48*, 72*) CK (0, 24, 48, 72)	10
(Beyer *et al.*, 2017)	Polyphenol Blend - 22±3 Placebo - 22±2	Untrained males (28 CK) (18 DOMS)	Parallel	6x10 @ 70% 1RM squat, 4x10 @70% 1RM leg press	Polyphenol blend of tea extracts	2000mg/day polyphenol blend, minimum 40% Polyphenols	4 weeks pre 4 days post	DOMS (0, 24, 48, 96) CK (24, 48, 96)	9
(Bowtell *et al.*, 2011)	28±2	Well trained males (10)	Crossover (2-week washout)	10x10 knee extensions @ 80% 1RM	Montmorency Tart Cherry	30ml x2/day of 9.117mg/ml anthocyanin	9 days (7 pre 2 post)	MVC (0, 24*, 48*) CK (0, 24, 48)	8

Authors	Age (mean years ± SD)	Participant cohort (training status, sex, sample size)	Study Design (parallel / crossover)	EIMD inducing exercise	PP intervention	Reported Dosage	Duration of ingestion (pre and post EIMD)	Outcome variable and time of outcome measurement post exercise (h)	PEDro score
(Brown et al., 2019)	19±1	Physically active females (20f)	Parallel	15x30m sprints w/ rapid 10m deceleration	Montmorency Tart Cherry	30ml x2/day of 73.5mg/L cyanidin-3-glucoside, 178.8 gallic acid equivalent	8 days (4 pre, day of, 3 post)	MVC (0, 24, 48, 72) DOMS (0, 24, 48, 72) CK (0, 24, 48, 72)	11
(Buchwald-Werner *et al.*, 2018)	Lemon Verbena - 32±9 Placebo - 31±9	Moderately active (44, 25f))	Parallel	CMJx200 plus 10% BW added	Lemon Verbena	400mg/day lemon verbena extract	15 days (10 pre, day of, 4 post)	DOMS (24, 48, 72, 96)	11
(Connolly, McHugh and Padilla-Zakour, 2006)	22±4	Male college students (14)	Crossover (6-day washout)	2x20 maximal eccentric elbow contractions	Tart Cherry blend	1200mg/day phenolic compounds	8 days (3 pre, day of, 4 post)	MVC (24*, 48*, 72*, 96*) DOMS (24, 48, 72, 96)	8
(Costello *et al.*, 2020)	30 ± 6	Recreational runners (20, 8f)	Parallel	Half Marathon	New Zealand Blackcurrant	600mg/day CurraNZ containing 210mg anthocyanin	9 days (6 pre, day of, 2 post)	DOMS (0, 24, 48)	10
(da Silva *et al.*, 2018)	Green Tea - 25±5 Placebo - 23±5	Non-Trained men (20)	Parallel	Maximal Calf raises	Green Tea Extract	500mg/day green tea extract	15 days (15 pre)	CK (0*, 48*) DOMS (0, 48)	9
(Herrlinger, Chirouzes and Ceddia, 2015)	Polyphenol blend - 24±5 Placebo - 23±4	Recreationally active males (25)	Parallel	40mins of downhill running @60% VO_{2max} and 10% decline	Polyphenol blend of tea extracts	2000mg/day polyphenol blend, minimum 40% Polyphenols	13 weeks (12 pre, 1 post)	MVC (24, 48*, 96*) CK (0, 24, 48, 96)	9
(Howatson et al., 2010)	Cherry juice - 37±13 Placebo - 38±5	Recreational Marathon Runners (20, 7f)	Parallel	Marathon	Tart Cherry blend	1200mg/day phenolic compounds	8 days (5 pre, day of, 2 post)	MVC (0, 24*, 48*) CK (0, 24, 48) DOMS (0, 24, 48)	7

Authors	Age (mean years ± SD)	Participant cohort (training status, sex, sample size)	Study Design (parallel / crossover)	EIMD inducing exercise	PP intervention	Reported Dosage	Duration of ingestion (pre and post EIMD)	Outcome variable and time of outcome measurement post exercise (h)	PEDro score
(Hutchison *et al.*, 2016)	Blackcurrant Nectar – 20±1 Placebo - 21±3	College Students (16, 3f)	Parallel	3x10 Eccentric squat @115% 1RM	Black currant nectar	Malvidan glucosides - 386.5mg/day Cyanidan glucosides - 351.38mg/day	8 days (4 pre, day of, 3 post)	CK (24, 48*, 96*) DOMS (24, 48, 96)	10
(Jajtner *et al.*, 2016)	Polyphenol Blend – 22±3 Placebo - 22±3	Recreationally active males (28)	Parallel	6x10 @ 70% 1RM squat, 4x10 @70% 1RM leg press and extension	Polyphenol blend of tea extracts	2000mg/day polyphenol blend, minimum 40% PP's	28 days	CK (24^, 48^, 96^)	9
(Jówko *et al.*, 2011)	Green Tea – 22±1 Placebo - 21±1	Healthy male PE students (35)	Parallel	Back squat and bench press to exhaustion @60% 1RM	Green Tea Extract	640mg/day polyphenols	4 weeks	CK (0, 24)	11
(Jówko *et al.*, 2015)	22±2	Male sprinters (16)	Crossover (4-week washout)	Repeated cycle sprint test	Green Tea Extract	980mg/day polyphenols	4 weeks	CK (0, 24)	11
(Lamb *et al.*, 2019)	Pomegranate – 24(IQR 21-33) Placebo – 24(IQR 23-32)	Non-trained men (24)	Parallel	5x10 Eccentric max elbow flexion	Pomegranate juice	1757.8mg/day phenolic compounds	9 days (4 pre, day of, 4 post)	MVC (0, 24, 48, 72, 96) DOMS (0, 24, 48, 72, 96) CK (0, 24, 48, 72, 96)	11
(Levers *et al.*, 2015)	21±3	Resistance Trained males (23)	Parallel	10x10 back squat @ 70% 1RM	Montmorency Tart Cherry	480mg/day of powdered tart cherries	10 days (7 days pre, day of 2 days post)	MVC (60min, 24, 48) CK (60min, 24, 48) DOMS (60min, 24*, 48*)	10
(Lima *et al.*, 2019)	22± 3	Healthy male PE students (30)	Parallel	30mins Downhill running (-15%) @ 70% VO_{2max}	Anthocyanin Rich juice	480ml anthocyanin rich juice per day	9 days (4 pre, day of, 4 post)	MVC (15min, 24, 48, 72, 96) CK (48, 96*) DOMS (24*, 48*, 72*, 96*)	10

Authors	Age (mean years ± SD)	Participant cohort (training status, sex, sample size)	Study Design (parallel / crossover)	EIMD inducing exercise	PP intervention	Reported Dosage	Duration of ingestion (pre and post EIMD)	Outcome variable and time of outcome measurement post exercise (h)	PEDro score
(Morgan *et al.*, 2018)	23±3	Recreationally Active males (10)	Crossover (2-week washout)	10x10 single leg knee extension @ 80% 1RM	Cacao Mucilage	154mg/day polyphenols	10 days (7 days pre, day of 2 days post)	MVC (0, 24, 48) CK (0, 24, 48) DOMS (0, 24, 48)	9
(O'Fallon *et al.*, 2012)	Quercetin Male - 21±2 Quercetin Female - 21±1 Placebo Male - 20±1 Placebo Female - 20±1	Healthy subjects (30, 15f)	Parallel	2x24 Max eccentric preacher curl exercise	Quercetin	1000mg/day quercetin aglycone	12 days (7 pre, 5 post)	MVC (0, 24, 48, 72, 96) CK (0, 24, 48, 72, 96) DOMS (0, 24, 48, 72, 96)	11
(Quinlan and Hill, 2020)	26±4	Team Sport Players (20, 12f)	Parallel	12x20m sprints, then LIST	Montmorency Tart-Cherry	60ml Tart cherry juice/day	8 days (5 pre, day of, 2 post)	MVC (0, 24*, 48*) CK (0, 24, 48) DOMS (0, 24, 48)	8
(Trombold *et al.*, 2010)	24±1	Recreationally active males (16)	Crossover (2-week washout)	2x20 maximal eccentric elbow contractions	Pomegranate extract	1300mg/day pomegranate polyphenols	9 days (4 pre, day of, 4 post)	MVC (0, 24, 48*, 72*, 96) CK (0, 24, 48, 72, 96) DOMS (0*, 24, 48, 72, 96)	9
(Trombold *et al.*, 2011)	22±2	Resistance Trained males (17)	Crossover (2-week washout)	3x20 eccentric elbow flexion, 6x10 eccentric knee extension	Pomegranate juice	1979mg/L tannins, 384mg/L anthocyanins	15 days (7 pre, day of, 7 post)	MVC (0, 24, 48, 72, 96) DOMS (0, 24, 48*^{e}, 72*^{e}, 96)	11

Pktq – Peak Torque, LIST – Loughborough Intermittent Shuttle Test, 1RM – One Repetition Maximum, BW – Bodyweight, PE – Physical education, f – Female, IQR – Interquartile range, * - Significant improvement observed with treatment, ^ - Significant detriment observed with treatment, *^{e} – significant improvement only in trial about elbow joint

Table 2. Bioactive characterisation of treatment products used in studies (n= 26) presented in this review

Author	Year	Food/Extract	Manner of ingestion	Food/Extract Dosage/day	Reported Polyphenol Content	Method of analysis	Dietary Control / Dietary Assessment	Intake Pattern	Compliance Assessment
(Arent *et al.*, 2010)	2010	Black Tea Extract	Capsule	1760mg Black tea extract	700mg Theaflavin/day, 30% catechins, ~95% total polyphenols	Not Reported	3-day dietary recall	2 capsules morning, 2 capsules early afternoon	Return of bottles
(Bazzucchi *et al.*, 2019)	2019	Quercetin	Capsule	1000mg quercetin aglycone	1000mg/day Quercetin aglycone	Not Reported	Requested to avoid Quercetin containing foods and other antioxidants and nutritional supplements	1 capsule breakfast, 1 12-hours later	Monitored by investigators daily
(Beals *et al.*, 2017)	2017	Tart Cherry	Beverage	60g Powdered Tart Cherries	733mg/day phenolic compounds	Not Reported	Avoid anti-inflammatory medication, 3-day food diary	1 serving morning, 1 serving evening	Not Reported
(Bell *et al.*, 2016)	2016	Tart Cherry	Beverage	60ml Tart Cherry Concentrate	178.8 GAE/L	Total phenolic content by Modified Folin-Ciocalteu colorimetric method	Follow low polyphenol diet throughout Daily Food diaries	1 serving 8:00am, 1 serving 6:00pm	Daily Food Diaries
(Bell *et al.*, 2015)	2015	Tart Cherry	Beverage	60ml Tart Cherry Concentrate	9.2mg/L anthocyanin	Not Reported	Follow low polyphenol diet throughout Daily Food diaries	1 serving 8:00am, 1 serving 6:00pm	Daily Food Diaries
(Beyer *et al.*, 2017)	2017	Polyphenol blend of tea extracts	Capsule	2000mg Tea extracts	Minimum 40% total polyphenols, 1.3% theaflavins, 5-8% EGCG	Not Reported	Dietary Recall	1 serving after testing, 1 serving 6 hours later	Return of containers
(Bowtell *et al.*, 2011)	2011	Tart Cherry	Beverage	60ml Tart Cherry Concentrate	9.117mg/ml anthocyanin	HPLC analysis	48h Food diary for each trial arm	1 serving morning, 1 serving afternoon	Not Reported
Brown et al. [30]	2019	Tart Cherry	Beverage	60ml Tart Cherry Concentrate	178.8 GAE/L	Total phenolic content by Modified Folin-Ciocalteu colorimetric method	Weighted food diary during all trial periods No consumption of supplements and anti-inflammatory drugs	1 serving before breakfast, 1 serving before evening meal	Not Reported
(Buchwald-Werner *et al.*, 2018)	2018	Lemon Verbena	Capsule	400mg lemon verbena extract	More than 18% polyphenols	Not Reported	Fruit and vegetable intake assessed. Refrain from intake of anti-inflammatory/oxidative drugs or supplements Standardised dinner and breakfast prior to trial	2 capsules morning	>85% required for inclusion (Assessment method not reported)

Author	Year	Food/Extract	Manner of ingestion	Food/Extract Dosage/day	Reported Polyphenol Content	Method of analysis	Dietary Control / Dietary Assessment	Intake Pattern	Compliance Assessment
(Connolly, McHugh and Padilla-Zakour, 2006)	2006	Tart Cherry	Beverage	24 fl oz cherry juice blend	1200mg/day GAE	Total phenolic content by Folin-Ciocalteu colorimetric method	No anti-inflammatory/oxidative drugs	1 serving morning, 1 serving evening	Not Reported
(Costello *et al.*, 2020)	2020	NZ Blackcurrant	Capsule	600mg CurraNZ	210mg anthocyanins/day	Not Reported	72h food diary, Habitual anthocyanin food frequency questionnaire	Twice per day	Not Reported
(da Silva *et al.*, 2018)	2018	Green Tea	Capsule	500mg Green tea extract	EGCG -1.60 mg/g, epicatechin -1.59 mg/g, epigallocatechin -16 mg/g, epicatechin gallate-17.80 mg/g	HPLC analysis	Not reported	1 serving morning	Not Reported
(Herrlinger, Chirouzes and Ceddia, 2015)	2015	Polyphenol blend of tea extracts	Capsule	2000mg Tea extracts	Minimum 40% total polyphenols, 1.3% theaflavins, 5-8% EGCG	Not Reported	Not reported	Twice per day	Weekly contact with researchers
(Howatson et al., 2010)	2010	Tart Cherry	Beverage	16fl oz tart cherry juice	1200mg/day GAE	Total phenolic content by Folin-Ciocalteu colorimetric method	Food diary, refrain from ingestion of nutritional supplements	1 serving morning, 1 serving afternoon	Not Reported
(Hutchison *et al.*, 2016)	2016	Black Currant Nectar	Beverage	16oz	Malvidin glucosides - 193.25mg/100g fruit Cyanidin glucosides - 175.69mg/100g fruit	Association of Official Agricultural Chemists (AOAC) Official pH Differential Method	Not reported	1 serving morning, 1 serving at night	Not Reported
(Jajtner *et al.*, 2016)	2016	Polyphenol blend of tea extracts	Capsule	2000mg Tea extracts	Minimum 40% total polyphenols, 1.3% theaflavins, 5-8% EGCG	Not Reported	Not reported	Twice per day	Return of empty containers
(Jówko *et al.*, 2011)	2015	Green Tea	Capsule	1000mg Green Tea extract	980mg/day polyphenols	Not Reported	Refrain from Tea, caffeinated beverages 24h pre-testing. No dietary supplements for duration	Two capsules twice per day	Return of remaining capsules

Author	Year	Food/Extract	Manner of ingestion	Food/Extract Dosage/day	Reported Polyphenol Content	Method of analysis	Dietary Control / Dietary Assessment	Intake Pattern	Compliance Assessment
(Jówko *et al.*, 2015)	2011	Green Tea	Capsule	1000mg Green Tea extract	980mg/day GAE	Epicatechin content by Folin-Ciocalteu colorimetric method	3-day food diary, refrain from other green tea and limit caffeine intake	Two capsules twice per day	Return of remaining capsules
(Lamb *et al.*, 2019)	2019	Pomegranate Juice	Juice	500ml pomegranate juice	1757.8mg/day ± 185.4mg GAE	Total phenolic content by Folin-Ciocalteu colorimetric method	Standardised post-exercise meal. Food diary for duration of study	1 serving morning, 1 serving evening	Return of empty bottles
(Levers *et al.*, 2015)	2015	Tart Cherry	Capsule	480mg Tart cherry skin powder capsules	600mg phenolic compounds per 290mg TC powder	Not Reported	4-day food diary	1 serving morning	Not Reported
(Lima *et al.*, 2019)	2019	Apple, Blueberry, Maqui berry, Raspberry, Cranberry	Juice	480ml Juice	116mg anthocyanins	Not Reported	Not Reported	Twice per day	Not Reported
(Morgan *et al.*, 2018)	2018	Cacao Mucilage	Beverage	330ml Cacao juice	154mg/day GAE	Total phenolic content by Folin-Denis reagent method	3-day food diary	1 serving per day	Daily supplementation log
(O'Fallon *et al.*, 2012)	2011	Quercetin	Bar	1000mg Quercetin Nutrition bar	1000mg/day Quercetin aglycone	Not Reported	Refrained from nutritional supplements and anti-inflammatory medication. Avoid consumption of foods with excessive quercetin	2 servings per day at 12h intervals	Return of supplement wrappers
(Quinlan et al 2020)	2020	Tart cherry	Juice	60ml per day	Not Reported	Not Reported	Food Diary for the duration of study	1 serving morning, 1 serving evening	Not Reported
(Trombold *et al.*, 2010)	2011	Pomegranate Juice	Beverage	500ml pomegranate juice	1,979 mg/L of tannins, 384 mg/L of anthocyanins, and 121 mg/L of ellagic acid derivatives	Not Reported	Refrained from antioxidant supplements and anti-inflammatory medication.	2 servings per day at 12h intervals	Not Reported

Author	Year	Food/Extract	Manner of ingestion	Food/Extract Dosage/day	Reported Polyphenol Content	Method of analysis	Dietary Control / Dietary Assessment	Intake Pattern	Compliance Assessment
(Trombold *et al.*, 2011)	2010	Pomegranate extract	Beverage	1000ml Pomegranate extract	1300mg/day GAE	Not Reported	Discontinue vitamin/mineral supplementation and pain medication	2 servings per day at 12h intervals	Email reminders

GAE – Gallic Acid Equivalent, EGCG – Epigallocatechin Gallate, HPLC – High Performance Liquid Chromatography, TC – Tart Cherry
*This characterisation is heavily dependent on the data provided. Data presented are as per published paper.

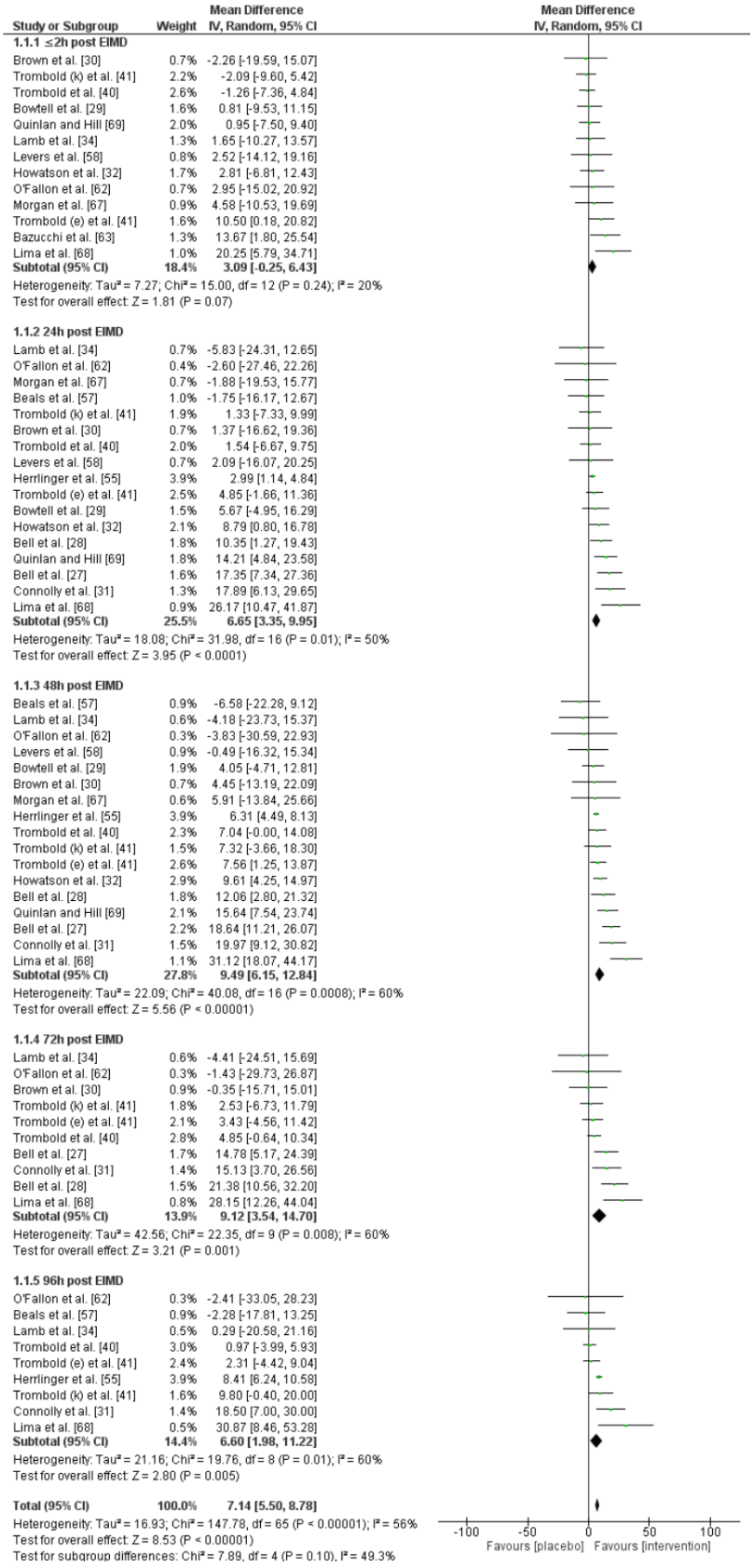


Fig. 2 Forest plot illustrating the effect of polyphenol treatments which contain flavonoids on recovery of maximum voluntary contraction muscle strength from ≤ 2h to 96h post EIMD in comparison to placebo treatment. EIMD – Exercise-Induced Muscle Damage, CI – Confidence Interval, Trombold (e) – Trombold et al. 2011 trial of muscle damage about the elbow joint, Trombold (k) – Trombold et al. 2011 trial of muscle damage about the knee joint.

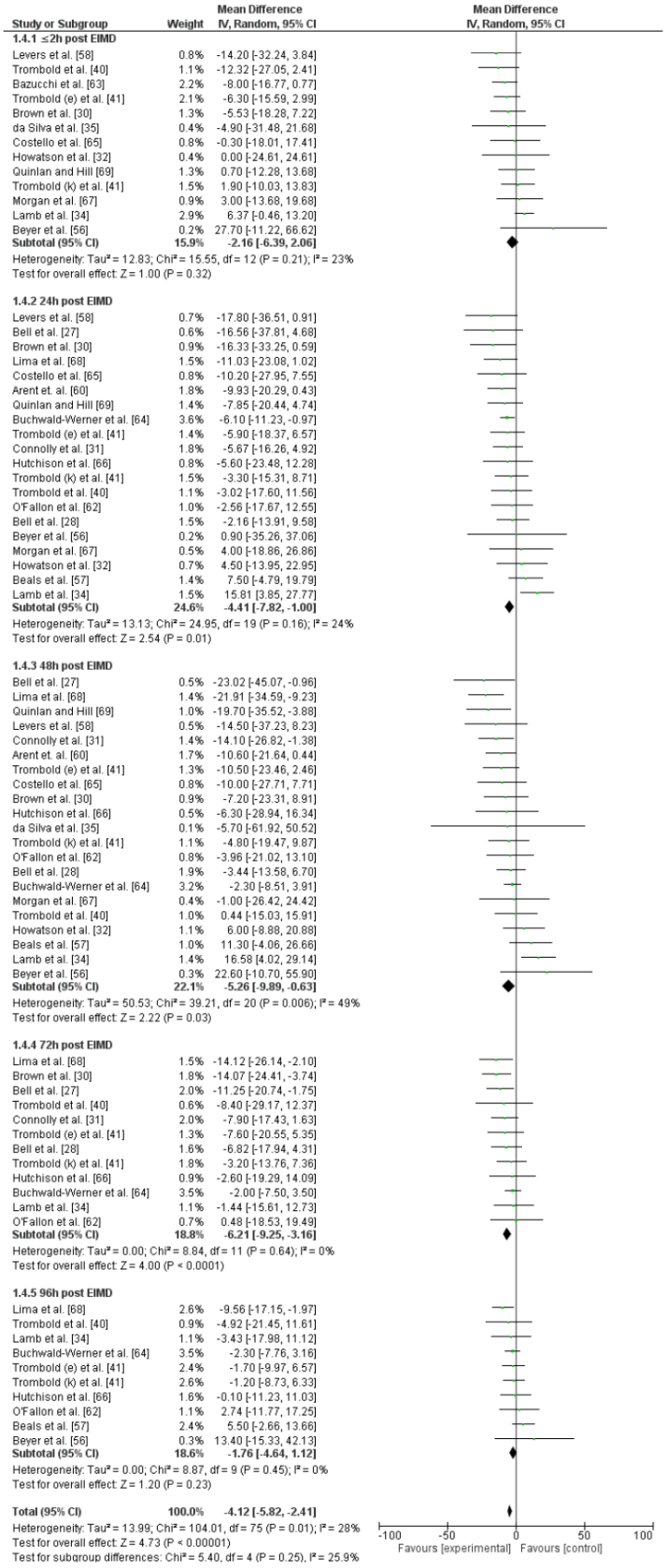


Fig. 3 Forest plot illustrating the effect of polyphenol treatments which contain flavonoids on recovery of delayed onset muscle soreness from ≤ 2h to 96h post EIMD in comparison to placebo treatment. EIMD – Exercise-Induced Muscle Damage, CI – Confidence Interval, Trombold (e) – Trombold et al. 2011 trial of muscle damage about the elbow joint, Trombold (k) – Trombold et al. 2011 trial of muscle damage about the knee joint.

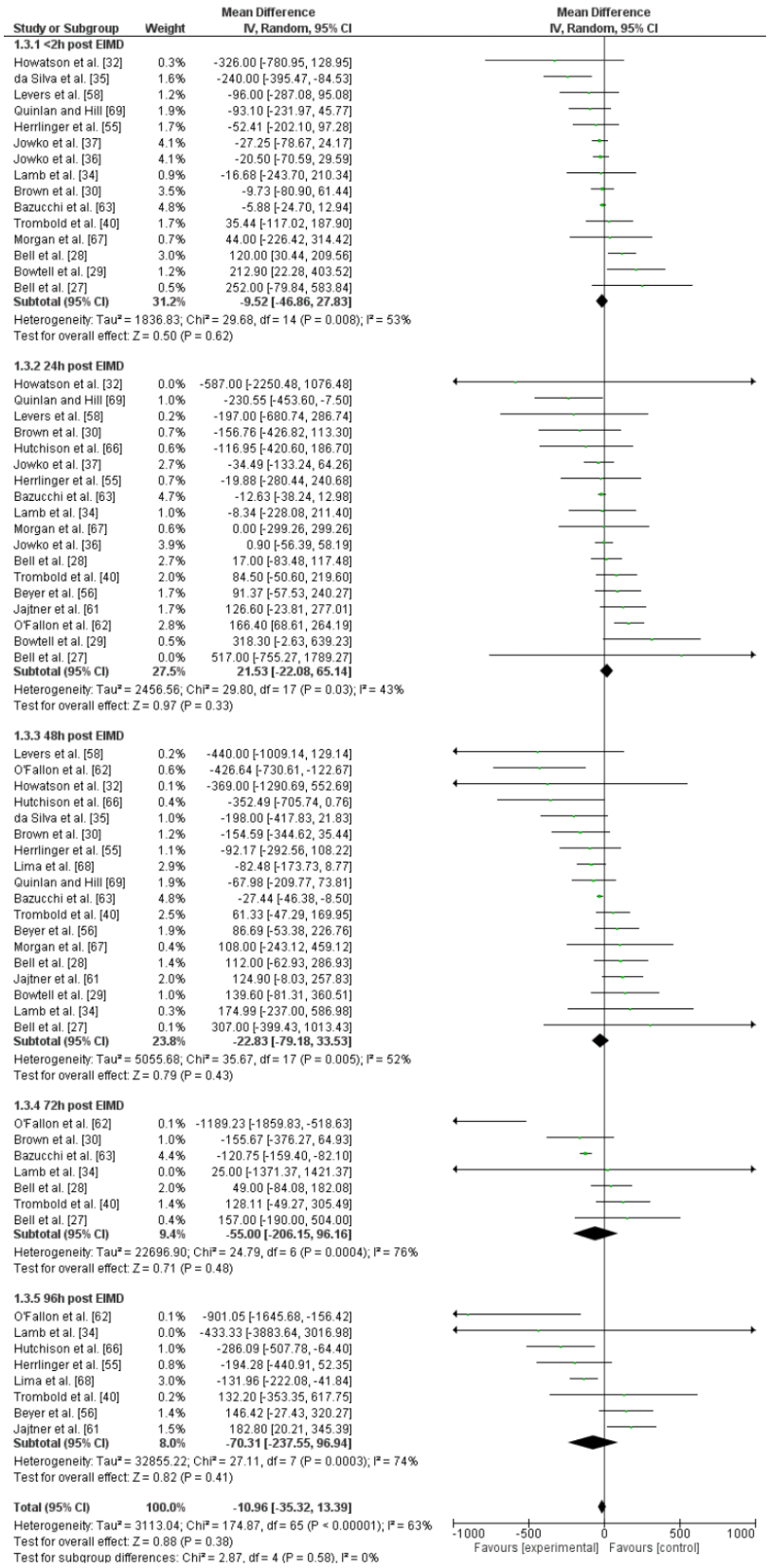


Fig. 4 Forest plot illustrating the effect of polyphenol treatments which contain flavonoids on recovery of creatine kinase response from ≤ 2h to 96h post EIMD in comparison to placebo treatment. EIMD – Exercise-Induced Muscle Damage, CI – Confidence Interval

4. Discussion

4.1 Overall Study findings

Of late, the development of functional foods for sport and exercise has become a priority for scientific research in sport and exercise nutrition, however few functional foods have gained as much scientific attention as polyphenols. A recent systematic review and meta-analysis has shown the potential for polyphenols to improve exercise performance (Somerville, Bringans and Braakhuis, 2017). In recent years a plethora of RCTs have been conducted investigating the effect of polyphenols on recovery from EIMD and a systematic review and meta-analysis has shown significant reductions in exercise-induced inflammation and oxidative stress with anthocyanin-rich whole fruit consumption (Bloedon *et al.*, 2019). However, to the best of the authors' knowledge, this is the first systematic review and set of meta-analyses to investigate the effect of flavonoid containing polyphenol ingestion on direct indices of recovery from EIMD. The results of these meta-analyses show that there is a significant beneficial impact of flavonoid polyphenol ingestion on recovery of both muscle soreness and muscle strength in the 4-day period following EIMD.

The first meta-analysis reports that muscle strength is significantly improved by an average of 7.14% in the 96-hour period following EIMD when flavonoid polyphenols are ingested for a minimum of 6 days in comparison to placebo. The greatest improvement in MVC muscle strength occurred at 48h post EIMD, with a mean improvement of 9.49% observed, although significant improvements were seen at all timepoints from 24h to 96h post EIMD.

There was an overall significant reduction of DOMS by 4.12% observed in the pooled analysis over the 4 days following EIMD when compared with placebo ingestion. The greatest magnitude of reduction occurs at 72 hours post EIMD, exhibiting a significant reduction of 6.21%. As a result of this it appears that flavonoid's may be most effective at shortening and lowering peak DOMS. This effect appears to have almost entirely subsided by 96 hours post exercise, suggesting they may not provide benefit to the residual soreness following peak DOMS.

In the current study no significant changes in CK were observed at any timepoint with flavonoid polyphenol ingestion. This may be a greater reflection of the sensitivity of

CK as a measure of EIMD changes in response to nutritional intervention than that of the efficacy of flavonoid polyphenol treatments, as discussed in section 4.1.3.

4.1.1 Recovery of Muscle Strength

Measurement of muscle strength is largely considered to be the most relevant measure of muscle damage, with MVC's of skeletal muscle the most reliable and sensitive measure of muscle strength following EIMD (Morton et al., 2005; Warren et al., 1999). Loss of muscle strength is suggested to be the only known quantifiable recovery outcome to span the entire time-course of muscle damage following eccentric contraction-based exercise (Clarkson and Hubal, 2001).

This meta-analysis observed a significant ~7% improvement in recovery of muscle function in the 4-day period post-EIMD associated with prolonged ingestion of polyphenol treatments which contain flavonoids. This suggests that in sports containing frequent eccentric muscle contractions, with competition events occurring within 4 days of one another, daily ingestion of such treatments has the potential to provide a key ergogenic effect to subsequent sporting performance. Significantly greater improvements were seen with tart cherry-based interventions when compared with pomegranate at both 24h and 72h post EIMD, suggesting flavonoids from tart cherry may provide greater benefit to recovery of muscle strength. This was concluded based on a test for subgroup differences. While seven tea-based polyphenol RCTs were included in this review only one of which assessed recovery of muscular strength (Herrlinger, Chirouzes and Ceddia, 2015).

Future research should endeavour to investigate whether other flavonoid sources such as tea can provide similar recovery of strength as tart cherry interventions. It was also observed that beverage-based interventions provided greater muscle strength recovery at 24h post EIMD. Only data for two studies were include in the capsule subgroup however at this timepoint and further data is required to confirm if it is indeed the manner of ingestion that is the cause for such differences. Training status also appeared to play a role in efficacy with studies utilising trained participants reporting significantly greater recovery of muscle strength 24-hours following muscle damage. As this was only apparent at one timepoint, further research is warranted comparing the effect of participant training status on the efficacy of polyphenol treatments which contain flavonoids.

One concern with MVC muscle strength measurements of recovery from EIMD, despite their reliability and accuracy, is whether results from such tests directly transfer to in-competition performance. Future studies should include measures of activity-specific muscle power measurements such as counter movement jumps (CMJ) and maximal sprints alongside MVC measurements to elucidate the potential direct ergogenic effects to competitive sport. Of the studies included in this meta-analysis, only 3 reported assessing measures of muscular power generation. Bell et al., (2016) conducted 20m sprint tests, 5-0-5 agility test and CMJ tests. Significant improvements were observed in all 3 performance tests. Bell et al. [28] measured 6-second maximal cycling power generation during a 72-hour post EIMD period and observed no significant change. Brown et al. (2019) assessed 30m sprint times, CMJ and reactive strength index (RSI) for 72 hours post EIMD. No significant difference was observed for RSI or 30m sprint however a significant improvement for CMJ height was observed following ingestion of tart cherry extract.

Overall, our key findings indicate that prolonged ingestion of polyphenols treatments which contain flavonoids improves the recovery timeline of muscle force generation capacity in MVC tests by ~7%. Further research is warranted to confirm whether the recovery improvement observed in MVC tests is directly applicable to sporting performance; emerging research findings indicate that improvements may transfer to jump height (Bell et al., 2016; Brown et al., 2019), and agility tests (Bell *et al.*, 2016), while the effect on sprint performance is unclear at present (Bell et al., 2016; Brown et al., 2019).

4.1.2 Recovery from Delayed Onset Muscle Soreness

VASs are reported as a reliable measure of musculoskeletal pain (Boonstra *et al.*, 2008). Pooled data shows that flavonoid polyphenol ingestion causes a mild analgesic effect on DOMS in the 96-hour period post EIMD. However, this was only significant at between 24- and 72-hours post EIMD when sub-grouped by time point. It has been shown that VAS measurements differ significantly from that of less subjective pressure pain threshold tests (PPT), thus the inclusion of more than one method would be inappropriate in a single pooled analysis (Yin Lau, Muthalib and Nosaka, 2013). Although VAS's have been shown to be the most accurate reflection of the time course of DOMS, it has been recommended that VAS measurement be included alongside PPT to assess the overall impact of DOMS (Yin Lau, Muthalib and Nosaka, 2013). Of

the 26 included studies in this review only 2 measured PPT. Brown et al., (2019) observed significant improvements to PPT alongside reductions in DOMS, observed with VAS measurement. Bowtell et al., (2011) used PPT as their only measure of DOMS and did not observe any significant improvement. The EIMD experienced in this study however was relatively mild and as such improvements may not have been detected.

Like that observed in the muscle strength meta-analysis, significantly greater recovery of muscle soreness was observed in studies using trained participants compared to untrained. This suggests training status may be a factor in the conferral of muscle recovery effects of polyphenol treatments which contain flavonoids.

Findings of the current meta-analysis suggest that six or more days of flavonoid ingestion can reduce the DOMS experienced over the 4 days' post EIMD. In particular, the greatest effect appears to occur at the latter end of peak DOMS ~72h post EIMD. Further studies utilising PPT measurement of muscle soreness are required to shed more light on the effect of flavonoids on DOMS.

4.1.3 Recovery of Creatine Kinase Concentrations

Ingestion of a flavonoid polyphenol treatment has no significant effect on CK in the 96-hour post exercise period when compared with placebo treatments. Despite its popularity, CK is largely criticised as a marker of EIMD. As far back as 1988, the reliability of CK has been discussed, with large inter-subject variability reported (Clarkson and Ebbeling, 1988). It has also been noted that CK correlates poorly with decreased muscular function following intense exercise (Warren, Lowe and Armstrong, 1999), and can be impacted by the enzymatic activity of muscle tissue, hydration status and ethnicity of subjects among other factors unrelated to the magnitude of muscle damage (Baird *et al.*, 2012). As such the results of this meta-analysis may be a greater reflection of the inability of CK to assess the impact of nutritional interventions on recovery from EIMD. When designing future RCTs investigating polyphenols and recovery from EIMD, unless every effort can be made to control these influencing factors, priority should be given to other, under-utilised indicators of EIMD such as PPT, muscle power and range of motion alongside MVC's and VAS pain measures which provide more reliable indications of the magnitude of EIMD over the time-course of muscle damage (Warren, Lowe and Armstrong, 1999).

4.2 Strength of the evidence

Overall the risk of bias, as assessed by the PEDro scale is low with all studies included in these meta-analyses reaching a score of 7 or higher and seven studies achieving the maximum score of 11 (de Morton, 2009). This is predominantly due to the highly controlled inclusion criteria set out for this meta-analysis requiring the use of a placebo arm and blinding to be included. However, despite these results it must be noted that the PEDro scale does not require reporting precise information regarding the protocols adopted in key areas such as randomisation and blinding. Many studies that score particularly high in the risk of bias analysis do not in fact provide detail beyond stating blinding and randomisation has occurred. This detail should be included in future RCTs in this area to allow for full and complete assessment of the risk of bias. While the PEDro scale has been shown to be a valid and reliable measure of methodological rigour in exercise RCTs, it does not specifically address methodological factors specific to nutrition RCTs. Some of these include monitoring of the composition of nutritional interventions, specific methods for monitoring compliance with nutritional interventions and assessment and control of broader diet during RCT protocols. As such these have been reviewed in Table 2.

The sample size of many of these studies is notably small indicating their individual power may be relatively weak. Of the 26 studies included in this review, only 9 referred to the use of any sample size calculations when designing RCTs. Although conducting a meta-analysis addresses the intrinsic issue of statistical power within these RCTs, it opens further issues due to the potential for introducing heterogeneity arising from variations in RCT design. This variation runs the risk of introducing high levels of heterogeneity into the analysis. Using the I^2 heterogeneity bands of (Higgins *et al.*, 2003), overall heterogeneity of MVC muscle strength analysis was moderate (I^2=56%), DOMS analysis was low (I^2=28%) and CK analysis was high (I^2=63%). High heterogeneity in CK meta-analysis is likely to have occurred as a combination the influencing factors shown to impact CK measurement such as ethnicity, hydration and enzymatic activity alongside the variability of CK response itself, as discussed in section 4.1.3.

Nine RCTs with a crossover design were included in these analyses. While crossover designs provide a good method to control for within-participant variation, the potential

for carryover from previous study arms in nutrition interventions is one which must be considered. All studies using this design reported using a washout period to account for this (Table 1.). Of the nine-crossover designed RCTs, 8 of these use a washout period of 2 weeks or over. Crossover EIMD trials, however, are also subject to the repeated bout effect particularly when maximal eccentric muscle contractions are used to cause EIMD (Howatson, Van Someren and Hortobágyi, 2007). This protective effect may induce a reduction in EIMD following a second bout of the same eccentric exercise, and thus distorting the impact of an intervention (McHugh, 2003). Despite these concerns subgroup analysis showed no significant difference between results obtained from parallel or crossover RCTs for any outcome at any timepoint.

Overall, despite some minor concerns the overall quality of evidence included in this systematic review is of high standard, as is highlighted by the results from the PEDro scale and moderate to low heterogeneity in key outcomes.

4.3 Flavonoid Containing Polyphenols and EIMD

While a full overview of the potential mechanisms of enhanced muscular recovery by polyphenols is beyond the scope of this review, it is worth noting that the understanding of the action of polyphenols has changed significantly in recent years (Bowtell & Kelly, 2019). Flavonoid polyphenols have traditionally been thought of as antioxidants, scavenging excess free radicals produced in the body. They, however, have poor bioavailability with <5% of phenolic compounds ingested reaching the bloodstream, with much of the other 95% fermented by the gut microflora (Clifford, 2004). This poor bioavailability means that the concentrations of phenolics which demonstrate radical scavenging activity in-vitro are rarely, if ever, reached *in-vivo* in relevant tissues. Recent research has attributed the ability of polyphenols to impact on recovery from EIMD to alterations in cell signalling stress response pathways, inducing upregulation of the production of endogenous antioxidant enzymes such as superoxide dismutase. The ability of polyphenols to upregulate the NRF2/electrophile response element pathway through interactions with Keap1 when exposed to ROS (Forman, Davies and Ursini, 2014; Huang *et al.*, 2015). Polyphenols have also been shown to reduce the production of ROS through inhibition of COX1 and COX2 subsequently downregulating NF-κB expression resulting in reduced release of pro-inflammatory cytokines (Esposito *et al.*, 2014). For a more detailed discussion of the

potential mechanisms of action please refer to the recent comprehensive review by Bowtell & Kelly, (2019).

4.4 Priorities for Future Research

4.4.1 Characterisation of Study Treatments

The current research process for the development of bioactive functional foods for use within the sport and exercise sector is largely in its infancy. This systematic review and meta-analysis has highlighted the potential of flavonoid polyphenols to enhance recovery from EIMD. While there is substantial evidence supporting a role of polyphenols in human health (Amiot, Riva and Vinet, 2016; Cory *et al.*, 2018), an understanding of their role in musculoskeletal systems is still at an early stage; however, findings such as those provided in the current review reinforce a need for further research in this area. Nevertheless, we are keen to acknowledge that the large variability in study protocol design and reporting hampers the ability for comparison across studies. Robust human studies are a fundamental cornerstone for establishing cause and effect relationships between polyphenols and health outcomes (Lucey, Heneghan and Kiely, 2016). A recent European Food Safety Authority (EFSA) guidance document for health claim substantiation has outlined ten key principles for the development of scientific evidence for functional foods and health (Lucey, Heneghan and Kiely, 2016); examples include: identification of primary outcomes for assessment, the inclusion of appropriate study populations, provisions for designing RCT protocols, the characterisation of treatments, ethical governance, among others. However, some of these key indicators of scientific quality are absent or poorly reported in the RCTs included in the current review. Characterisation of study treatments, flavonoid dosage and composition was particularly weak in many of the RCTs included in this systematic review. A quantitative analysis of the effect of dosage was not possible due to this poor characterisation. While all studies reported dosage of treatment substance, specific quantification of the polyphenol content by total phenolic content assay (TPC) using either Folin-Ciocalteu or Folin-Denis method was only reported in 7 of 26 studies. Furthermore, characterisation of treatments in terms of dosage of specific polyphenol subclasses is also required to enable identification of specific phenolic compounds with the most potent impact on skeletal muscle. The reporting of such critical detail in studies going forward would

significantly augment the quality of evidence in this area and would facilitate a more accurate comparison across studies particularly for the establishment of polyphenol efficacy and dose. On this note, Bowtell & Kelly, (2019) have postulated that the dosage required for improved EIMD recovery is likely to be higher than that required for acute performance benefits, however further investigation is duly warranted.

To discern an effective polyphenol dosage, a clearer understanding of the precise mechanisms of action must also be garnered. To achieve this there is a need for RCTs to include muscle biopsy samples to specifically assess the impact of dosing regimens, treatment protocols and polyphenol composition on relevant stress signalling pathways and how these may impact outcomes of recovery from EIMD.

Another fundamental criterion to augment RCT quality is the knowledge of habitual dietary intakes and the control of polyphenol consumption during interventions. Ideally RCTs should assess polyphenol exposure prior to and where possible during RCTs. Twelve out of 26 studies reported the analysis of habitual diet using either food diaries or dietary recall. Six studies also instructed participants to reduce or avoid foods high in polyphenols for the duration of the RCT. Nine studies reported instructing participants to refrain from ingestion of any supplementation or anti-inflammatory medications. Furthermore, to ensure all participants received the intended daily dosage, it is important to carry out compliance checks or assessment. While most studies reported participant compliance with the study treatment, this was not reported for twelve studies included in this review.

Twenty-two studies included in this systematic review and meta-analysis reported treatment regimens of the daily dosage being split twice per day, mostly with between 10 and 14-hour periods between ingestion. Polyphenol pharmacokinetic studies have reported peak plasma polyphenols in the initial 1-2 hours post ingestion and have reported elevated concentrations for up to 12 hours post-acute ingestion (Mertens-Talcott *et al.*, 2008; Seymour *et al.*, 2014; Zhong *et al.*, 2017). Sandhu et al., (2018) has reported that plasma polyphenols may remain elevated for up to 15h post ingestion following a period of chronic ingestion of a strawberry polyphenol treatment, while de Ferrars et al., (2014) has shown multiple peaks of plasma polyphenol levels throughout a 48-hour post ingestion period due to the role of enterohepatic recirculation, hepatic recycling and microbial metabolism during anthocyanin metabolism. Given the peaks and troughs observed in plasma polyphenol levels noted

by de Ferrars et al., (2014) it appears entirely appropriate that a twice daily ingestion regimen be used for muscular recovery studies however, further research is required to elucidate whether this extends to effects in muscle tissue. An interesting observation from both Zhong et al., (2017) and (Martínez-Huélamo *et al.*, 2016) was the biphasic release of polyphenols associated with fat ingestion. This biphasic release is suggested to result from the process of enterohepatic circulation causing storage and subsequent release of flavonoids in bile from the gall bladder (Kalt *et al.*, 2014). The pattern of macronutrient ingestion, particularly fat ingestion may play a significant role in the metabolism of anthocyanins and potentially other polyphenols and govern the additional peaks observed by de Ferrars et al., (2014). This influence of fat ingestion resulting in secondary peaks in polyphenol concentrations may represent an avenue for further research into the effect of co-ingestion of polyphenols with other nutrients. Further research should be undertaken to investigate how specific food matrices impact on polyphenol stability and ultimately the response to treatment.

4.4.2 Long Term Intake of High-Dose Polyphenol & Antioxidant treatments

The current body of research supports the use of polyphenols for periods of intense and frequent competition due to the presented recovery benefits and previously proven performance benefits (Somerville, Bringans and Braakhuis, 2017). However, the long-term use of antioxidant vitamin supplementation during prolonged training blocks has been largely scrutinised due to the potential blunting effect which has been observed on training adaptation (Ristow *et al.*, 2009; Paulsen *et al.*, 2014; Bjørnsen *et al.*, 2016). This has largely been attributed to the importance of ROS as signalling molecules for adaptation from cellular stress such as that caused by exercise. Resulting from this, it is thought that the use of traditional nutritional antioxidants such as vitamin C and E may negate some of the adaptation from exercise training (Gomez-Cabrera *et al.*, 2015). However, due to the antioxidant effect associated with polyphenol ingestion being recently attributed to upregulation of the human body's endogenous antioxidant response system rather than the direct radical scavenging ability as is the case with traditional vitamin antioxidants, it seems plausible that prolonged ingestion of polyphenols during training blocks may yield different results (Bowtell & Kelly, 2019). This has been substantiated by a small number of training RCTs using flavonoid treatments which have shown no attenuation of training adaptation

(Braakhuis, Hopkins and Lowe, 2014; Kuo *et al.*, 2015; Beyer *et al.*, 2017). However, RCTs utilising the non-flavonoid polyphenol resveratrol have observed some blunting of training effect in older populations (Gliemann *et al.*, 2013; Scribbans *et al.*, 2014). There is a clear need for further empirical data to discern the potential risks of attenuation of the training response with prolonged polyphenol ingestion. For further detail of how dietary supplements affect exercise training adaptation see Rothschild and Bishop's recent review (Rothschild and Bishop, 2020).

5 Conclusion

In conclusion, to the best of the authors' knowledge this is the first systematic review and meta-analysis investigating the effect of flavonoid polyphenols on recovery from EIMD. The results of these analyses provide convincing evidence that ingestion of flavonoid polyphenols for a period of 6 days or more can improve the recovery of muscle strength by ~7% and reduce muscle soreness by ~4% in the 96-hour window following exercise-induced muscle damage. When coupled with the results of the comprehensive meta-analyses conducted by Bloedon et al., (2019) who observed small but significant reductions in exercise-induced inflammation and oxidative stress with anthocyanin-rich whole fruit consumption, flavonoid containing polyphenol ingestion appears to be an extremely attractive addition for many athletes and active individuals in the quest to improve muscular recovery post exercise. A systematic review and meta-analysis by Somerville et al., (2017) showed a clear moderate benefit to exercise performance with polyphenol ingestion over a 7-day period. The scientific evidence surrounding the use of polyphenols in sport and exercise is now sufficiently strong to recommend the inclusion of flavonoid-rich foods in the daily diet of athletes and active individuals for a minimum of 7 days surrounding competition given their propensity to improve both acute exercise performance and recovery from EIMD.

Future research should prioritise the precise characterisation of polyphenol treatments. This will allow for the development of more specific recommendations for the creation of future evidence-based functional food products and guidelines for the inclusion of polyphenol rich whole-foods in the diets of populations engaging in physically demanding exercise.

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Randomised Controlled Trial Preface

Contextualising the Collaborative Effort of the RubusElite RCT

1. *Introduction*

The following two chapters will outline and report the results of key outcomes from a collaborative, multi-disciplinary, multi-institution proof of concept human randomised controlled trial (RCT) investigating the recovery and performance efficacy of the RubusElite prototype beverage, a culmination of research efforts on the RubusElite project. This RCT is a result of close collaboration between the School of Food and Nutritional Sciences UCC and the Department of Sport and Exercise Science SETU Waterford.

This preface will provide further information about the design of the RCT and will also inform on the research findings and future analyses led by other research groups as part of the RubusElite Project. This research was critical to support and facilitate the current PhD but are not explicitly presented as results of this PhD thesis.

2. *RubusElite Beverage Design*

The RubusElite beverage designed as part of this project was ultimately created by the RubusElite food science team. The nutritional specifications resulted from multiple iterations of a '*Paper Prototype'* provided to the Food Science team following findings from Chapters 2, 3 and 4 of this PhD thesis. This paper prototype acts as an ideal nutritional profile with key targets for the food science team to meet within their product stability and acceptability challenges. An example of one of these iterations is presented in Table 1. Further factors related to beverage stability, digestion, bioavailability and bioacessibility were accounted for in further preclinical trials led by the nutritional biochemistry team and the food science team. Much of the results of these investigations have been recently published peer reviewed journals (van de Langerijt, O'Mahony and Crowley, 2022; van de Langerijt *et al.*, 2023) and in the PhD thesis of Dr Tessa van de Langerijt of the UCC RubusElite Food Science Team.

Protein boluses of 30g have been suggested to be sufficient to maximally stimulate muscle protein synthesis in both young and old healthy males (Symons *et al.*, 2009). It has been suggested that >1000mg of polyphenols is required to elicit recovery enhancing effects (Bowtell and Kelly, 2019). As a result, during the collaborative development of PHPM, the UCC food science team were provided a nutritional target of a dosage of 30g protein and 750mg polyphenols per 500ml, which would be

consumed twice per day. This was compared with a commercial HPM containing 30g protein and a milk with negligible protein content.

RubusElite

Characteristic	Current Specifications	Considerations	Supporting literature	Literature Justification
Beverage Volume	250ml per serving 2 servings per day 1 container per day	Potential for high viscosity given small volume and high protein content. Small volume easier to consume if astringency is an issue	Lemieux and Simard 1994, McManus et al 1981, Lesschaeve and Noble 2005	Lemieux - Astringency from tannins perceived as a defect in dairy McManus - Polyphenols precipitate natural macromolecules esp. proteins causing astringency Lesschaeve - Astringency poorly received by many but may be improved w/ exposure and overcome w/ promise of positive outcomes such as health benefits
Beverage Container	500ml Container	To ensure safety and stability of RubusElite beverage a 500ml container must be used. Two 250ml doses to be divided using measuring cup/beaker.		
PP Dose	750mg/serving 1500mg/day 1500mg/container	Potential of astringency of high dose especially within dairy.	Herrlinger et al 2015, Somerville et al 2017, Bowtell and Kelly 2019	Herrlinger - 2000mg/day Tea PP ↓ strength loss & DOMS post ex > than 1000mg and placebo Bowtell - Review recommending >1000mg/day PP to enhance recovery from EIMD Somerville - Review suggesting ↑ performance benefits may be observed w/ greater intake
Protein Type	Milk Protein Concentrate (80% casein 20% whey) Potential to adjust casein:whey ratio to enhance leucine content	Casein provides greater stabilisation for polyphenols. A balance is needed as whey provides greater leucine which reduces total protein and therefore viscosity.	Norton and Layman., 2006, Tang et al., 2009, Witard et al., 2016	Norton - Leucine is key to stimulating muscle protein synthesis (MPS) following exercise. Tang - Whey induced ↑ level of MPS compared to casein and soy at rest and after exercise Witard - Leucine provides substrate for MPS and anabolic signal for mTOR pathway
Protein Dose	Must achieve minimum 2-3g of leucine/serving, 4-6g leucine per container. Total protein content depends on casein and whey % above. ~7.5-10% of total beverage to avoid excessive viscosity	Higher casein % will result in lower leucine content which may not have desired muscle building effect. Potential to supplement with excessive leucine if a significant issue	Schoenfeld et al 2018 van Vliet 2015 Churchward-Venne et al 2013	Schoenfeld - 0.4-0.55g/kg/meal target to maximise to maximise muscle anabolism van Vliet - 2-3g of Leucine required to maximally trigger MPS Churchward-Venne - 6.25g whey w/ 5g supp leucine stimulates MPS = 25g whey
Fat Content	Data from in vitro/ cell culture studies suggest fat within matrix may enhance bioavailability. To be considered in Task 3/4	Investigate in the future the impact of fat on bioavailability. Full fat dairy may be a possibility	Guo et al., 2013 Bohn 2014 Ortega et al., 2009	Guo - 15.4g of fat coingested w/ quercetin enhanced bioavailability. Bohn - Lipids may influence hydrophobic PP bioavailability such as Flavonoid aglycone Ortega - Cocoa PP digestibility enhanced w/ 50% fat content through enhanced micellarization

Abbreviations - EIMD - Exercise Induced Muscle Damage, PP - Polyphenol, ↓ - decrease, ↑ - increase, DOMS - Delayed Onset Muscle Soreness, MPS - Muscle Protein Synthesis, w/ - with, TEAC - Trolox Equivilant Antioxidant Capacity

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3. *Subclassification and Characterisation of Study Beverages*

3.1 Polyphenol Subclassification

The polyphenol subclasses present in the FDBP used to create the RubusElite prototype were identified by Dr Dilip Rai and team at Teagasc Food Research Centre Ashtown. The methodology used is presented below and the full polyphenol subclassification is presented in Table 3.

HPLC-QTOF mass spectrometry data revealed the presence of fifteen dominant phenolic compounds in the freeze-dried blackberry purée (Table 3). The polyphenols were tentatively identified using literature sources, MS/MS fragmentation patterns and accurate mass measurements. These polyphenols can be classified in to five distinctive categories, namely anthocyanins, proanthocyanidins, ellagitannins, flavonoids, and phenolic acids. The identification of the twelve polyphenols in the purée was achieved using commercial standards. The primary polyphenolic subclasses identified within the PHPM intervention were the anthocyanin Cyanidin-3-O-glucoside at 218.28 ± 16.02mg/100g DW and the ellagitannins Sanguiin H-6 and Lambertianin C which were present in concentrations of 105.96 ± 16.83mg/100g DW and 102.71 ± 18.00mg/100g DW respectively.

3.2 Importance of Subclassification and Comparison to Previous Performance Nutrition RCTs

This RCT is the first of its kind to investigate the effects of a polyphenol rich nutritional intervention on recovery from EIMD that has monitored the stability of the phenolic content throughout the duration of the RCT and provides the full polyphenol subclassification of the intervention product. The anthocyanin Cyanidin-3-O-glucoside, and the ellagitannins Lambertianin C and Sanguiin H-6 were identified as the predominant polyphenols in the FDBP used in PHPM (See RubusElite RCT Preface Table 3.). There is an incredibly wide range of compounds which fall under the classification of polyphenolic compounds, within the flavonoid subclass alone there are over 6000 compounds identified to date (Panche, Diwan and Chandra, 2016). Different polyphenol compositions can result in differing bioavailability and differing

physiological responses and as a result it is vital to carry out appropriate classification of subclasses present to identify potential active compounds which provide ergogenic properties (Pandey and Rizvi, 2009; Bowtell and Kelly, 2019). However, this is rarely included in published post exercise recovery RCTs using polyphenol interventions as evidenced by the systematic review and meta-analysis presented in Chapter 4 (Carey, Lucey and Doyle, 2021).

A considerable proportion of the FDBP used in PHPM this study was the anthocyanin compound, cyanidin-3-O-glucoside. From the literature, the most researched anthocyanin rich polyphenolic intervention is Montmorency tart cherry juice and its associated extracts (Beals et al., 2017; Bell et al., 2016; Connolly et al., 2006; Howatson et al., 2010; Ortega et al., 2020). A comprehensive systematic review and meta-analysis of 14 muscle recovery RCTs showed a significant benefit of tart cherry products on recovery following strenuous exercise (Hill *et al.*, 2021). Tart cherry juice has been shown to contain a high proportion of anthocyanins in its phenolic profile, however a full characterisation of other subclasses present has yet to be published (Keane *et al.*, 2016). A RCT displayed an anthocyanin rich blackcurrant nectar was effective at improving recovery from EIMD in 24 healthy participants (Hutchison *et al.*, 2016). It appears that anthocyanin rich polyphenol interventions may be effective at reducing the impact of EIMD however further full classification of polyphenol-based interventions is required. This aligns well with the results of the present RCT.

The PHPM also contained a significant ellagitannin content as well as anthocyanins. A RCT that provided 16 recreationally active males with 1300mg/day of an ellagitannin rich pomegranate extract containing 95.5% ellagitannins demonstrated enhanced recovery of muscle strength post exercise (Trombold *et al.*, 2010). The precise compounds and method of characterisation of this intervention product was not reported however so it is unclear whether this contained the same ellagitannin subclasses as were abundant in the current intervention. The high anthocyanin and ellagitannin content of the FDBP used in the PHPM in the current RCT suggests a favourable polyphenolic composition to support post exercise recovery from EIMD.

Other polyphenolic subclasses have shown efficacy for post exercise recovery. Morgan et al., (2018) investigated the role of polyphenol rich cacao mucilage on post exercise recovery in 10 recreationally active males and reported characterisation including predominantly catechin and flavanol polyphenols present in their

intervention however their total phenolic dosage was considerably lower than the current study (154mg/day) (Morgan *et al.*, 2018). Another RCT reported that daily consumption of three 500mg capsules of a polyphenol rich supplement containing predominantly ellagic acid and the xanthone α-mangostin lowered muscle pain post exercise in 13 recreationally active athletes (Romain *et al.*, 2017).

4. RubusElite Beverage Polyphenol Quantification

The total phenolic content of the freeze-dried blackberry puree (FDBP) used in the RubusElite beverage was assessed by the RubusElite Food Science team and monitored monthly using the Folin Ciolcateu method during the RCT to assess for polyphenol degradation due to long term storage of the FDBP (Singleton, Orthofer and Lamuela-Raventós, 1999). The stability of this FDBP is presented in Figure 1.

There was no significant change in total measured polyphenol content of the FDBP across timepoints as assessed by repeated measures ANOVA ($P = 0.298$). Average of all TPC replicates across all timepoints during the study showed an average polyphenol content of 28.3 ± 2.9 mg/g of freeze-dried powder which results in an effective polyphenol intervention of 1413 ± 147 mg per day (50g of FDBP).

The evidence underpinning dosage recommendations to elicit recovery benefits is largely equivocal and based on narrative assessment of the literature (Bowtell and Kelly, 2019; Carey, Lucey and Doyle, 2021). Even when polyphenol content has been quantified in previously published polyphenol interventions it is not monitored for stability despite the clear need to monitor dosage stability during RCTs due to the potential for polyphenols to degrade during storage or for there to be batch differences in phenolic content (Srivastava *et al.*, 2007; Howard *et al.*, 2012; Oracz, Zyzelewicz and Nebesny, 2015). In the current RCT there is a slight downward trend across time of total phenolic concentration (TPC) of FDBP, there was no significant difference of polyphenol content month to month across the duration of the study and all study participants randomised to the intervention group were provided a theoretical effective dosage of active compounds (RubusElite Preface Figure 1.). TPC analysis of the FDBP shows an average dosage provided across the study of a total phenolic content of ~1400mg per day for the PHPM group.

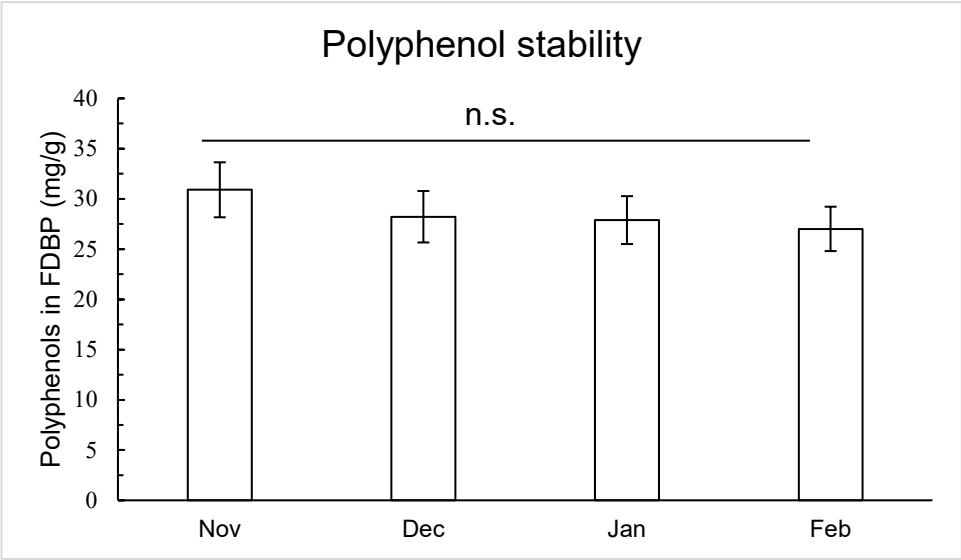


Figure 1. Stability of polyphenol content in FDBP contained in PHPM as assessed by total phenolic content by Folin-Ciocalteu method. Data presented as mean ± SD. Statistical significance set at $P < 0.05$ as assessed by repeated measures ANOVA. n.s. - Not significantly different

5. Beverage composition conclusion

The extensive characterisation of the polyphenol content in PHPM during this study is a much-needed addition to the evidence surrounding polyphenols and post exercise recovery. In particular, the combination of complete subclassification, quantification of polyphenol content and the monitoring of the stability of this polyphenol content throughout the RCT sets this RCT apart from much of the published RCTs to date. This will help considerably with informing future recommendations for polyphenol intake to support post exercise recovery.

6. Further RCT Outcomes

In addition to the outcomes reported in chapters 5 and 6; due to the emerging role of polyphenols on the gut microbiome and the clear links between the microbiome and central stress processing and exercise related outcomes, further samples and measurements were collected as part of this RCT to investigate the impact of the RubusElite beverage prototype on central stress processing and gut microbiome composition. This also impacted on the inclusion criteria of the RCT with it being required that participants not be consuming prebiotics or probiotics, have a digestive disorder or to have taken antibiotics in the previous two months. The analysis of these outcomes is being led by APC Microbiome Ireland and as a result are not being presented as core outcomes of this PhD thesis (see Figure 2).

During this RCT stool samples were collected prior to the intervention period and at the test visit following 7 days of beverage consumption to assess for changes in gut microbial composition. Saliva samples were collected at waking on the morning of the baseline visit and at the test visit to assess for changes in the cortisol awakening response. Saliva samples were also collected immediately following exercise to assess for differences in post exercise cortisol response. Blood samples were collected at baseline, test visit, 24 hours and 48 hours post exercise to assess changes in inflammatory response. Various validated questionnaires and visual analogue scales were administered to participants to assess for changes in levels of anxiety and psychological stress prior to and at the end of the intervention period. All these outcomes are currently being analysed at APC Microbiome Ireland with results pending.

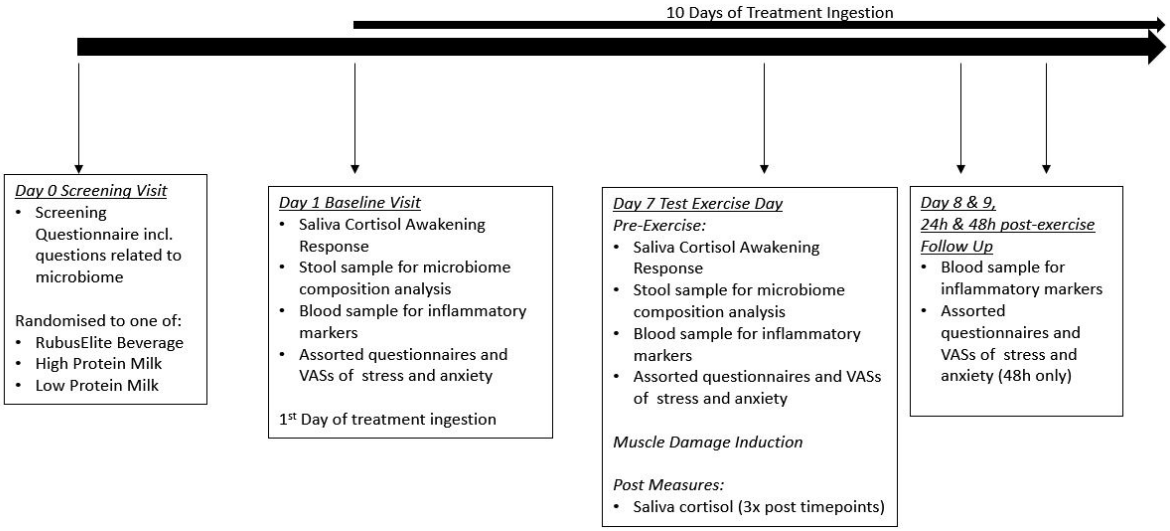


Figure 2. Collection of samples and data related to further RCT outcomes, the analysis of which is being led by APC Microbiome Ireland

Table 3. Characterisation of major polyphenolic compounds in freeze-dried blackberry purée by HPLC-ESI-QTOF mass spectrometry

Compound	Class of Polyphenols	Empirical Formula	Observed [M-H]- *m/z*	Calculated $[M-H]^-$ *m/z*	MS/MS fragment ions (*m/z*)	Freeze dried puree mg/100g DW	Reference
Gallic acid	Phenolic acids	$C_7H_6O_5$	169.0143	169.0137	111.03; 96.98; 155.03	13.66 ± 1.02	Chen et al. (2016); Singh et al. (2016) [1]
Protocatechuic acid	Phenolic acids	$C_7H_6O_4$	153.0185	153.0188	109.03; 91.02	1.74 ± 0.25	D'Angelo et al. (2020) [1]
Chlorogenic acid	Phenolic acids	$C_{16}H_{18}O_9$	353.0866	353.0873	191.09; 155.05; 179.07		D'Angelo et al. (2020) [1]
Lambertianin C	Ellagitannins	$[C_{123}H_{80}O_{78}]^{2-}$	1401.1112	1401.1068	301.01; 633.09; 1250.64; 935.11	102.71 ± 18.00	Heger et al. (2008)
Sanguiin H-6	Ellagitannins	$C_{82}H_{54}O_{52}$	1869.1470	1869.1503	935.09; 1235.16; 1567.15; 633.08	105.96 ± 16.83	Heger et al. (2008)
Cyanidin-3-O-glucoside	Anthocyanins	$[C_{21}H_{21}O_{11}]^+$	447.0929	447.0927	285.05; 269.06; 125.03	218.28 ± 16.02	Chen et al. (2016) [1]
Cyanidin-3-O-rutinoside	Anthocyanins	$[C_{27}H_{31}O_{15}]^+$	593.1597	593.1506	285.06; 284.05; 269.06; 287.07	5.29 ± 0.22	Vagiri et al. (2012); De Gomes et al. (2019) [1]
Epicatechin	Flavonoids	$C_{15}H_{14}O_6$	289.0723	289.0712	245.09; 203.08; 125.04	4.80 ± 0.91	Yuzuak et al. (2018); Mikulic-Petkovsek et al. (2021) [1]
Procyanidin B2	Proanthocyanidins	$C_{30}H_{26}O_{12}$	577.1339	577.1346	289.15; 407.18; 125.07; 408.19	8.65 ± 0.24	de Pascual-Teresa et al. (2000); Bystrom et al. (2008) [1]
Procyanidin trimer C	Proanthocyanidins	$C_{45}H_{38}O_{18}$	865.1976	865.1980	287.13; 125.06; 407.18; 243.10		Rockenbach et al. (2012); Czyzowska et al. (2020)
Ellagic acid pentoside	Phenolic acids	$C_{19}H_{14}O_{12}$	433.0403	433.0407	300.98; 153.03; 78.98		Oszmiański et al. (2015); Kolniak-Ostek et al. (2015) [1]
Rutin	Flavonoids	$C_{27}H_{30}O_{16}$	609.1430	609.1456	301.05; 271.04; 255.05; 151.02	2.96 ± 0.24	Oszmiański et al. (2015) [1]
Ellagic acid	Phenolic acids	$C_{14}H_6O_8$	300.9998	300.9984	301.01; 117.05; 284.02; 229.03	26.92 ± 2.44	Hager et al. (2008) [1]
Quercetin-3-O-glucoside	Flavonoids	$C_{21}H_{20}O_{12}$	463.0867	463.0877	301.03; 271.02; 255.03		Oszmiański et al. (2015) [1]
Quercetin	Flavonoids	$C_{15}H_{10}O_7$	301.0357	301.0348	178.05; 125.05; 137.05; 147.00	1.33 ± 0.25	Kolniak-Ostek et al. (2015) [1]

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Chapter 5 – Effect of a Blackberry Polyphenol Enriched High Protein Dairy Beverage on Recovery from Exercise Induced Muscle Damage and Oxidative Stress: a Randomised Controlled Trial

1. Introduction

1.1 What is Exercise Induced Muscle Damage?

Exercise induced muscle damage (EIMD) is the temporary decline in muscle function that occurs following intensive exercise, particularly that which includes considerable eccentric loading of the muscle (Clarkson and Tremblay, 1988; Owens *et al.*, 2018). This reduction in muscle function results in meaningful declines in key performance outcomes such as reduced muscle strength and power which typically lasts between five- and seven-days post exercise depending on the magnitude of the insult (Owens et al., 2018; Sayers & Clarkson, 2001; Warren et al., 1999). EIMD is also associated with considerable increases in post exercise muscle soreness which typically peaks between 24- and 48-hours post exercise, often referred to as delayed onset muscle soreness (DOMS) (Cheung et al., 2003; Nosaka et al., 2002).

These deleterious post exercise outcomes occur as a result of microtrauma to the structure of the muscle fibre, following mechanical force placed on the muscle fibre (Clarkson and Sayers, 1999; Markus *et al.*, 2021). This damage results in an inflammatory cascade which can also result in secondary damage due to excessive production of reactive oxygen species (Owens *et al.*, 2018). This damage, both mechanical and biochemical, results in considerable harm to the efficiency of excitation contraction coupling of the muscle meaning considerable reduction to its performance capabilities (Owens *et al.*, 2018).

Nutritional solutions are well established to be one of the prime candidates to reduce the negative impact of EIMD (Urso and Clarkson, 2003; Howatson and Van Someren, 2012; Harty *et al.*, 2019; Markus *et al.*, 2021). Many of these nutritional interventions have been proposed due to their antioxidant and anti-inflammatory action which has been hypothesised to reduce the negative biochemical impact of excess reactive oxygen species production on the muscle post exercise (Harty *et al.*, 2019).. Other interventions supporting post exercise recovery apply a different mechanism, focussing on the provision of amino acid substrate to support muscle repair and regeneration following EIMD such as protein and BCAA supplementation (Howatson *et al.*, 2012; Harty *et al.*, 2019; Pearson, Hind and Macnaughton, 2022).

1.2 Role of Polyphenols in Recovery from EIMD

Polyphenols are non-nutritive bioactive phytochemicals found in fruits, teas and other plant-based food sources (Manach *et al.*, 2004). They are well known for their potent antioxidants effects and as a result have been linked with various health related outcomes such as improved lipid profiles, reduced blood pressure and insulin resistance among others (Scalbert, Johnson and Saltmarsh, 2005; Kanti and Syed, 2009; Sureda *et al.*, 2014; Cory *et al.*, 2018; Heneghan *et al.*, 2018)**.** They have also been suggested to improve recovery from EIMD (Myburgh, Kruger and Smith, 2012; Myburgh, 2014; Bowtell and Kelly, 2019). The antioxidant action of polyphenols has been linked with upregulation of cell signalling stress response pathways which control the endogenous antioxidant response such as the NRF2 – Electrophile Response Element pathway (Kanner, 2020; Zhou et al., 2019). The digestion and absorption of polyphenols compared to other antioxidants is notably poorer, and as a result the interaction with cell signalling pathways such as NRF2 have been attributed to interaction with the gut microbiome (Bowtell and Kelly, 2019; Sorrenti *et al.*, 2020).

There have been numerous randomised controlled trials (RCTs) which have investigated the role of polyphenol rich interventions on recovery from EIMD such as the use of tart cherry juice, tea extracts and berry fruits and extracts (Beals et al., 2017; Connolly et al., 2006; Howatson et al., 2010; Herrlinger et al., 2015; McAnulty et al., 2004; McLeay et al., 2012; Romain et al., 2017; Yarahmadi et al., 2014). As part of this PhD thesis a meta-analysis of polyphenol interventions on recovery from EIMD is presented earlier in this thesis (Carey*,* Lucey and Doyle 2021) (chapter 4). Further systematic reviews and meta-analyses also reinforce the role for fruit derived polyphenol rich interventions to support post exercise recovery from EIMD (Doma, Gahreman and Connor, 2020; Ortega *et al.*, 2020; Rickards *et al.*, 2021). Another meta-analysis has shown that anthocyanin rich whole fruit including pomegranates, tart cherries and wild blueberries among others can improve exercise induced oxidative stress and inflammation (Bloedon *et al.*, 2019). While there appears to be scientific evidence supporting the use of polyphenols for recovery from EIMD, the most appropriate polyphenol source and ingestion regimen is required to achieve such benefits to recovery remains unclear. One narrative review of RCTs suggested that a dosage of at least 1000mg/day, at least three days prior to and three days following exercise is required to elicit recovery advantages, however empirical evidence

underpinning this claim is limited (Bowtell and Kelly, 2019). While polyphenol sources such as tart cherry juices and tea extracts are some of the most extensively researched, it is unclear whether these sources contain preferential subclasses of polyphenols required to support post exercise recovery (Bowtell and Kelly, 2019; Carey, Lucey and Doyle, 2021). The precise characterisation of many of these interventions is distinctly lacking as is evidenced in the previous chapter in this thesis (chapter 4) (Carey, Lucey and Doyle, 2021). It is clear that the explicit defining of polyphenol subclasses and dosages is a trial feature which requires further investigation and greater reporting to allow for a greater understanding of the precise role of the specific polyphenol subclasses in post exercise recovery (Bowtell and Kelly, 2019).

The source of polyphenols in this RCT was selected as blackberries (Rubus Fruticosus). Blackberries contain a unique polyphenolic composition predominantly containing anthocyanins cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside, as well as considerable ellagitannin and catechin polyphenols (Tzima *et al*., 2023).

Anthocyanins are a class that have been noted particularly for their ability to augment post exercise recovery with the anthocyanins from Montmorency tart cherries shown to consistently improve post exercise recovery from following strenuous exercise (Hill *et al*., 2021). Whole fruits rich in anthocyanins were recently shown to also reduce exercise induced oxidative stress and inflammation in a large meta-analysis of RCTs (Bloedon *et al*., 2019).

Pomegranate ellagitannin polyphenols have also been investigated for their ability to enhance post exercise recovery in RCTs, showing positive effects (Trombold *et al*., 2010, 2011). Tea catechins from green tea have also been shown to improve post exercise recovery of muscle soreness as well as oxidative stress and inflammation (Herrlinger *et al*., 2015, Jówko *et al*., 2015, Sugita *et al*., 2016). Despite this favourable polyphenolic composition, blackberry polyphenols have yet to be investigated within the context of post exercise recovery.

1.3 Dairy Protein and Exercise

Dietary protein is established as playing a considerable role in the muscular response to exercise (Phillips, 2007; Burd *et al.*, 2009; Morton, McGlory and Phillips, 2015). The two primary methods by which muscle protein breakdown and synthesis can be

shifted to a balance favouring synthesis are feeding of dietary protein and exercise (Burd *et al.*, 2009). Essential amino acids provide essential substrate for the formation and repair of muscle tissue post exercise (Church *et al.*, 2020). The amino acid leucine has been shown to not only act as a substrate to support post exercise muscular remodelling but also to act as a key signalling molecule in the process of muscle protein synthesis through its interaction with the mTor pathway (Holowaty *et al.*, 2023). Dairy protein has been shown to be particularly rich in essential amino acids and leucine in particular compared to other protein sources (Vliet, Burd and van Loon, 2015). Along with this, the digestibility of dairy proteins has been shown to be superior to many other protein sources, particularly non-animal-based sources (Vliet, Burd and van Loon, 2015; Dupont and Tomé, 2020). As such dairy protein represents one of the most effective sources of protein to support muscle remodelling and repair post exercise. While investigations into the role of protein supplementation on the rate of recovery from EIMD have been mixed (Pasiakos, Lieberman and McLellan, 2014; Pearson, Hind and Macnaughton, 2022), the carbohydrate-to-protein blend present in bovine milk has been suggested to be an effective tool to support post exercise recovery with a number of randomised controlled trials reporting benefits for post exercise recovery from EIMD (Cockburn *et al.*, 2008; Cockburn, Bell and Stevenson, 2013; Rankin, Stevenson and Cockburn, 2015).

1.4 Combining Protein with Polyphenols

In theory there is considerable merit for the combination of polyphenols and milk to support post exercise recovery given the previously discussed efficacy of both compounds to improve recovery from EIMD and the added benefit of dairy protein on muscle protein synthesis to support the post exercise repair and remodelling of muscle tissue. It is also plausible that protein rich food matrices such as milk may act as an effective delivery vehicle for polyphenols to the gut due to the potential protective effect of dairy protein on polyphenols through encapsulation of polyphenols within the protein structure (van de Langerijt et al., 2023). Despite this, the combination of protein and polyphenols in recovery from EIMD has only been studied in two RCTs to date (Nieman *et al.*, 2013; Pavis *et al.*, 2021). One of these RCTs investigated the impact of a blueberry and tea-based polyphenol treatment with soy protein on inflammation and oxidative stress surrounding a three-day intensive running period in

31 long distance runners (Nieman *et al.*, 2013). Twenty grams of the polyphenol enriched soy protein powder was consumed twice per day for a total of 17 days. The total polyphenol concentration was 2136mg per day. This RCT observed no impact of the treatment on markers of inflammation and oxidative stress during or following this period. The other RCT investigated the impact of a pomegranate polyphenol enriched protein beverage containing 211mg of polyphenols and 20g protein once per day for 14 days surrounding a bout of 300 eccentric leg extensions (Pavis *et al.*, 2021). This RCT observed significant improvements in post exercise recovery of muscle strength, soreness and creatine kinase over the 168 hours post exercise in 18 healthy recreationally active males (Pavis *et al.*, 2021).

While only two studies have investigated polyphenol protein combinations on exercise induced muscle damage, other RCTs have investigated different post exercise outcomes. One such RCT investigated the role of the 2136mg polyphenol and 20g soy protein powder twice per day for 17 days on outcomes for immune function post exercise, showing delayed virus replication but no effects on bacterial infections in 31 long distance runners (Ahmed *et al.*, 2014). Another RCT investigated the impact of a pre-exercise bolus of a 20g blend of whey, casein and pea protein combined with 650mg pomegranate polyphenols and pre-sleep bolus 18g protein blend alongside 480mg tart cherry juice to enhance post exercise myofibrillar protein synthesis rates and functional adaptation to resistance training over an 11-week period in 15 recreationally active healthy adults (Pavis et al., 2022). This study showed improvements to early functional adaptation to the first 10 resistance training sessions and increases in muscle protein synthesis associated with ingestion of their polyphenol protein beverage (Pavis *et al.*, 2022).

No study to date has investigated a milk polyphenol blend on post exercise recovery from EIMD. However, one study has investigated the role of bovine milk enriched with 490mg of flavonoid polyphenols on exercise induced intestinal injury, permeability and inflammation in 12 recreational cyclists (Kung *et al.*, 2022). This study showed no impact of the beverage which contained a polyphenol blend derived from blueberry, cocoa and green tea on post exercise intestinal and digestive outcomes.

There has also been research suggesting that using a protein rich food source for polyphenol delivery, may enhance the bioavailability of polyphenols with one study

showing in-vitro protection of the polyphenol epigallocatechin gallate (EGCG) when delivered in a complex with soy protein (Zhou *et al.*, 2020). A recent review of studies investigating polyphenol protein interactions has suggested that protein polyphenol complexes and compounds provide potential for stronger antioxidant action however this depends on the protein polyphenol ratio and type of polyphenol (Sun, Sarteshnizi and Udenigwe, 2022).

This current RCT aims to investigate the role of a blackberry polyphenol enriched high protein milk on post exercise recovery as assessed by muscle strength and muscle soreness when compared to an equivalent commercial high protein milk and a low protein milk control. Secondary outcomes include the impact of this polyphenol enriched high protein milk on exercise induced oxidative stress and antioxidant capacity.

2. Methods

2.1 Study Overview & Recruitment

The RubusElite RCT was designed as a parallel proof of concept single blind randomised controlled trial. This RCT encompassed a collaborative approach which incorporated multiple departments across multiple institutions. Supportive evidence which provides valuable context for this RCT but is not presented as work specific to this PhD thesis is presented in RubusElite RCT Preface.

Moderately active male participants between the ages of 18 and 45 were recruited by a convenience sampling approach. This included advertisement on social media platforms, word-of-mouth, and electronically distributed flyers. Sample size was determined in line with previously published parallel designed polyphenol RCTs (Beals et al., 2017; Connolly et al., 2006; Howatson et al., 2010; Herrlinger et al., 2015; McAnulty et al., 2004; McLeay et al., 2012; Romain et al., 2017; Yarahmadi et al., 2014). A study protocol overview figure is presented in Figure 1. The study took place at the human performance laboratories of the South East Technological University, Waterford, Ireland, between June 2022 and May 2023. This RCT was granted ethical approval by the research ethics board of South East Technological University, Waterford (ref: WIT2021REC044, January 2022) (Appendix 13). This RCT protocol was registered with www.clinicaltrials.gov, ID number NCT05463835.

2.2 Participant Screening

Prospective participants were sent a pre-screening questionnaire to determine their initial eligibility following first contact (Appendix 14). In this questionnaire participants were asked various questions regarding their eligibility to take part in the study. Eligible participants were required to be biologically male, between 18 and 45 years of age, free from chronic illness or current injury, free from landing related lower limb injury history, not currently undertaking a plyometric training programme, not have a history of international sporting competition and not having taken either probiotic supplements or antibiotics in the past two months. They were also asked about their ability and willingness to comply with various key study protocols including avoidance of selected polyphenol rich foods for the duration of the study (Appendix 19), avoidance of non-medically prescribed nutritional supplements and

nutraceuticals for the duration of the study, avoidance of strenuous exercise in the 48 hours prior to and following the study test day. If a participant met each of the above criteria, they were invited to attend an onsite study screening visit.

During the follow-on onsite screening visit, participants received a verbal overview of the study along with a participant information leaflet (PIL) (Appendix 15). Participants were given the opportunity to ask questions, they were then asked to fill out a brief health questionnaire to ensure they could exercise at maximal intensity. Participants were then asked to provide their written consent prior to any study protocols being undertaken (Appendix 16). Participants then undertook a graded exercise test-to-exhaustion where peak oxygen uptake (VO_2peak) was assessed. The test was carried out on a Lode Corvial CPET cycle ergometer (lode B.V. Groningen, Netherlands) which was pre-programmed to start at 80W and increase by 20 watts every minute until volitional exhaustion. At each stage, heart rate, and Rate of Perceived Exertion (Borg, 1970) were recorded. Participants were instructed to cycle until volitional exhaustion while keeping their cadence above 65rpm throughout the test. Strong verbal encouragement was provided throughout the test. Oxygen consumption was measured using a MOXUS Modular Metabolic System (AEI Technologies, Texas, USA). Eligible participants were required to be moderately active with a VO_2peak between 35.0 and 55.0 ml/kg/min which corresponded to having a baseline level of aerobic fitness sufficient to complete the study protocol but not having underwent high level endurance exercise training. This was determined based on normative data from males aged between 18 and 45 (Myers *et al.*, 2017). Once the participant achieved the required score in the VO_2peak graded exercise test they were deemed eligible and enrolled on the study. Participants were randomly assigned in a parallel fashion to one of three groups using a block randomisation method via the online tool www.sealedenvelope.com (Sealed Envelope, London, United Kingdom).

2.3 Study Beverages

All participants were instructed to consume two 500ml dosages of their assigned study beverage per day for 10 days, evenly spaced between 10 and 14 hours apart. The three treatment arms were:

- **Polyphenol Enriched High Protein Milk (PHPM)** – 500ml high protein milk containing 30g protein, 25g freeze dried blackberry powder (FDBP) containing750mg polyphenols. Consumed twice per day for a total daily consumption of 1000ml containing 60g protein and 50g FDBP with 1500mg polyphenols
- **High Protein Milk (HPM)** – 500ml high protein milk containing 30g protein. Consumed twice per day for a total consumption of 1000ml containing 60g protein.
- **Low Protein Milk (LPM)** – 500ml low protein milk containing 1g protein. Consumed twice per day for a total consumption of 1000ml containing 2g protein.

This PHPM prototype was developed by the RubusElite UCC Food Science research team in accordance with target nutritional specifications provided by the nutrition team. The resulting prototype was characterised for the presence of polyphenol subclasses by the RubusElite team at Teagasc Food Research Centre Ashtown. Polyphenol subclasses were identified using HPLC-QTOF mass spectrometry. Total polyphenol content was monitored monthly for the duration of the RCT by the UCC Food Science team and was repeated monthly to assess for degradation of the active ingredient during storage using the Folin-Ciocalteu method (RubusElite RCT Preface Figure 1.). The commercial high protein milk (North Cork Creameries, Cork, Ireland) was identical to that which was provided to the PHPM group without the added FDBP. This HPM contained 30g milk protein with no polyphenol content. The third beverage, which acted as a control beverage, was a commercially available low protein dairy beverage (Taranis Dalia Liquid, Taranis Nutrition, France) with comparable total energy content to that of the high protein milk also with no polyphenol content. For macronutrient and energy profile of each study beverage see Table 1.

Table 1. Energy and macronutrient characteristics of all study beverages per 500ml dosage

Per 500ml dosage (twice per day)	Polyphenol & High Protein Milk	High Protein Milk	Low Protein Milk
Energy (Kcal)	341	265	250
Carbohydrate (g)	43	24	32
Fat (g)	5.9	5.5	13
Protein (g)	31.7	30	1

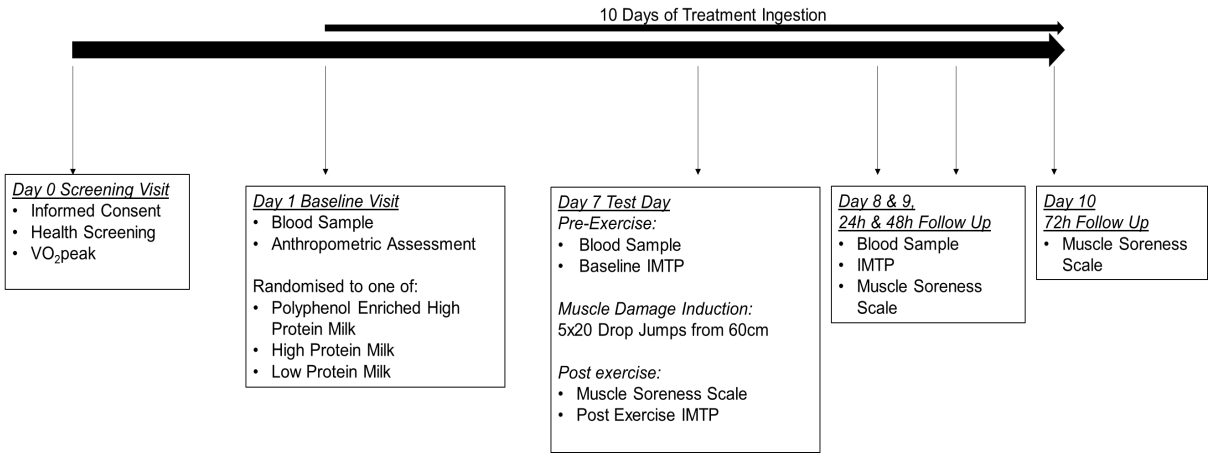


Figure 1. Overview of RCT structure and all study procedures

2.4 Baseline Study Visit

During the baseline study visit, participants attended the exercise lab following a 12-hour fast to provide a baseline venous blood sample taken by a trained phlebotomist. Following this, participants were provided with a breakfast voucher for the university canteen and instructed to purchase breakfast ad libitum however they would be required to repeat this breakfast for each of the following study visits (test visit, 24h post exercise and 48h post exercise). Participants were then provided with 6 days of their assigned beverage, instructions outlining the beverage consumption protocol and a custom designed compliance form on which participants were requested to mark off the time of each beverage consumption or whether a dose was missed (Appendix 17). All participants were required to have an adherence of at least 80% to be included in study analysis.

2.5 Test Study Visit

On day 7 of beverage consumption, participants returned to the laboratory in a fasted state and provided a venous blood sample. Following this they consumed an identical breakfast to the baseline day. Upon arrival back to the lab following breakfast they underwent a baseline 24-hour interview administered dietary recall with a trained nutritionist according to a multi pass technique (DAPA Measurement Toolkit)**.** This resulting dietary data was analysed using Nutritics Research Edition v5.87 software (Nutritics, Dublin, Ireland) to generate nutrient intake data.

Participants then underwent a baseline muscle strength assessment using an isometric mid-thigh pull (IMTP) protocol. Following the IMTP participants underwent a muscle damage induction protocol involving 100 drop jumps from a height of 0.6m. Full muscle damage and muscle strength protocols are described below. Immediately post muscle damage a repeat IMTP protocol was completed, and participants ranked their level of lower limb muscle soreness on a 0-10 visual analogue scale while undertaking a bodyweight squat movement to approximately 90° knee angle. Participants were then provided with their study beverages for that study day and were asked to consume their first beverage within 1 hour of the completion of the study visit.

2.6 Muscle Damage Protocol and Strength Measurement

The exercise-induced muscle damage induction protocol consisted of five sets of twenty drop jumps from a height of 0.6m. Participants were provided with a 2-minute rest period between each set and participants were given 10 seconds to perform each repetition. Wooden boxes with rubber gripping on top, were constructed into a 0.6m platform with a 0.22m intermediate step to the rear. The participant ascended to the platform using the intermediate step and stepped off from the box landing on the floor with both feet. Upon landing the participant was instructed to immediately and fluidly descend to an approximate knee angle of 90° followed by an immediate maximal vertical jump. Two trained assessors provided verbal feedback during the entire protocol based on visual observations from two differing angles to ensure both safe and consistent completion of the study protocol. All participants completed the protocol with the same flooring conditions. This protocol was first described in (Miyama and Nosaka, 2004) and has since been used in multiple intervention RCTs

investigating exercise induced muscle damage which have shown it to be a reliable, repeatable and sufficient methodology for the generation of sufficient muscle damage (Miyama and Nosaka, 2004; Goodall and Howatson, 2008; Howatson, Goodall and Someren, 2009; Howatson *et al.*, 2012; Clifford *et al.*, 2016). This protocol is demonstrated in Figure 3.

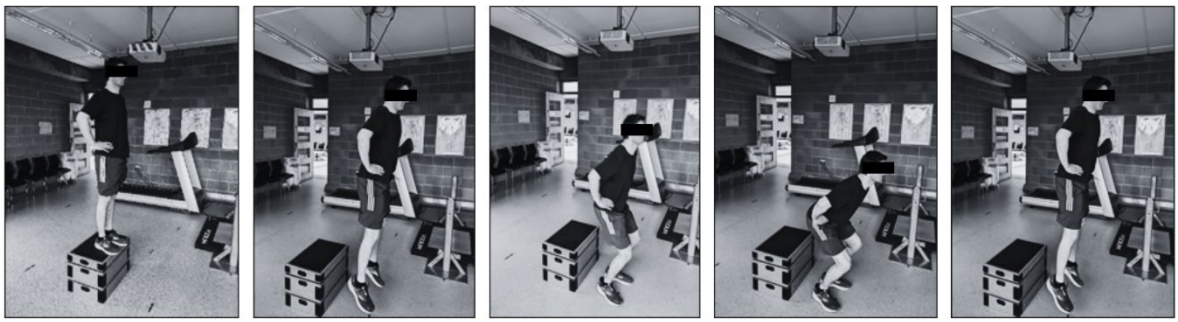


Figure 3. Muscle damage induction protocol, drop jumps from 0.6m, 5x20 repetitions.

Muscle strength was measured using an IMTP protocol on BTS Bioengineering Force Podiums (BTS Bioengineering, Garbagnate Milanese MI, Italy), with data capture completed by 3Digivec software (BTS Bioengineering, Garbagnate Milanese MI, Italy). Isometric mid-thigh pulls followed the protocol proposed by Comfort et al., (2019). Participants were initially assessed to determine the appropriate bar height demonstrating the IMTP exercise form and then measuring hip and knee angles using a digital goniometer. Hip angles were required to be between 140° and 150° while the knee angle was required to be between 125° and 145°. Bar height and participant position was manipulated until a comfortable position meeting the required angles was obtained. Participants then underwent a standardised warm-up procedure which involved 10 bodyweight squats and 20m of walking bodyweight lunges. The second phase of the warm-up involved 3 sets of 3 repetitions of 3 second duration IMTPs at increasing intensity which simultaneously acted as a familiarisation protocol to make the participants comfortable with the procedure and equipment. The first set was completed at 50% of perceived maximal effort, the following at 75% and 90% respectively. Participants were provided 1 minute of passive recovery between each warm-up set. Following completion of the warm-up, participants were given 2.5 minutes passive recovery prior to the first maximal IMTP. For all warm-up and maximal IMTPs participants were strapped securely to a fixed barbell using Figure-8

weightlifting straps and were provided with powdered chalk to enhance grip. A standardised coaching cue was used for all participants to "drive your feet into the ground as hard and fast as possible". Participants were instructed to avoid pretension on the bar during the pre-pull phase and to avoid a countermovement during the start of the pull. Participants provided 3 seconds of 'quiet standing' prior to the initiation of the maximal IMTP to ensure a clear baseline prior to commencing force production. Participants were counted down from 3 and given strong verbal encouragement throughout a 5-second-long pull. Participants were given 2.5 minutes rest following each IMTP. Participants completed at least 2 IMTPs, if a participant improved by greater than 250N between their first and second IMTP a third repetition was completed. The IMTP measurements were taken at baseline, immediately post-muscle damage, 24h post-muscle damage, and 48h post-muscle damage (Figure 1).

2.7 Soreness and Biomarker Assessment

Subjective measures of muscle soreness have been proposed as appropriate supportive evidence to that of test-retest performance parameters in accordance with EFSA guidance for substantiation of food claims related to muscle recovery (Turck *et al.*, 2018). Visual analogue scale measurements of muscle pain have been suggested to be a better representative of muscle pain during delayed onset muscle soreness (DOMS), than pressure pain tests (Wing Yin Lau, Muthalib and Nosaka, 2013). Muscle soreness was assessed using a visual analogue scale, immediately, 24 hours, 48 hours and 72 hours post exercise (Appendix 18.). On each occasion the participant was asked to rate their muscle soreness following a bodyweight squat to an approximate knee angle of 90° on a scale from zero to ten, where zero was labelled as 'no pain' and ten was labelled as 'extreme pain'.

Secondary outcomes included blood biomarkers of muscle damage and oxidative stress, such as protein carbonyls, 8-iso PGF2α (8-isoprostane), FRAP and malondialdehyde (MDA). Fasting intravenous blood samples were collected by a trained phlebotomist at baseline, test exercise day, 24 hours and 48 hours post exercise. Plasma protein carbonyls was analysed by using Protein Carbonyl Colorimetric Assay Kit (Elabscience Biotechnology Co., Ltd., Wuhan, China). Serum MDA was measured using a CheKine Lipid Peroxidation Assay Kit (Abbkine, Wuhan, China). Plasma 8-iso PGF2α was measured using RayBio Isoprostane (8-iso PGF2α) Enzyme Immunoassay Kit (RayBiotech, Georgia, USA). All samples were measured in

duplicate within the same batch. Inter-assay variability and intra-assay variability were determined to ensure consistency and reproducibility of biomarker measurements was <15%.

The ferric reducing power of serum samples was quantified using the FRAP assay adapted for a 96 well plate. For the standard curve, a stock solution of 3.4mM FeSO4.7H2O was prepared and serial diluted in a final volume of 40µL. Samples (20µL) were diluted with 20µL distilled water in a 96 well plate. FRAP standard reagent, 300µL (20mL of 300mM acetate buffer, 2mL 10mM TPTZ and 2mL H2O) was added to each of the standards and, for samples, 300µL (20mL 300mM acetate buffer, 2mL 10mM TPTZ and 2mL 20mM FeCl3.6H2O) was added. Samples were left in the dark for 20 minutes, and absorbance was measured at 595nm using a plate reader (Varioskan Flash, ThermoScientific).

2.8 Data Analysis

All statistical procedures were carried out in SPSS v.28.0.0 (Armonk, NY: IBM Corp). Descriptive analysis was conducted for all outcomes at each timepoint. All variables underwent normality testing using the Shapiro-Wilk test. One-way ANOVAs were used to assess baseline comparability of demographic factors and dietary data across treatment groups. As part of one-way ANOVA protocols, Levenes test for homogeneity of variances was conducted. Linear mixed models repeated measures analysis of variance (MMRM) were applied to examine the effects of treatment, time and treatment*time in outcomes of recovery from exercise induced muscle damage, including recovery of muscle strength, soreness and biomarkers of oxidative stress. In the application of this model several assumptions were assessed, and adjustments made when violations were observed. Within-subject observations were assessed for correlation using intraclass correlation coefficients. Residuals were assessed for normal distribution using a Shapiro-Wilk test and log transformation conducted where this was violated. Mauchly's sphericity test was conducted, and a Greenhouse-Geisser correction was applied where appropriate. Additionally, various covariates were integrated into the model, with Akaike's Information Criterion (AIC) scores being used to evaluate the model fit as each covariate was introduced. Data adjusted for covariates is presented as mean ± SEM, while unadjusted data is presented as mean ± SD. All data that was adjusted is presented in unadjusted form in Appendix 20. Bonferroni

adjustments for multiple comparisons were employed for all MMRM analyses. Univariate general linear model analysis of variance was used in a post hoc manner to identify significant changes at specific timepoints post exercise. Statistical significance was set at $P<0.05$ for all analysis.

3. Results

3.1 Subjects

One-hundred and forty-nine participants underwent the initial pre-screening procedure. Following this, seventy-five participants were screened for eligibility using a VO_2peak graded exercise test. Of this seventy-five, thirty-two were deemed eligible and proceeded to beverage allocation. Following beverage allocation all participants completed all study procedures and there were no dropouts. This represented twelve participants in the PHPM and ten participants in both the HPM and LPM groups respectively. Full participant recruitment details are presented in the CONSORT diagram (Figure 3.). Baseline participant demographic data is presented in Table 1.

There was no significant difference in participant baseline age ($P = 0.13$), height ($P = 0.20$), BMI ($P = 0.10$) or VO_2peak ($P = 0.49$) across treatment groups. There was a significant difference between treatment groups in bodyweight at baseline with the HPM (96±17kg) weighing significantly more than the LPM group (80±13kg) ($P = 0.03$). This difference in bodyweight was accounted for in all hypotheses testing by adjusting for bodyweight as a covariate.

Table 2. Baseline demographic characteristics of participants overall and groups according to their randomised study group (n=32)

	Overall (n=32)		**Polyphenol & High Protein Milk (n=12)**		**High Protein Milk (n=10)**		**Low Protein Milk (n=10)**		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*
Age (years)	30.2	8.9	27.6	8.9	28.5	6.9	34.9	9.5	0.13
Body mass (kg)	86.9	14.7	85.2[ab]	10.0	96.2[a]	17.3	79.8[b]	13.1	0.03
Height (cm)	179.9	7.3	178.3	4.8	183.3	6.5	178.3	4.8	0.20
BMI (kg/m^2)	26.8	4.0	26.7	2.5	28.7	5.6	25.0	2.6	0.10
VO_2peak (ml/kg/min)	46.5	5.2	45.0	5.4	47.1	5.0	47.5	5.1	0.49

BMI – body mass index, [a] - Superscripts denote significant difference $P<0.05$ assessed by one-way ANOVA.

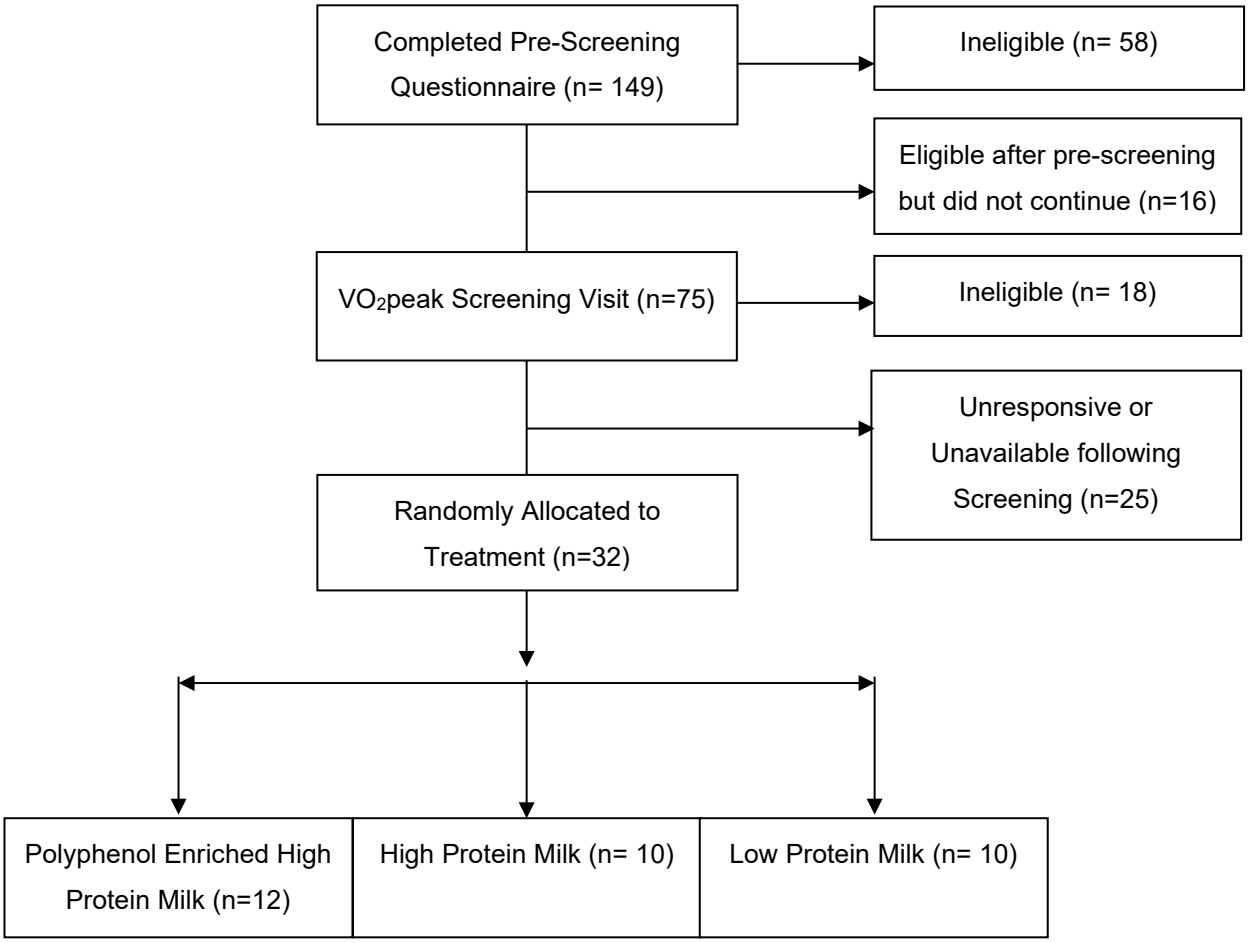


Figure 3. CONSORT diagram outlining participant recruitment, eligibility and randomisation.

3.2 Dietary Intake

The dietary intakes of participants in the 24-hours prior to undergoing EIMD are presented in Table 5. One notable observation from the data was the protein intake based on the beverage consumed. There was a statistically significant difference in the protein consumed in the last 24 hours prior to EIMD depending on the study beverage group suggesting that study intervention had an impact on daily protein intake as expected ($P < 0.01$). Specifically, participants who consumed the PHPM had a significantly high protein intake (186±40g) than those in the LPM group (120±29g) ($P < 0.01$). Similarly, those who consumed the high-protein milk (HPM) reported a protein intake of 196±42g, which was also significantly higher than the LPM group's

intake ($P < 0.01$). However, when comparing the protein intakes of the PHPM and HPM groups, they were similar and thus showed no significant difference ($P = 0.81$).

The beverage consumed did not show a significant effect on the energy (P = 0.64), carbohydrate (P = 0.99), or fat intakes (P = 0.55) respectively, during the 24-hour period prior to EIMD.

Adherence to the treatment was high across all study groups. Overall, participants were 98.1±4.5% compliant with their respective treatments. The breakdown for each group was as follows: PHPM at 98.3±4.9%, HPM at 97.0±6.3%, and LPM leading with 99.0±3.2%. Statistical analyses confirmed that these differences in compliance between the groups were not significant (P = 0.62).

Table 3. 24-hour Dietary Recall Mean energy and macronutrient intake prior to test day including the nutrients provided by treatment beverage.

	Polyphenol & High Protein Milk (n=12)	**High Protein Milk (n=10)**	**Low Protein Milk (n=10)**	**Significance *P***
Energy (Kcal)	2958 ± 863	3205 ± 827	2890± 597	0.64
Protein (g)	186 ± 40[a]	196 ± 42[a]	120 ± 29[b]	<0.01
Carbohydrate (g)	321 ± 77	327 ± 125	321 ± 76	0.99
Fat (g)	103 ± 66	123 ± 30	125 ± 48	0.55

[ab] - Superscripts denote statistical significance between treatment groups $P<0.05$ assessed by one-way ANOVA

3.3 Recovery of Muscle Strength

Recovery of muscle strength is presented in Figure 3. A consistent drop in muscle strength after exercise across all time points was observed when compared to baseline levels in the overall sample. Immediately after exercise, there was a mean decrease of 215.0N or 21.9kg in muscle strength ($p<0.001$). This decline in muscle strength persisted to 24 hours (mean difference= -273.3N or -27.9kg, $p<0.001$) and 48 hours post-exercise (mean difference= -295.7N or -30.2kg, $p<0.001$). This indicates that in the overall group, the exercise was sufficient to significantly generate EIMD.

The type of beverage consumed had a significant impact on muscle strength recovery over the 48-hour post-exercise period ($P = 0.006$). Participants who consumed PHPM

had significantly greater relative muscle strength over the 48-hour period post exercise compared to those who consumed LPM with a mean difference of 222.4N or 22.7kg (P=0.005). However, there were no significant differences between PHPM and HPM groups (P=1.00), or between HPM and LPM groups (P=0.074).

The influence of beverage type at each time point in isolation was investigated using post-hoc univariate analyses. Immediately post exercise, no beverage appeared to significantly influence muscle strength recovery (P = 0.269). At 24 hours post-exercise, however, there was a significant effect of treatment group (P = 0.045), specifically, both the PHPM (2283±416N) and HPM (2231±452N) groups showed a significant improvement in muscle strength when compared to the LPM (2081±523N) group (P=0.035 and P=0.027) respectively. Yet, no significant difference was noted between PHPM and HPM at this 24-hour mark (P = 0.663). By 48 hours, there was no significant effect of one beverage over another at this specific timepoint (P = 0.323).

In terms of the effect of time on muscle strength recovery, we did not observe any significant changes to post exercise strength suggesting that participants had not fully recovered 48 hours following exercise (P = 0.406), nor did we find any interaction between time and the type of beverage consumed suggesting that while the magnitude of muscle strength decrement was impacted by the study beverage, the rate of recovery was not (P = 0.478).

In summary, muscle strength decreased across all groups after exercise, but consuming PHPM showed an advantage in retaining muscle strength over LPM in aiding within the 48-hour post exercise period. This effect was particularly apparent at 24 hours post-exercise. HPM may improve recovery of strength at 24 hours post exercise compared with LPM.

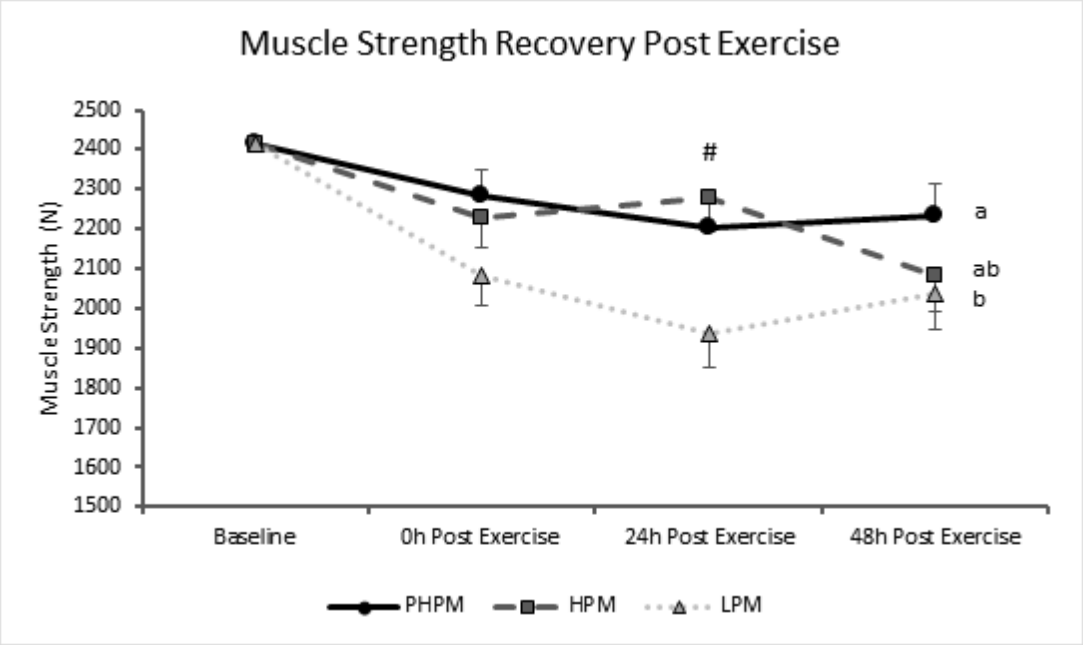


Figure 3. Recovery of muscle strength post exercise as assessed by isometric mid-thigh pull (IMTP). Data is adjusted for covariates and presented as mean ± SEM.
[a] – Superscripts denote statistically significant treatment effect across all post exercise timepoints identified by linear mixed models repeated measures. $P < 0.05$
- Significant treatment effect at specific timepoint between PHPM and LPM identified univariate post hoc analysis. $P < 0.05$

3.4 Recovery of Muscle Soreness

The muscle soreness response post-exercise is presented in Figure 4. Muscle soreness was present during the 72-hour post exercise period for all participants. The type of beverage consumed post-exercise had a significant impact on muscle soreness over the 72 hours post exercise, as confirmed by a significant overall treatment effect (P = 0.007).

There was significantly lower muscle soreness in those who consumed PHPM compared with those in the LPM group (P = 0.001) over the entire 72-hour post exercise period. Similarly, when compared to HPM, PHPM also led to significantly less muscle soreness (P < 0.001). However, when comparing the HPM and LPM groups, there was no statistically significant difference in post-exercise muscle soreness (P = 0.794) (Figure 4.).

The influence of beverage type at each time point in isolation showed that the beverage type significantly influenced muscle soreness immediately post exercise (P = 0.044). Specifically, PHPM (2.3±1.7) was associated with significantly less soreness

compared to both HPM (4.0±2.2) (P = 0.038) and LPM (3.9±1.6) (P = 0.036). However, there was no difference in soreness between the HPM and LPM groups immediately after exercise (P = 0.932). As participants continued their recovery, when each timepoint was taken in isolation there was no significant difference in soreness associated with treatment beverage at 24h (P = 0.158), and 48 hours (P = 0.291) and 72 hours (P = 0.114).

There was a significant time effect on muscle soreness suggesting that participants were significantly more recovered at 72 hours than at 24 hours post exercise (P <0.001). This suggests that regardless of the treatment beverage consumed, the passage of time itself influenced the muscle soreness experienced by participants. However, it's interesting to note that there was no interaction effect between the time and the type of beverage consumed (P = 0.914). This suggests that the rate of muscle soreness recovery over time was consistent, irrespective of the type of beverage taken, even though the magnitude experienced by the PHPM group was lower than both LPM and HPM.

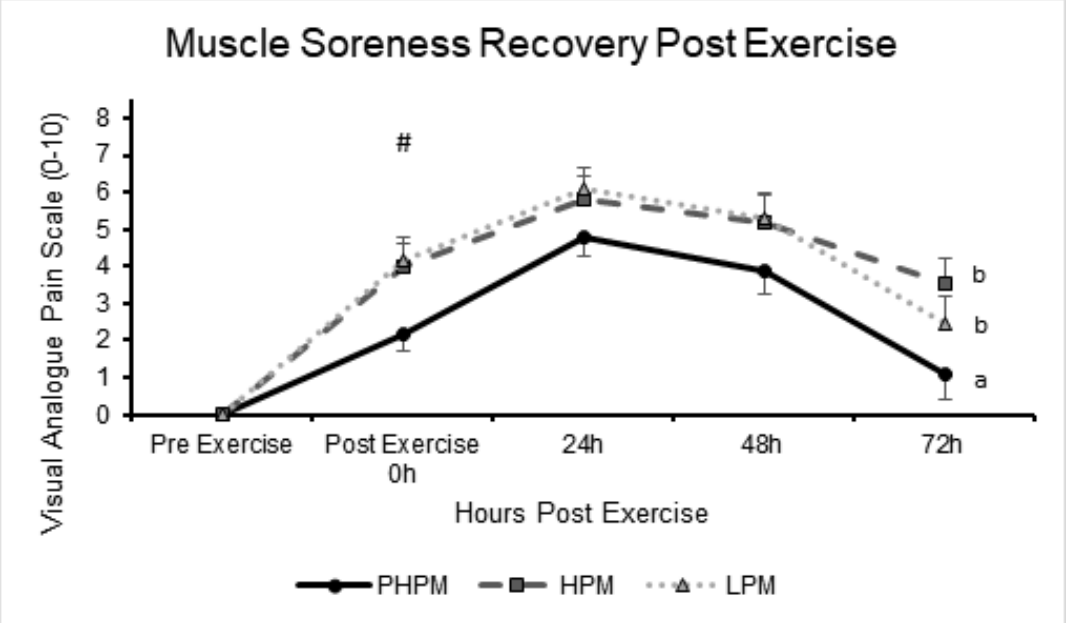


Figure 4. Muscle soreness response post exercise as assessed by visual analogue scale, adjusted for covariates. Data is adjusted for covariates and presented as mean ± SEM. Statistical significance set at P<0.05

[a] – Superscripts denote statistically significant treatment effect across post exercise timepoints identified by linear mixed models repeated measures. P<0.05

- Significant treatment effect at specific timepoint between PHPM and LPM, and between PHPM and HPM identified univariate post hoc analysis. P<0.05

3.5 Blood Biomarkers of Oxidative Stress

Blood markers associated with oxidative stress can be found in Table 4.

Malondialdehyde (MDA) and F2 8 Isoprostane: Neither the beverage consumed nor the progression of time, or the interaction of beverage and time after exercise had a significant impact on the levels of MDA or F2 8 isoprostane, markers of lipid oxidation (all $P > 0.05$).

Protein Carbonyls: There was a significant treatment effect associated with the beverage consumed and protein carbonyl concentrations post exercise ($P = 0.02$). Specifically, those who consumed HPM showed lower concentrations of protein carbonyls than PHPM ($P = 0.02$). However, when comparing PHPM to low protein milk (LPM) or comparing HPM to LPM, no significant differences were noted (both $P>0.05$). Time also played a role in influencing protein carbonyls, with a significant change noticed between the test exercise day and 48 hours post-exercise ($P = 0.03$). Again, the interaction of treatment and time didn't appear to have any significant combined effect on the levels of protein carbonyls within the 48 hours post-exercise ($P=0.34$).

FRAP: For FRAP, a treatment effect was observed ($P < 0.001$). There was a significant difference in the FRAP levels between the PHPM and LPM groups, with those consuming PHPM exhibiting lower circulating antioxidant capacity than LPM ($P < 0.001$). However, comparisons between PHPM and HPM or between HPM and LPM did not reveal any significant differences (both $P>0.05$). The progression of time post-exercise and the combined influence of treatment and time didn't significantly impact FRAP levels within the analysed period (both $P>0.05$).

While the type of post-exercise beverage had minimal to no impact on lipid oxidation biomarkers MDA and F2 8 isoprostane, it did have a noticeable effect on protein carbonyls and FRAP.

Table 4. Biomarkers of oxidative stress. Data is adjusted for covariates and presented as mean ± SEM. Statistical significance set at $P<0.05$

	All Groups	**Polyphenol & High Protein Milk (n=12)**			**High Protein Milk (n=10)**			**Low Protein Milk (n=10)**					
	Adjusted Baseline	Test Day	24h post exercise	48h post exercise	Test Day	24h post exercise	48h post exercise	Test Day	24h post exercise	48h post exercise	Treatment *P*	Time *P*	Treatment* Time *P*
FRAP (microM FeSO4/L serum)	1241	1058 ± 62[a]	1141 ± 74[a]	1097 ± 64[a]	1177 ± 70[ab]	1210 ± 83[ab]	1192 ± 72[ab]	1324 ± 68[b]	1455 ± 81[b]	1288 ± 70[b]	<0.01	0.31	0.86
MDA (nmols/mL serum)	0.78	0.70 ± 0.04	0.73 ±0.04	0.81 ± 0.06	0.78 ± 0.05	0.82 ± 0.05	0.74 ± 0.07	0.69 ± 0.05	0.71 ± 0.05	0.72 ± 0.07	0.37	0.67	0.65
F_2 8-Isoprostane (ng/mL plasma)	0.47	0.57 ± 0.06	0.60 ± 0.07	0.62 ± 0.09	0.43 ± 0.07	0.48 ± 0.07	0.52 ± 0.09	0.54 ± 0.07	0.51 ± 0.07	0.54 ± 0.09	0.18	0.73	0.97
Protein Carbonyls (nmol/mg prot)	0.47	0.49 ± 0.01[a]	0.48 ± 0.02[a]	0.49 ± 0.01[a]	0.48 ± 0.02[b]	0.43 ± 0.02[b]	0.43 ± 0.01[b]	0.48 ± 0.02[ab]	0.49 ± 0.02[ab]	0.47 ± 0.01[ab]	0.02	0.03	0.34

FRAP – Ferric reducing antioxidant power, MDA – malondialdehyde, [a] – Superscripts denote statistically significant treatment effect across post exercise timepoints identified by linear mixed models repeated measures. $P<0.05$

4 Discussion

This single blind, parallel proof of concept RCT is the first to demonstrate that 10 days of ingestion of a blackberry polyphenol enriched high protein milk has the propensity to improve post exercise recovery following an intensive exercise bout. Consuming PHPM twice per day at a daily dosage of ~1400mg polyphenols and ~60g milk protein resulted in significantly improved recovery of skeletal muscle strength compared to a control low protein milk product. In addition to this, PHPM significantly attenuated the muscle soreness response post exercise compared to both a commercial HPM and LPM control. HPM showed isolated effect on recovery of muscle strength compared to LPM during peak EIMD.

4.1. Induction of Muscle damage

This RCT used the method to 5x20 drop jumps from a height of 60cm to induce EIMD as first reported by Miyama and Nosaka (2004). This brought about significant muscle damage as is shown by the increases in muscle soreness in the 48 hours post exercise and the reductions of muscle strength post exercise in all three treatment groups. This muscle damage was shown to be significant in pooled data with muscle strength significantly reduced compared with baseline at all timepoints post exercise. In the LPM control group muscle strength decrements on average peaked at the 24-hour post exercise timepoint with this group on average 16.8% weaker than their pre muscle damage IMTP muscle strength measurement. This overall decrement is broadly comparable to that of the placebo/control groups of other RCTs using this method to induce muscle damage (between 15% and 23% reductions) however many of these observed the greatest post exercise strength decrements immediately post exercise rather than 24 hours post exercise which was shown in this study (Goodall and Howatson, 2008; Howatson, Goodall and Someren, 2009; Clifford *et al.*, 2016). These studies did use other methods of isometric muscle strength measures and not IMTP, the variation in lower limb muscle recruitment may account partly for this.

4.2 Recovery of Muscle Strength

4.2.1 RCT Design and muscle strength measurement

Outcomes in this study were derived with reference to the published guidance on the scientific requirements for European Food Safety Authority (EFSA) health claims related to muscle function and performance (Turck *et al.*, 2018). They recommend test-retest assessment on performance parameters such as muscle strength after an initial strenuous bout as the most appropriate method to assess muscle recovery claims (Turck *et al.*, 2018). Muscle strength has been suggested to be the most appropriate method of post exercise assessment of EIMD following eccentrically loaded exercise (Warren et al., 1999). IMTP measurements of muscle strength has been shown to be valid, reliable and sensitive method of assessing muscle strength when appropriate protocols are undertaken (Comfort *et al.*, 2019).

4.2.2 Effect of treatments on recovery of muscle strength

In the current RCT, PHPM significantly improved overall recovery of post exercise muscle strength when compared with LPM control however there was no significant difference in recovery of muscle strength observed between PHPM and HPM groups. Overall, there was no significant difference between HPM and LPM however when taken in isolation HPM improved muscle strength at 24h post exercise suggesting possible benefit of HPM during the peak of EIMD.

4.2.3 Previous polyphenol RCTs on recovery of muscle strength

Polyphenol rich interventions of various dosages from numerous sources have been shown to improve post exercise recovery of muscle strength however this is the first study to demonstrate this ergogenic effect is at least maintained when delivered as part of a high protein dairy matrix (Myburgh, Kruger and Smith, 2012; Herrlinger, Chirouzes and Ceddia, 2015; Romain *et al.*, 2017). The current RCT is the first to show that when provided in a dairy matrix, the muscle strength recovery benefits are at minimum maintained. The only RCT to date to provide a polyphenol intervention in a dairy matrix surrounding exercise provided 12 cyclists with a dosage of 490mg of polyphenols from mixed flavonoid rich sources with 240ml of shelf stable milk for 15 days in the lead up to 60 minutes of intensive laboratory cycling (Kung *et al.*, 2022). This study however showed no significant effect of treatment on post exercise intestinal permeability or intestinal injury (Kung *et al.*, 2022).

4.2.4 Previous dairy protein RCTs on recovery of muscle strength

HPM alone showed no significant improvement in recovery of muscle strength when compared with LPM apart from at 24h post exercise in isolation. Milk has been suggested to be a strong candidate as a post exercise recovery drink due to its approximate isotonic properties and water content for rehydration, source of carbohydrate for replenishment of glycogen stores and a good protein source for post exercise muscle protein synthesis and recovery from EIMD (James *et al.*, 2018). One RCT showed that consumption of 500ml of milk may limit the decrements of muscle function after damaging exercise however their sample size was small (n=14) and as such only used inferential statistics (Cockburn, Bell and Stevenson, 2013). This study also did not report the precise protein content of the milk used in the intervention. Another RCT investigating milk on post exercise recovery showed that ingestion of 500ml of milk containing 17g of protein consumed post eccentric exercise attenuated losses of muscle strength compared with an energy matched carbohydrate solution in 32 team sport players (Rankin, Stevenson and Cockburn, 2015). While the effects of milk on post exercise recovery have shown promising results in previous RCTs no such study has investigated high protein milk in the range of 30g of protein on recovery of muscle function post exercise.

4.2.5 Recovery of muscle strength conclusion

In summary the current study showed that PHPM enhanced post exercise recovery of muscle strength greater than that of a low protein milk control. However, there was no significant difference in recovery of muscle strength between polyphenol rich high protein milk and high protein milk alone. High protein milk may provide isolated benefits to recovery of muscle strength 24h post exercise.

4.3 Recovery of Muscle Pain

4.3.1 RCT Design and Muscle Soreness Measurement

EFSAs guidance for health claims substantiation to support muscle recovery states that subjective measures of perceived muscle soreness are appropriate to be used as supportive evidence to that of muscle performance parameters such as muscle strength (Turck *et al.*, 2018). Visual analogue scales such as the one used in this study have been shown to be valid and reliable measures of musculoskeletal pain (Boonstra *et al.*,

2008). Visual analogue scales have also been suggested to be more appropriate measures of the time course of changes in delayed onset muscle soreness post exercise compared with pressure pain threshold tests (Lau, Muthalib and Nosaka, 2013).

4.3.2 Effect of treatments on recovery of muscle soreness

In this study PHPM showed a significant effect on recovery of muscle soreness post exercise with significantly reduced perceived muscle soreness compared with both LPM and HPM. No significant difference was observed between HPM and LPM. This suggests that the protein carbohydrate blend of HPM alone had no impact on post exercise recovery of muscle pain however enriching this HPM with blackberry polyphenols significantly improved post exercise pain response.

4.3.3 Comparison with previous studies on muscle soreness

Polyphenol rich interventions have been shown to significantly improve post exercise muscle soreness in multiple meta-analysis (Doma, Gahreman and Connor, 2020; Carey, Lucey and Doyle, 2021; Rickards *et al.*, 2021). This study is the first study to demonstrate that this effect is at minimum maintained when the polyphenolic intervention is provided in a high protein matrix. Interestingly there was no impact of high protein milk on muscle soreness post exercise. This contrasts with the findings of Rankin and colleagues who observed a likely beneficial effect of milk on post exercise recovery of muscle soreness over a similar 72-hour period post exercise, this was despite a smaller dosage of milk protein (17g) and similar carbohydrate content (24g) (Rankin, Stevenson and Cockburn, 2015). However, another study investigating the role of milk on post exercise saw an unclear effect of milk on post exercise recovery of muscle pain despite observing likely beneficial effects in recovery of functional performance parameters (Cockburn, Bell and Stevenson, 2013).

4.3.4 Recovery of muscle soreness conclusion

Blackberry polyphenols when added to high protein milk confer added benefits to the magnitude of post-exercise muscle soreness. When combined with the well-established benefits of dairy protein for enhancement of muscle protein synthesis, and the effect of PHPM to enhance recovery of muscle strength, PHPM represents a strong candidate to be included in post exercise recovery regimens.

4.4 Biomarkers of Oxidative Stress and Antioxidant Status

4.4.1 RCT Design and Outcome measurement

Due to the role of polyphenols in mediating reactive oxygen species and the propensity for EIMD to be exacerbated by excessive production of reactive oxygen species, it was deemed pertinent to monitor the both the antioxidant activity (FRAP) and the oxidative stress (MDA, Protein carbonyl and 8-iso PGF2α) response surrounding EIMD during this study. MDA and 8-iso PGF2α represent measures of lipid oxidation while protein carbonyls determine protein oxidation.

4.4.2 Treatment effect on lipid and protein oxidation

No significant impact of any treatment was observed for either MDA or 8-iso PGF2α surrounding the intensive exercise bout. Across all measures of oxidative stress, there was no significant effect of PHPM compared with LPM control. This is despite previous research reporting that anthocyanin rich whole fruit were effective at reducing oxidative stress across multiple biomarkers including protein carbonyls (Bloedon *et al.*, 2019). However it must be noted that this effect was shown to be small in a meta-analysis containing 366 participants and as such this comparatively small RCT may have been underpowered to identify an effect (Bloedon *et al.*, 2019).

Interestingly however, there was a significant reduced protein carbonyl response associated with HPM compared with that of the PHPM group. While some dairy products have been shown to exhibit antioxidant properties associated with milk proteins (Khan *et al.*, 2019), it is unclear why these would elicit an effect on the HPM group and not the PHPM group, particularly since enhanced recovery of muscle strength and soreness was observed in PHPM group and not HPM in comparison to LPM. This also contrasts with another study which has investigated the effect of 500ml of milk on post exercise protein carbonyl response to repeated cycling sprints but found no effect (Rankin *et al.*, 2017). Curiously there was also a significant difference suggesting a greater antioxidant activity of the LPM when compared with the PHPM group. While polyphenols are well known for their antioxidant ability it has been previously observed that polyphenols may in certain circumstances exhibit pro-oxidant effects, predominantly associated with high dosages or interactions with metal ions (Yordi *et al.*, 2012). Considering the high dosage of polyphenols used in this study, it is not beyond the realms of possibility that a pro-oxidant effect was elicited

by the PHPM. It has also been shown that total antioxidant capacity assays such as FRAP only assess the effect of small molecular antioxidants in plasma and do not account for intracellular antioxidant enzymes such as superoxide dismutase and peroxiredoxin, which are regulated by key targets of polyphenols such as the NRF2 electrophile response element (Bowtell and Kelly, 2019). This may explain the lack of enhancement of antioxidant action exhibited in FRAP assays associated with PHPM yet positive improvements in muscle strength and muscle soreness recovery although further research is required to confirm this.

4.5 Limitations

Due to the exploratory proof of concept nature of this RCT, the sample size used was relatively small. This may have been underpowered to detect a treatment effect in biomarkers of oxidative stress which have been noted to be small in a previous meta-analysis (Bloedon et al., 2019). Despite this, the sample size used in this study is in line, and in many cases larger than many other RCTs investigating the impact of polyphenols and dairy protein on recovery from EIMD due to the costly and burdensome nature of exercise related randomised controlled trials.

Due to the food-based design of this study it was not possible to use an identical placebo control as part of this study design. Despite this, due to the parallel nature of this RCT participants were not aware of the characteristics of other study beverages, only that three "dairy-based beverages" were being assessed. No further details of the nutritional composition of any intervention arm were provided to participants until all study procedures had been concluded. Due to the non-identical nature of the intervention beverages and the requirement for researchers to prepare study beverages during certain participant visits it was impossible to employ a double-blind procedure during this RCT.

While the protein content (between HPM and PHPM) and the total energy content were approximately matched across treatments, it is possible that the variation in carbohydrate content (table 1.) may have influenced performance in the EIMD induction protocol and thus influenced the damage induced. However, 24-hour dietary recall data from the period immediately prior to EIMD induction protocol shows

almost identical CHO intake across groups (table 3), and as such it is unlikely to have influenced findings.

The parallel design of this study may have introduced additional heterogeneity to the results of this study due to baseline differences across groups. However, crossover designed RCTs which normally are undertaken to account for this variation run into considerable issues due to the repeated bout effect when used in EIMD recovery RCTs. The repeated bout effect is the phenomena post intensive unaccustomed exercise where the body undergoes pronounced adaptation in a super-compensatory manner to protect itself from a future similar bout of exercise (McHugh, 2003; Hyldahl, Chen and Nosaka, 2017). This poses considerable issues due to the inability to standardise muscle damage induction between study arms due to the adaptation of the body following the first bout of exercise and as such a parallel design is the most appropriate design for this type of RCT.

Although procedures were used to standardise exercise instruction, demonstration and equipment used during the muscle damaging protocol, no quantifiable method was used to ensure standardised muscle damage was induced for every participant. Future studies may consider camera-based systems to assess squat depth at the bottom of landing or force plate systems to assess landing forces exerted where resources allow.

All participants in this study were instructed to abstain from strenuous exercise for 48 hours prior to muscle damage induction to ensure muscle soreness and strength decrements were only a result of study protocols. However, muscle soreness was not assessed at baseline and as such it is possible that there was residual soreness that may have occurred prior to the study protocols.

While the original study to propose this muscle damage induction protocol suggested to complete the exercise barefoot due to the effect of shoes on landing mechanics (Miyama and Nosaka, 2004), pilot testing in the present facilities observed considerable foot pain following 100 drop jumps barefoot due to the rigid nature of the concrete flooring and determined it to be more appropriate to allow footwear to be used. This may however have reduced total muscle damage experienced and added variation to the forces experienced across participants due to varying cushioning effect of shoes worn.

4.6 Future Directions and Research Priorities

One of the strengths of this RCT is the comprehensive characterisation of study intervention beverages particularly the polyphenol content of the PHPM intervention. This is a particular weakness in previous RCT protocols investigating polyphenol interventions and future research investigating other polyphenol sources should aim to adopt similar characterisation protocols (Carey, Lucey and Doyle, 2021). This will allow for greater understanding of indicative polyphenol dosage and subclass recommendations. This RCT has focussed on the complementary effect of polyphenols and milk protein on post exercise recovery from EIMD, however future research is required to evaluate the effect of long-term consumption of polyphenol interventions on exercise induced adaptation. Other antioxidant supplementation such as vitamin C and E during longer term exercise training has shown propensity to blunt training adaptation in certain population groups (Gomez-Cabrera *et al.*, 2015; Bjørnsen *et al.*, 2016). While early research suggests this may not extend to polyphenols, this needs further clarification to inform recommendations for use of polyphenols during intense training periods (Beyer *et al.*, 2017; Pavis *et al.*, 2022). Should the use of polyphenols during training not blunt adaptation, further research will be warranted to investigate the impact polyphenol protein blends such the PHPM beverage on muscular adaptation. One study has investigated the role of a protein polyphenol blend during exercise training showing improvements in muscle protein synthesis but have suggested this may only apply to early onset of training and may not be ergogenic for longer term adaptation (Pavis *et al.*, 2022). Further investigations in this realm, while dependant on confirming no blunting of training adaptation, could provide fascinating insight into the co-consumption of polyphenols and protein to support exercise performance and adaptation in the longer term.

5. Conclusion

This proof-of-concept RCT is the first to observe that 10 days of consumption of a high protein milk enriched with blackberry polyphenols can provide enhanced recovery from EIMD following a single intensive exercise bout. In particular, PHPM showed significantly improved maintenance of muscle strength compared to LPM over the 48h post exercise and reductions in muscle soreness over the 72 hours post

exercise when compared with both a high protein milk alone and a control low protein milk. PHPM also showed improved recovery of muscle strength over the 48-hour period post exercise compared with a low protein control. This polyphenol rich intervention was particularly rich in the anthocyanin cyanidin-3-O-glucoside, and the ellagitannins lambertianin C and sanguiin H-6. The synergistic effects of berry polyphenols and milk protein for skeletal muscle and exercise provide an exciting area for future research and a promising avenue to support athletes following intensive exercise.

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Chapter 6 – Effect of a Blackberry Polyphenol Enriched High Protein Dairy Beverage on Performance in a 15km Cycling Time Trial: a Randomised Controlled Trial

1. Introduction

Appropriate nutritional practices are vital in supporting endurance exercise performance, particularly fuelling strategies providing carbohydrate intake prior to and during prolonged endurance events (Ravindra *et al.*, 2022). Recent studies have shown that certain bioactive functional food components enhance endurance performance. For example, consistent performance benefits have been observed with the ingestion of caffeine and dietary nitrate (Sökmen *et al.*, 2008; Hoon *et al.*, 2014; Pickering and Kiely, 2018; Silva *et al.*, 2022). Improving the endurance performance of exercising skeletal muscle can potentially be achieved through the modulation of reactive oxygen species (ROS) (Durand *et al.*, 2015). ROS are produced during repetitive muscle contraction and when produced in excess have been implicated in impaired blood flow and vasodilatory capacity during exercise (Donato *et al.*, 2010). Supplementation with antioxidants have been proposed as a viable method to improve endurance exercise performance. This has been confirmed in studies reporting that antioxidants such as N-acetyl cysteine can improve endurance exercise performance by improving blood flow and vasodilation in time to exhaustion and simulated cycling race events (Medved *et al.*, 2004; Slattery *et al.*, 2014).

Polyphenols are non-nutritive bioactive phytochemicals which are found in various plant-based food sources such as fruits, vegetables, beans, and teas (Manach *et al.*, 2004; Sureda *et al.*, 2014). There is a large array of polyphenol subclasses, many of which have exhibited differing biological functionality and bioactivity (Panche, Diwan and Chandra, 2016; Manach *et al.*, 2018). Polyphenols have been shown to exhibit antioxidant properties through interactions with endogenous antioxidant pathways such as the nuclear factor erythroid 2 related factor 2 – electrofile response element (NRF2-ERE) pathway (Zhou *et al.*, 2019; Kanner, 2020). This effect has recently been attributed to interactions between polyphenols and the gut microbiome (Sorrenti *et al.*, 2020). NRF2 is a transcription factor that has been strongly associated with performance in endurance events with stronger expression observed in endurance athletes compared with control and strength trained athletes (Eynon *et al.*, 2009). As such, polyphenols have been suggested to potentially attenuate the deleterious effects of exercise induced ROS such as impaired vasodilation through upregulation of the production of endogenous antioxidant enzymes resulting from interactions with NRF2-ERE pathways (Bowtell and Kelly, 2019). This antioxidant activity has been

suggested to preserve nitric oxide (NO) bioavailability and as a result improve vascular responses to exercise and improve oxygen availability for working muscle (Cosby *et al.*, 2003). Polyphenols have been shown in-vivo to improve flow mediated dilation in non-exercise focussed studies (Kiyimba *et al.*, 2023) and have also been shown to improve muscle oxygenation during exercise (Morgan, Barton and Bowtell, 2019).

To date, several randomised controlled trials (RCTs) have been conducted which investigate the effect of acute dosages of polyphenols to enhance endurance exercise performance. Findings indicate mixed results, with some RCTs exhibiting ergogenic benefits while others have observed little or no effect (Motard-Bélanger *et al.*, 2013; Trexler *et al.*, 2014; Crum *et al.*, 2017; Deley *et al.*, 2017). Two systematic reviews and meta-analyses of RCTs have shown that consumption of polyphenol intervention provide clear benefit to endurance exercise performance in various endurance exercise modalities (Somerville, Bringans and Braakhuis, 2017; d'Unienville *et al.*, 2021). Somerville et al. (2017) has shown that polyphenol supplementation for at least 7-days increases performance by 1.9% predominantly measured in time trial (TT) and time to exhaustion (TTE) trials. D'Unienville et al (2021) observed a significant but trivial performance enhancement in TT and TTE trials, however a significant moderate enhancement in graded exercise test protocols in an analysis of mixed acute and chronic ingestion RCTs. These meta-analyses did not observe a significant difference in performance enhancement between acute and chronic intervention protocols.

RCTs investigating the role of anthocyanin-rich New Zealand Blackcurrant ingestion have shown significant benefits to endurance exercise performance with a meta-analysis of nine RCTs showing a small significant benefit (Braakhuis, Somerville and Hurst, 2020). However, a further meta-analysis of polyphenol interventions, a subgroup analysis of New Zealand Blackcurrants has shown no such effect (d'Unienville *et al.*, 2021).

Other anthocyanin rich intervention Montmorency tart cherry juice, as well as ellagitannin rich pomegranate interventions have shown significant benefits during subgroup analysis within this meta-analysis (d'Unienville *et al.*, 2021). The variance in the response to polyphenol treatment source likely is a result of a number of factors either alone or in isolation. These may include phenolic composition, timing and duration of polyphenol ingestion, control of background diets and fitness level of

the participants. To date however, blackberries have yet to be investigated for their effect on endurance exercise performance despite their high anthocyanin and ellagitannin content (Tzima, Putsakum and Rai, 2023).

Bovine milk is suggested to be a good source of fluid intake surrounding intensive exercise for various reasons (Roy, 2008). The protein content of milk has been shown to promote muscle protein synthesis during exercise training, providing support in the process of repair and adaptation of skeletal muscle (Kanda *et al.*, 2016). Milk has been shown to be an effective hydration beverage and may even perform better than both sports drinks and water at improving net fluid balance post exercise (Shirreffs, Watson and Maughan, 2007; Desbrow *et al.*, 2014). Milk has also been shown to be similar in its provision of carbohydrate to commercial sports drinks in its ability to offset the carbohydrate dependent fatigue in endurance exercise (Loureiro *et al.*, 2021).

Proteins have also been suggested to positively impact the digestion of polyphenols through the formation of protein-polyphenol aggregates which are formed by non-covalent hydrophobic interactions (Jakobek, 2015; van de Langerijt, O'Mahony and Crowley, 2023). Research has shown that casein micelles present in milk have a particular affinity for bonding with polyphenols (Hasni *et al.*, 2011; van de Langerijt, O'Mahony and Crowley, 2023). Recent research has shown that addition of freeze-dried blackberry polyphenols to milk has a protective effect on polyphenols, particularly anthocyanins during in vitro digestion through the formation of such protein polyphenol aggregates which may improve the overall bioaccessibility of anthocyanins (van de Langerijt *et al.*, 2023).

Despite the observed positive independent effects of polyphenols and milk, the effect of a milk polyphenol blend on endurance exercise performance remains to be investigated in a human intervention RCT. While the effects of polyphenol-protein blends on post exercise factors such as muscle protein synthesis, oxidative stress response, immune response and intestinal permeability and injury have been investigated; the body of evidence supporting the combination of polyphenols and protein on exercise outcomes is largely in its infancy despite the plethora of theoretical synergistic benefits (Nieman *et al.*, 2013; Ahmed *et al.*, 2014; Pavis *et al.*, 2022). The aim of this study was to investigate whether 7-days of ingestion of a high protein milk enriched with blackberry polyphenols enhances 15km cycling time trial performance compared with both a high protein milk and low protein milk control beverage.

2. Methods

2.1 Study Overview & Recruitment

This study took the form of a parallel proof of concept, single blind, randomised controlled trial. All study procedures took place at the human performance laboratories of the South East Technological University, Waterford, Ireland between June 2022 and May 2023. Moderately active male participants between the ages of 18 and 45 years were recruited by a convenience sampling approach. Sample size was determined in line with previously published parallel designed polyphenol RCTs (Motard-Bélanger *et al.*, 2013; Trexler *et al.*, 2014; Crum *et al.*, 2017; Deley *et al.*, 2017, Morgan, Barton and Bowtell, 2019). This included advertisement on social media platforms, word-of-mouth, and electronically distributed flyers. This study was granted ethical approval by the ethics board of South East Technological University, Waterford (ref: WIT2021REC044, January 2022) (Appendix 13). All participants provided informed consent prior to any study procedures (Appendix 16). This RCT protocol was registered with www.clinicaltrials.gov, ID number NCT05463835.

2.2 Participant Screening

Individuals who expressed interest in taking part in the research study were requested to complete a pre-screening questionnaire to determine their initial eligibility following first contact (Appendix 14). To be eligible to take part in the RCT, participants were required to be biologically male, between 18 and 45 years of age, free from illness or current injury, not have a history of international sporting competition and not having taken either probiotic supplements or antibiotics in the past two months. Participants were also required to confirm their willingness to comply with study protocols including abstention from consumption of selected polyphenol rich foods for the duration of the study, avoidance of non-medically prescribed nutritional supplements and nutraceuticals for the duration of the study, cessation of exercise training in the 48 hours prior to and following both study test days. If a participant met each of the above criteria, they were booked in to complete the study screening visit. Participants were required to achieve a VO_2peak of between 35.0 and 55.0 ml/kg/min to be eligible to take part. This fitness level was deemed to be sufficient to complete the 15km cycling time trial while not representative of high-level endurance exercise training. Participants were provided a list of polyphenol rich

foods to avoid for 7 days prior to baseline visit and throughout the study intervention period based on the details of polyphenol rich foods presented in Pérez-Jiménez et al., (2010) (Appendix 19.).

Participants were assessed for baseline anthropometric characteristics including height using a stadiometer and weight using a scale. VO_2peak was assessed using a graded exercise test to volitional exhaustion. The test was conducted on an electronically braked cycling ergometer which was programmed to start at 80W and increase by 20W every 1-minute stage (Lode Corival CPET cycle ergometer B.V. Groningen, Netherlands). Exhaustion was determined as the point at which the participant could not continue any further or they were unable to maintain a cadence of at least 65rpm despite verbal prompts to do so. Strong verbal encouragement was provided throughout. VO_2peak was measured using a MOXUS Modular Metabolic System (AEI Technologies, Bastrop, TX USA).

2.3 Study Beverages

Once confirmed to be eligible, participants were randomly assigned to one of three groups using a block randomization method via the online tool sealedenvelope.com. Following their baseline visit all participants were instructed to consume two 500ml dosages of their assigned study beverage per day for 7 days, evenly spaced between 10 and 14 hours apart. One dosage of polyphenol enriched high protein milk beverage (PHPM) contained 500ml high protein milk enriched with 25g freeze dried blackberry powder (FDBP). This FDBP was formulated with the aim of providing a target dosage of 750mg polyphenols twice per day. Total polyphenol content (TPC) was measured prior to and monthly to assess stability of polyphenol concentrations during storage. Polyphenol subclasses were qualitatively and quantitatively identified using HPLC-QTOF mass spectrometry. Full methodology and results for polyphenol concentration analysis and characterisation is presented in the RubusElite RCT Preface.

Once all study procedures were completed, all participants who were randomised to the PHPM group were asked to fill out a product acceptability questionnaire. This questionnaire was designed based on a previous study assessing the acceptability of a polyphenol rich beverage (Orjuela-Palacio, Zamora, and Lanari, 2014). This involved an overall ranking of experience of consuming the beverage on a 9-point hedonic scale

where 0 represented “dislike very much”, 5 represented “neither like nor dislike” and 9 represented “like very much”. Participants were then asked to rank their opinion of the beverage on 5 diagnostic attributes: sourness, sweetness, astringency, aroma and viscosity. These were ranked on a “just about right” scale which ranged from -4 = too little, to +4 = too much anchored about a central point of 0 denoting “optimum”.

A commercial high protein milk (HPM) (North Cork Creameries, Cork Ireland), identical to that which was provided to the PHPM group without FDBP, contained 30g milk protein and contained no polyphenol content. The third beverage, which functioned as a control beverage, was a commercially available low protein dairy beverage (Taranis Dalia Liquid) (LPM) with comparable total energy content to that of the high protein milk also with no polyphenol content. For the macronutrient and energy profile of each beverage see the RubusElite RCT Preface. To assess compliance participants returned a beverage ingestion log which noted the time of and confirmed the consumption of study beverages over the treatment period (Appendix 17.). Participants were only included in analysis where they consumed 80% or more of their assigned study beverages.

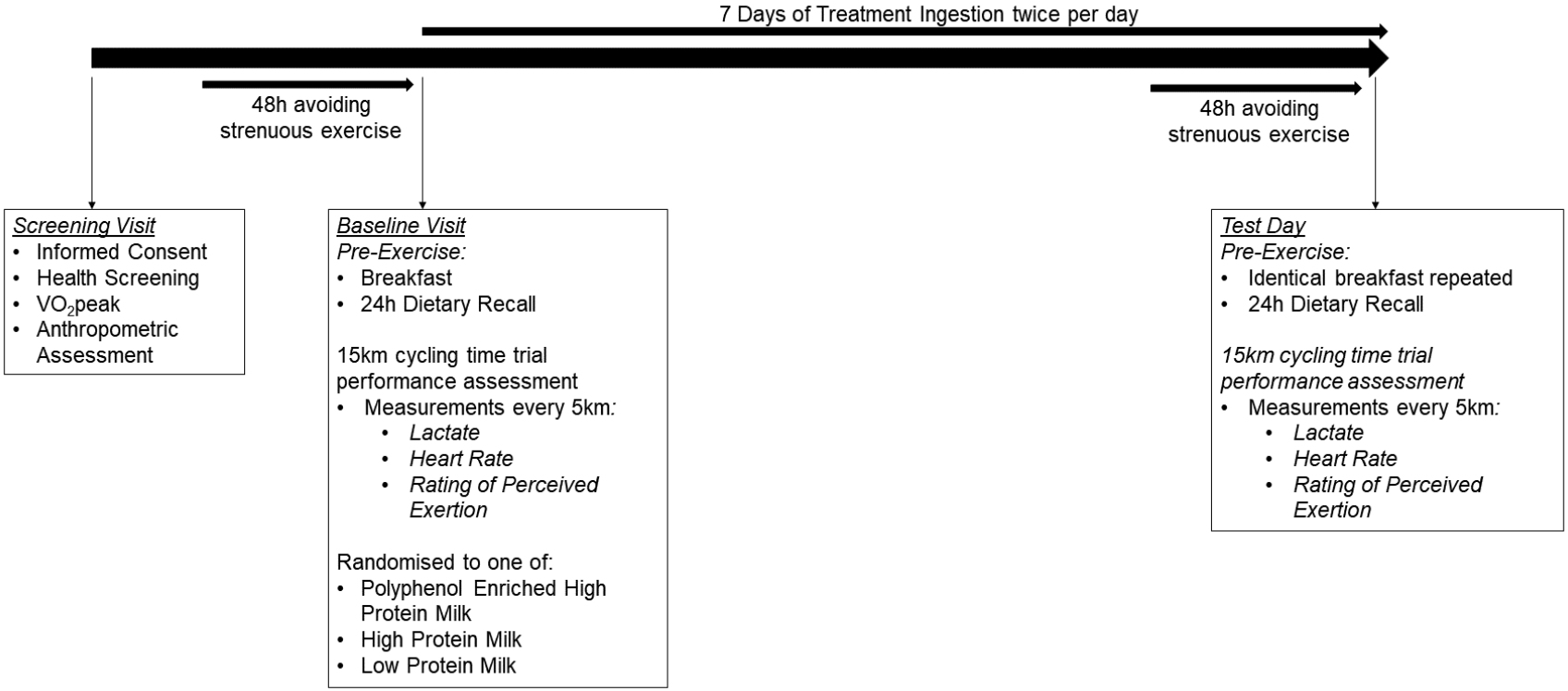


Figure 1. RubusElite endurance performance randomised controlled trial study design.

2.4 Experimental design

The overall study design is presented in Figure 1. This RCT comprised of two experimental study visits, a baseline visit (Day 0) and a test visit (Day 7). On the morning of each experimental visit, participants arrived at the laboratory following an overnight fast and a 48-hour period of abstention from strenuous exercise. Overnight participants were allowed to drink water ad libitum and instructed to arrive to the laboratory in a fully hydrated state. Participants were provided with a voucher to purchase a breakfast of their choice from the university restaurant and were instructed that they would be required to repeat this breakfast at the subsequent test visit. Participants were instructed to avoid caffeine intake in this breakfast. Following breakfast participants returned to the laboratory and underwent an interviewer administered 24-hour dietary recall. The 24-hour food recall was completed by a trained assessor according to a multi pass technique (DAPA Measurement Toolkit). This data was analysed using Nutritics Research Edition v5.87 software (Nutritics, Dublin, Ireland).

2.5 Exercise protocol

Participants were required to attend the laboratories on two occasions for performance testing. Acute endurance exercise performance was assessed during a 15-kilometre cycling time trial on an indoor cycling ergometer. A 15km cycling time trial was chosen as it has been successfully applied in a similar randomised controlled trial investigating the role of Montmorency tart cherry juice (Morgan, Barton and Bowtell, 2019). It was also shown that time trial events with a duration of between 10-30 minutes had the greatest mean difference associated with polyphenol intake in a recent meta-analysis sub-grouped by duration and exercise type (d'Unienville *et al.*, 2021).

Participants completed two 15km time trials, one following 7-days of washout from polyphenol rich foods, prior to study beverage consumption (TTpre), and one on the seventh day of study beverage ingestion (TTpost). Following a standardised breakfast and dietary assessment as described above, participants were positioned on the ergometer in a position of their liking. Saddle height, handlebar reach, and handlebar height were recorded during the first trial and was repeated exactly in the same manner for the second time trial. All time trial procedures were performed on the same

Wattbike pro cycle ergometer (Wattbike ltd., Nottingham, England). During all procedures, the default Wattbike display was visible to all participants with information including current cadence, speed and distance remaining in the time trial. Participants completed a 5-minute warm-up at intensities corresponding to intensities represented on Borgs rating of perceived exertion (RPE) (Borg, 1982). This warm-up consisted of 2 minutes of cycling at a RPE of 10 followed by 1 minute cycling at RPE's of 11,12 and 13. Participants were then instructed to complete the fifteen kilometres as quickly as possible and were allowed to set their own pace and control the resistance on the flywheel. Participants were informed of an expected duration of the test between 20 and 30 minutes and to set their pacing strategy accordingly. Heart rate (HR), stage time, RPE and blood lactate were recorded every 5km during the test. Blood lactate was measured from the earlobe using a handheld Lactate Plus Meter 40828 (Nova Biomedical GmbH, Mörfeldon-Walldorf, Germany). Heart rate was measured using a Polar H10 heart rate sensor (Polar Electro Oy, Kempele, Finland). The time trial was conducted at the same time each day to account for diurnal variation in the same laboratory with controlled environmental conditions.

2.6 Data Analysis

All statistical procedures were conducted in IBM SPSS v.28.0.0 (Armonk, NY: IBM Corp). Statistical significance was set at $P<0.05$ for all analyses.

One-way ANOVA was used to assess differences in demographic variables across treatment groups at baseline. Repeated measures ANOVA was used to determine the interaction between treatment group and time across dietary intake values in the 24-hours prior to the two time trials. One-way ANOVAs were conducted on the calculated differences in dietary intake to ascertain the change between supplement groups from TTpre to TTpost. Paired sample T tests were undertaken to examine changes in dietary intake within treatment groups between TTpre and TTpost

A one-way repeated measures ANOVA was used to assess for differences in time to completion of the 15km cycling task. Two-way repeated measures ANOVA was used to assess for differences in lactate concentration, rating of perceived exertion and heart rate.

Shapiro-Wilk tests were used to assess the normality of all variables. Log transformations were conducted where a variable violated the assumption of normality. Bonferroni adjustments for multiple comparisons were used where appropriate. Levenes test for homogeneity of variances was used for all one-way ANOVAs. Tukey post hoc tests were used for one-way ANOVAs where a significant difference ($P < 0.05$) was observed. In repeated measures ANOVAs, sphericity was assessed using Mauchleys test for sphericity, and a Greenhouse-Geisser correction was used in cases where this assumption was violated.

3. Results

3.1 Subjects

One-hundred and forty-nine participants underwent the initial pre-screening procedure. Seventy-five then completed the graded exercise test to exhaustion to measure VO_2peak. Thirty-two participants achieved the target range of a VO_2peak between 35 and 55 ml/kg/min and proceeded to randomised beverage allocation and completed all study procedures. Following allocation to study beverages there were no dropouts, and all completed the study protocol. This represented twelve in the PHPM group and ten in both the HPM and LPM groups, respectively. Full participant recruitment details are presented in the CONSORT diagram (Figure 2). Participant baseline demographic data is presented in Table 1. There were no significant differences in participant baseline age, height, BMI, VO_2peak or treatment compliance across treatment groups. There was a significant difference between treatment groups in bodyweight at baseline with the HPM weighing significantly more than the LPM group.

Table 1. Baseline demographic characteristics of participants overall and groups according to their randomised study group (n=32)

	Overall	**Polyphenol & High Protein Milk (n=12)**	**High Protein Milk (n=10)**	**Low Protein Milk (n=10)**	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	*P*
Age (years)	30.2±8.9	27.6±8.9	28.5±6.9	34.9±9.5	0.13
Weight (kg)	86.9±14.7	85.2±10.0[ab]	96.2±17.3[a]	79.8±13.1[b]	0.03
Height (cm)	179.9±7.3	178.3±4.8	183.3±6.5	178.3±4.8	0.20
BMI (kg/m^2)	26.8±4.0	26.7±2.5	28.7±5.6	25.0±2.6	0.10
VO2peak (ml/kg/min)	46.5±5.2	45.0±5.4	47.1±5.0	47.5±5.1	0.49

[a]- denotes a significant difference with [b] Significance set at $P<0.05$ as assessed by one-way ANOVA

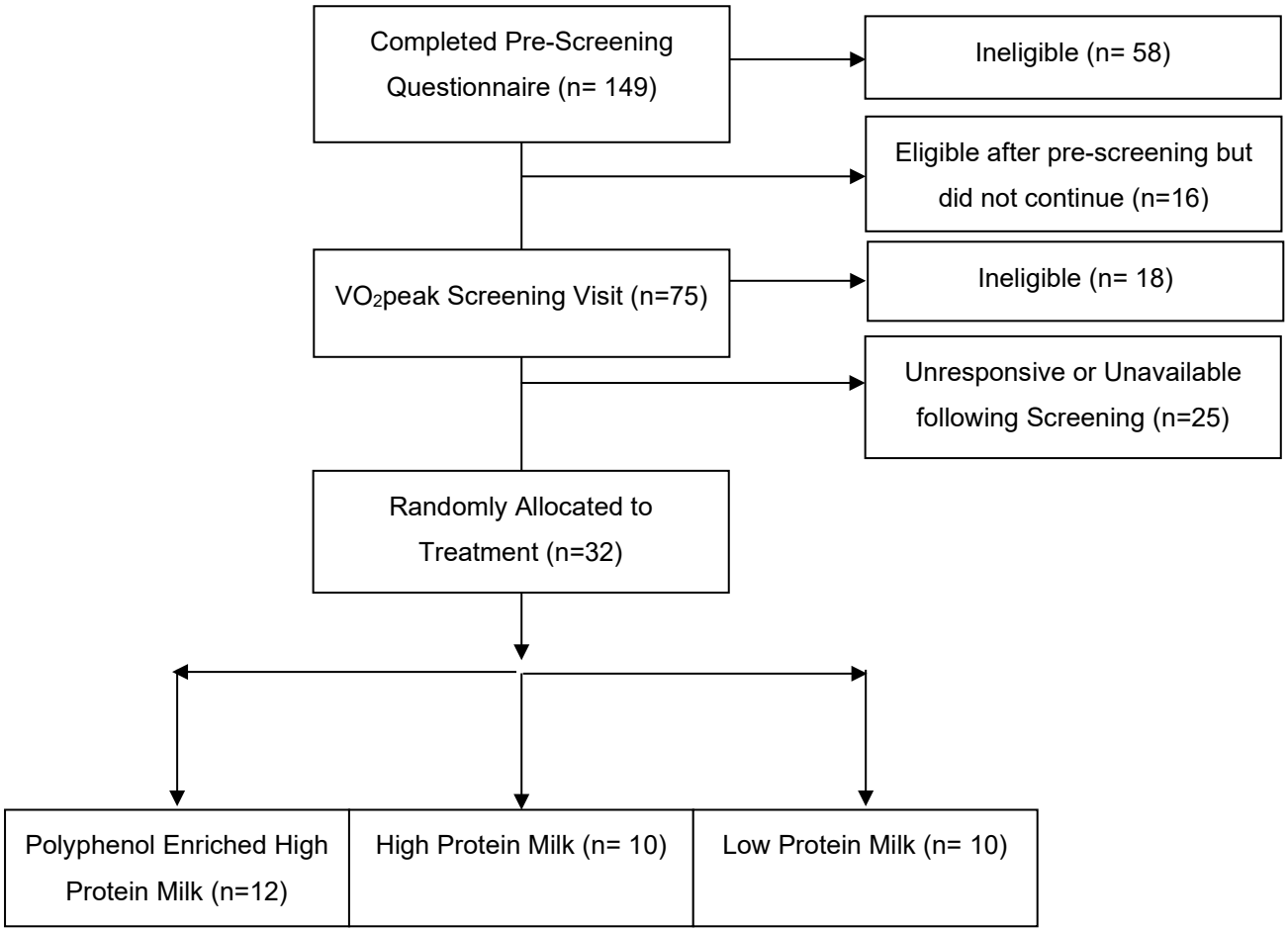


Figure 2. CONSORT diagram outlining participant recruitment, eligibility and randomisation.

3.2 Treatment compliance, acceptance and 24h Dietary Recall, Macronutrient and Energy Intakes

Compliance with consumption of study treatments was high. There was no significant difference in compliance between the intervention groups ($P = 0.90$). Compliance was 97.6±5.4% in the PHPM group, 97.9±4.7% in the HPM group and 98.6±4.4% for the LPM group, respectively. Overall study treatment compliance was 98.0±4.8%.

Participants randomised to the PHPM group (n=12) rated their overall experience of consuming their treatment beverage at 5.4±1.4 out of 9, denoting a score just above "neither like nor dislike". Participants ranked sensory diagnostic attributes, sourness (0.6±1.2), aroma (0.4±1.7), astringency (0.6±0.7) and viscosity (0.1±2.0) close to the optimum value of 0, while sweetness was ranked further on the "too little" side (-1.25±1.3).

The intake of energy was observed to be significantly higher in the 24 hours prior to TTpost compared to TTpre ($P = 0.047$) for all treatment groups (Table 2a). This may be partly due to the significant increase in protein consumed in the 24 hours prior to TTpost compared with TTpre ($P < 0.001$), likely due to two out of three interventions containing a high protein content as expected (Table 1). There was no significant change in overall group dietary intakes between TTpre and TTpost for carbohydrate ($P = 0.07$) or fat intakes ($P = 0.72$) (Table 2a).

In the 24-hours leading to TTpre, there was no significant difference between any treatment groups for intake of total energy ($P = 0.99$), protein ($P = 0.99$), carbohydrate ($P = 0.89$) or fat ($P = 0.80$) (Table 2b). In the 24 hours prior to TTpost, participants in both the PHPM and HPM groups has significantly higher protein intakes compared with the LPM group ($P < 0.01$). Both PHPM and HPM groups also had a significantly higher percentage of their total energy consumption come from protein in the 24-hour period prior to TTpost than LPM ($P < 0.01$) (Table 2b). There was no significant difference in total energy ($P = 0.64$), carbohydrate ($P = 0.99$) or fat ($P = 0.55$) consumed between the treatment groups in the 24-hours leading to TTpost (Table 2b).

The change in protein intake between TTpre and TTpost was also significantly different between beverage groups ($P < 0.01$) (Table 2c). PHPM and HPM groups increased their protein intake significantly more than LPM in TTpost. There was no significant difference in the change of protein intake between HPM and PHPM. There was no significant difference in the change of nutrient intakes between treatment

groups from TTpre to TTpost in terms of energy ($P = 0.72$), carbohydrate ($P = 0.80$) or fat ($P = 0.07$) (Table 2c).

Table 2a. Overall group 24-hour Dietary Recall energy and macronutrient intake prior to TTpre and TTpost including nutrients provided by treatment beverage. All data presented as mean ± SD

	Overall Group (n=32)			
	TTpre	**TTpost**	**TTpre-TTpost *P-value***	**Change**
Energy (Kcal)	2705 ± 838	3014 ± 765	0.047	309 ± 829
Protein (g)	130 ± 37[a]	169 ± 49[b]	<0.01	38 ± 58
Protein (% of total Kcal)	20 ± 5[a]	23 ± 5[b]	<0.01	3 ± 6
Carbohydrate (g)	286 ± 125	323 ± 91	0.07	37 ± 111
Carbohydrate (% of total Kcal)	42 ± 9	43 ± 8	0.56	1 ± 11
Fat (g)	114 ± 45	116 ± 51	0.72	3 ± 53
Fat (% of total Kcal)	38 ± 8	34 ± 9	0.08	-4 ± 11

[a, b] superscript denotes a significant difference in TTpost nutrient intakes across treatment groups as assessed by paired samples T-test.

Table 2b. Beverage group 24-hour Dietary Recall energy and macronutrient intake prior to TTpre and TTpost including the nutrients provided by treatment beverage. All data presented as mean ± SD

	Polyphenol & High Protein Milk (n=12)			**High Protein Milk (n=10)**			**Low Protein Milk (n=10)**				
	TTpre	**TTpost**	**TTpre-TTpost *P-value***	**TTpre**	**TTpost**	**TTpre-TTpost *P-value***	**TTpre**	**TTpost**	**TTpre-TTpost *P-value***	***TTpre P-value***	***TTpost P-value***
Energy (Kcal)	2686 ± 927	2958 ± 863	0.16	2725 ± 904	3205 ± 827	0.16	2710 ± 740	2890 ± 597	0.09	0.99	0.64
Protein (g)	129 ± 39	186 ± 40[a]	0.51	131 ± 49	196 ± 42[a]	0.50	131 ± 23	120 ± 29[b]	0.76	0.99	<0.01
Protein (% of total Kcal)	21 ± 7	26 ± 4[a]	0.11	19 ± 3	25 ± 4[a]	0.63	20 ± 3	17 ± 4[b]	0.80	0.83	<0.01
Carbohydrate (g)	288 ± 168[x]	321 ± 77[y]	0.047	271 ± 86[x]	327 ± 125[y]	0.04	298 ± 105	321 ± 76	0.18	0.89	0.99
Carbohydrate (% of total Kcal)	42 ± 12	45 ± 10	0.26	40 ± 4	40 ± 6	0.33	44 ± 9	45 ± 8	0.56	0.64	0.34
Fat (g)	110 ± 48	103 ± 66	0.08	122 ± 45	123 ± 30	0.43	110 ± 44	125 ± 48	0.44	0.80	0.55
Fat (% of total Kcal)	37 ± 10	30 ± 11	0.08	40 ± 4	35 ± 5	0.49	36 ± 9	38 ± 8	0.90	0.54	0.66

[a, b] superscript denotes a significant difference in TTpost nutrient intakes across treatment groups. Significance set at $P < 0.05$.

[x, y] superscript denotes a significant difference between TTpre and TTpost intakes within treatment group. Significance set at $P < 0.05$.

Table 2c. Beverage group 24-hour Dietary Recall energy and macronutrient intake changes between TTpre and TTpost. All data presented as mean ± SD

	Polyphenol & High Protein Milk (n=12)	High Protein Milk (n=10)	Low Protein Milk (n=10)	Effect of Beverage on change TTpre to TTpost
	Change	**Change**	**Change**	***P***
Energy (Kcal)	272 ± 958	482 ± 886	180 ± 637	0.72
Protein (g)	57 ± 50[a]	65 ± 56[a]	-11 ± 39[b]	<0.01
Protein (% of total Kcal)	5 ± 6[a]	6 ± 4[a]	-3 ± 5[b]	<0.01
Carbohydrate (g)	34 ± 139	56 ± 95	23 ± 97	0.80
Carbohydrate (% of total Kcal)	3 ± 13	0 ± 6	0 ± 13	0.77
Fat (g)	-7 ± 58	2 ± 47	15 ± 56	0.64
Fat (% of total Kcal)	-7 ± 10	-5 ± 5	3 ± 13	0.07

[a, b] - superscript denotes significant difference in the change in nutrient intake between treatment groups. Significance set at $P < 0.05$.

3.3 Performance in 15km Time Trials

Participants' time to complete the 15km did not significantly change between TTpre and TTpost ($P = 0.54$). On average for the full sample, the time taken during TTpre was 1516 ± 89 seconds, and during TTpost, it was 1519 ± 97 seconds (Figure 3).

Furthermore, there was no effect of treatment group on the total time taken to complete the 15km trial. The 15km time trial performance remained consistent from TTpre to TTpost across all three beverage groups ($P = 0.21$) (Figure 3).

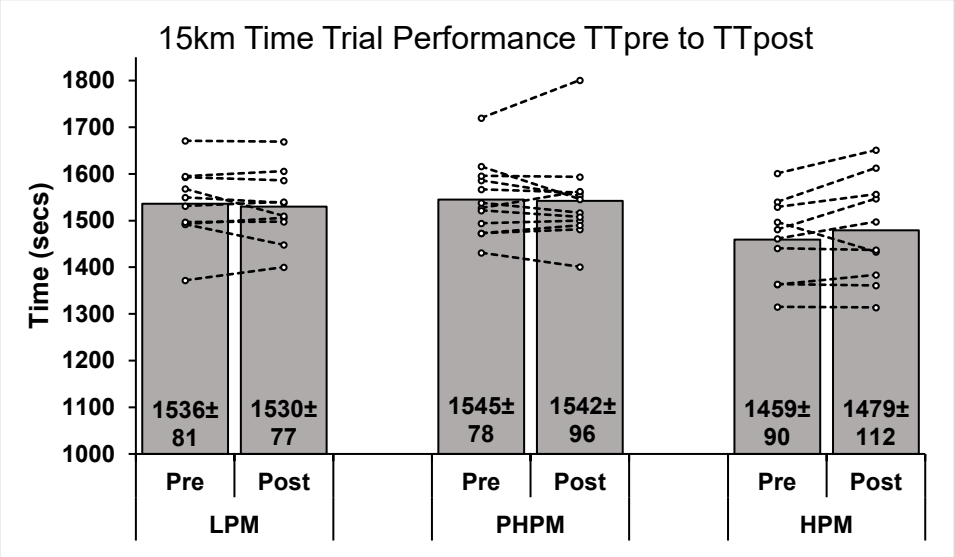


Figure 3. Total time to complete 15km from TTpre to TTpost. Data presented as individual data and mean ± SD, statistical significance set at $p<0.05$. No significant difference in change in time trial performance between treatment groups.

3.4 Rating of Perceived Exertion

The rating of perceived exertion was measured by the Borg RPE scale (Borg, 1982). In the overall sample, as participants progressed through the 15km time trials, there was a significant change in RPE ($P < 0.001$). This indicates that participants' feeling of exertion changed as they covered more distance. Participants felt they were exerting themselves more at the 15km point than at both the 5km ($P < 0.001$) and 10km marks ($P < 0.001$). Moreover, their perceived effort at the 10km point was also notably higher than at the 5km mark ($P < 0.001$) (Table 3.).

The type of beverage consumed did not significantly affect the change in participants' RPE between TTpre and TTpost ($P = 0.29$). This means that regardless of the beverage

consumed, participants felt similarly about their effort in TTpre to that of TTpost (as seen in Figure 4).

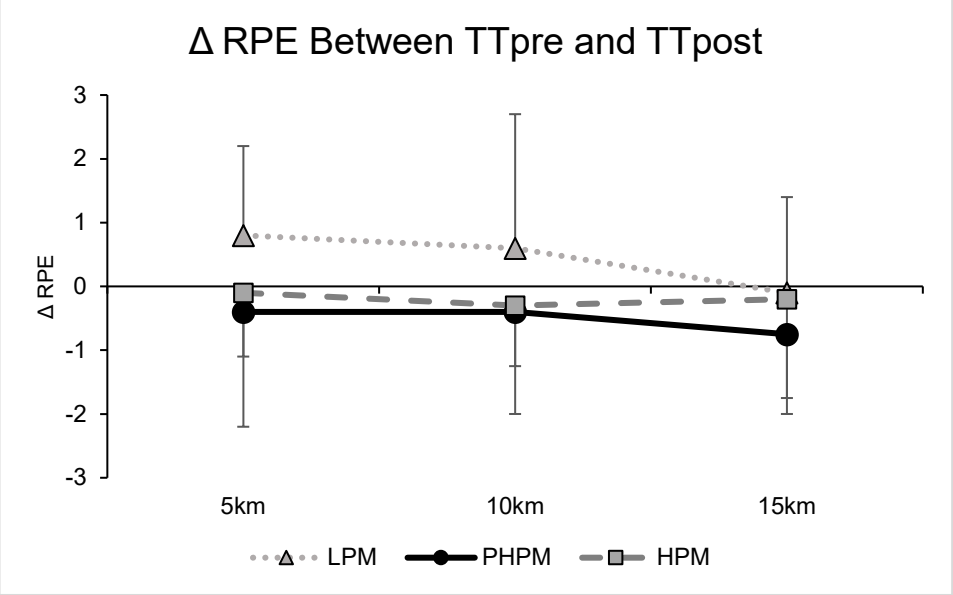


Figure 4. Change in rating of perceived exertion between TTpre and TTpost at 5km, 10km and 15km during 15km cycling time trial. Data presented as individual data and mean ± SD, statistical significance set at p<0.05. No significant difference in change in RPE between treatment groups at any timepoint.

3.5 Heart Rate

In the overall group, as participants covered more distance during the 15km time trial, there was a significant change in HR (P <0.001). Participants' heart rate was notably higher at the 15km point compared to both the 5km (P < 0.001) and 10km intervals (P < 0.001). However, there was no significant difference in heart rate when comparing the 10km and 5km points (P = 0.13) (Table 3).

The treatment group did not significantly influence the participants' change in HR between TTpre and TTpost (P = 0.39). This means the shift in heart rate before and after supplementation was consistent across all three beverage groups, as shown in Figure 5.

While there was no effect of treatment, there was an overall difference in HR between TTpre and TTpost in the full group. Participants had a lower heart rate during TTpost compared to TTpre (P =0.01).

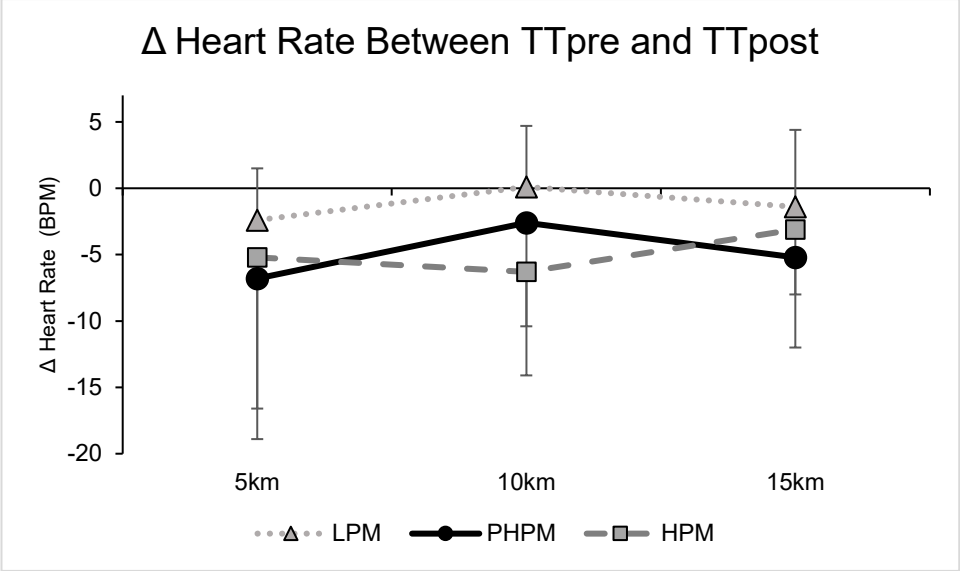


Figure 5. Change in heart rate (bpm) between TTpre and TTpost at 5km, 10km and 15km during 15km cycling time trial. Data presented as individual data and mean ± SD, statistical significance set at $p<0.05$. No significant difference in change in heart rate between treatment groups at any timepoint.

3.6 Blood Lactate Concentration

The type of beverage consumed did not significantly impact the participants' blood lactate concentrations. There was no notable interaction between the type of beverage consumed and the change in lactate levels between TTpre and TTpost ($P = 0.822$), as depicted in Figure 6. However, similar to HR, the specific time trial had a significant impact on lactate levels. Participants had higher lactate levels during TTpre than during TTpost ($P = 0.024$) (Table 3).

Additionally, the distance covered during the 15km time trial significantly influenced lactate levels. The data indicates that lactate concentrations shifted notably ($P < 0.001$) as the trial progressed. Post hoc analysis revealed that the lactate concentrations were considerably higher at the 15km point than at the 5km ($P < 0.001$) and 10km points ($P = 0.001$). Also, lactate levels at the 10km point were higher than at the 5km mark ($P = 0.005$).

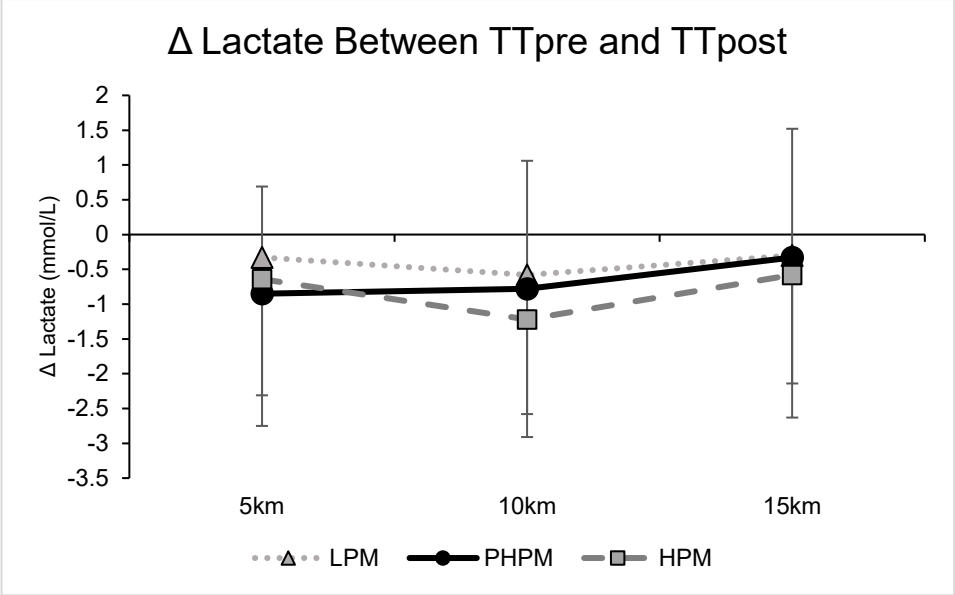


Figure 6. Change in lactate (mmol/L) between TTpre and TTpost at 5km, 10km and 15km during 15km cycling time trial. Data presented as individual data and mean ± SD, statistical significance set at $p<0.05$. No significant difference in change in blood lactate concentration between treatment groups at any timepoint.

Table 3. 5km split performance, ratings of perceived exertion, heart rate and lactate responses during 15km cycling time trials in TTpre and TTpost. All data mean ± SD.

	Overall (n=32)			PHPM (n=12)			HPM (n=10)			LPM (n=10)		
	5km	10km	15km	5km	10km	15km	5km	10km	15km	5km	10km	15km
5km split times (secs)												
TTpre	499 ± 31	510 ± 31	506 ± 34	501 ± 27	520 ± 31	524 ± 28	486 ± 30	491 ± 32	482 ± 33	510 ± 33	517 ± 24	509 ± 28
TTpost	508 ± 36	510 ± 33	498 ± 34	511 ± 34	517 ± 35	509 ± 37	498 ± 41	499 ± 38	482 ± 37	515 ± 36	513 ± 24	501 ± 25
RPE												
TTpre	13.7±1.7[a]	16.0±1.8[b]	18.7±1.5[c]	14.0 ± 1.5	16.5 ± 1.5	19.5 ± 1.0	14.5 ± 1.5	16.5 ± 1.4	18.2 ± 1.5	12.6 ± 1.8	14.9 ± 2.3	18.1 ± 1.6
TTpost	13.9±1.3[a]	15.9±1.3[b]	18.3±1.5[c]	13.9 ± 1.2	16.1 ± 1.2	18.8 ± 0.9	14.4 ± 1.4	16.2 ± 1.6	18.0 ± 1.8	13.4 ± 1.4	15.5 ± 1.3	18.0 ± 1.8
HR (bpm)												
TTpre	161 ± 18[a, x]	166 ± 17[a, x]	178 ± 15[b, x]	164 ± 19	168 ± 18	181 ± 18	160 ± 17	166 ± 16	176 ± 17	160 ± 21	164 ± 18	177 ± 12
TTpost	156 ± 18[a, y]	163 ± 17[a, y]	175 ± 16[b, y]	158 ± 18	165 ± 16	176 ± 17	154 ± 17	159 ± 17	173 ± 17	157 ± 20	164 ± 19	176 ± 15
Lactate (mmol/L)												
TTpre	5.9 ± 2.4[a, x]	7.4 ± 2.8[b, x]	8.8 ± 2.8[c, x]	7.1 ± 2.5	8.4 ± 2.3	9.5 ± 1.8	4.9 ± 2.0	6.5 ± 2.9	8.0 ± 3.2	5.4 ± 2.3	7.2± 3.3	8.7 ± 3.3
TTpost	5.3 ± 2.2[a, y]	6.6 ± 2.2[b, y]	8.4 ± 2.8[c, y]	6.2 ± 2.2	7.6 ± 1.5	9.2 ± 2.1	4.3 ± 1.8	5.3 ± 2.0	7.5 ± 3.4	5.1 ± 2.1	6.7 ± 2.5	8.4 ± 2.9

[a, b, c] – superscript denotes a significant difference between distance-point (5km, 10km and 15km) during the same time trial. Statistical significance set at $P < 0.05$

[x, y, z] – superscript denotes a significant difference between time trial (TTpre and TTpost) at the same distance point (5km, 10km and 15km). Statistical significance set at $P < 0.05$

4. Discussion

4.1 Study Key Findings

This single blind, parallel proof of concept randomised controlled trial is the first to the authors knowledge to investigate whether ingestion of a polyphenol-rich intervention delivered as part of a high protein bovine milk matrix (PHPM) for 7-days can improve endurance exercise performance in moderately active males. Consuming PHPM twice per day at a daily dosage of ~1400mg polyphenols and ~60g milk protein did not appear to enhance time to completion of a 15km cycling time trial when compared with both a high protein milk and a low protein milk control beverage. Similarly, there was also no evidence for an effect of this PHPM treatment or indeed the HPM treatment to influence physiological measures of exercise intensity such as blood lactate concentration and heart rate. There was also no effect of this intervention on participants level of perceived exertion throughout the 15km time trial.

4.2 Total Time to Complete 15km

This RCT showed no significant improvement in the time to completion of a 15km cycling time trial following ingestion PHPM compared with LPM or HPM for 7 days. This is in contrast to the meta-analysis of 14 RCTs conducted by Somerville and colleagues, who observed a significant moderate benefit to endurance performance associated with polyphenol consumption following similar ingestion durations (Somerville, Bringans and Braakhuis, 2017). It must be noted that this meta-analysis included a variety of measurements of performance and modes of exercise from time to exhaustion trials, graded exercise tests and fixed duration maximum distance trials along with time trials and also only included chronic ingestion studies of 7-days or longer (Somerville, Bringans and Braakhuis, 2017). One study investigating the effect of 7-days of ingestion of anthocyanin rich Montmorency tart cherry pills on performance in a 15km cycling time trial observed an average reduction in time taken to complete 15km by 4.6% associated with tart cherry supplementation (Morgan, Barton and Bowtell, 2019). While this is the most comparable protocol in terms of exercise mode, the population was well trained cyclists which may account for part of the difference in results. Based on previously reported RCTs, further factors which may have impacted the results of this RCT have been identified. These include the polyphenol dosage and composition, the timing of intake, the dairy matrix the

polyphenols were delivered in, and the population studied. These factors are discussed in depth below.

4.3 Polyphenol Dosage & Subclass

The average daily dosage used in the included studies of the meta-analysis by Somerville and colleagues was in general considerably lower compared to the polyphenol dosage used in this RCT (~688mg vs ~1413mg) (Somerville, Bringans and Braakhuis, 2017). While the higher daily dosage employed in this study is a notable difference to many of the RCTs previously published in this area, it seems unlikely that a higher dosage would negate beneficial effects. A previous RCT has shown that there was a positive dose response effect when increasing dosage between 300mg and 900mg per day of polyphenols derived from New Zealand blackcurrant extract on physiological response to prolonged cycling in endurance trained males (Cook *et al.*, 2017). While not in the realm of endurance exercise performance, another study has also shown that by increasing daily dosage of a polyphenol blend, muscle function during a post exercise recovery period is further enhanced, providing further evidence to support a dose response relationship to ergogenic effects for polyphenols (Herrlinger, Chirouzes and Ceddia, 2015).

D'Unienville et al (2021) have carried out extensive meta-analyses of on bioactive functional foods which may support endurance exercise performance. Among these analyses are specific polyphenol analyses which included 59 polyphenol interventional human RCTs. This study included a meta-regression to assess the effect of dosage on the endurance enhancing effect of polyphenols. However, of the 59 included polyphenol RCTs, 26 did not report the quantified polyphenolic content of the intervention (d'Unienville *et al.*, 2021). The meta-regression of those that reported a polyphenol dosage showed no dose response relationship on the ergogenic effects of polyphenols. However, it must be noted that there was a very wide range of polyphenols sources included in this analysis which resulted in considerable heterogeneity in the resulting data (d'Unienville *et al.*, 2021). The vast majority of polyphenol interventions included in this meta-regression included dosages below 1000mg per day, so the results of this should be interpreted exclusively for these lower dosage ranges (d'Unienville *et al.*, 2021). There is one RCT to date which investigated the effect of high dosage polyphenols on endurance exercise performance, similar to

the current RCT. Trinity et al (2014) investigated the effect 1800mg pomegranate polyphenols per day for 7 days and failed to show an effect on a 10-minute cycling time trial performance following a preceding period of 50 minutes of moderate intensity cycling (Trinity *et al.*, 2014). The current evidence suggests that it is possible there may be a point of diminishing returns when it comes to polyphenol dosages, however, further research is required to understand whether this may be the case for high dosage polyphenol or whether it is the subclasses and timing frequently used in high dosage RCTs that has contributed to these results.

Polyphenols have a large variety of subclasses due to the definition of a polyphenol being any compound containing two or more phenolic ring structures (Panche, Diwan and Chandra, 2016). As such there are thousands of compounds separated into multiple subclasses, the precise composition of which has been shown to affect their physiological function (Pandey and Rizvi, 2009; Panche, Diwan and Chandra, 2016). Somerville and colleagues observed that quercetin supplementation, a flavonoid polyphenol found in onions, berries and green tea offered superior benefits to other sources of polyphenols (Somerville, Bringans and Braakhuis, 2017). Quercetin is a polyphenol subclass that is only present in small quantities in the PHPM used in this study which may have impacted the efficacy potential of the product. However, the PHPM was rich in the anthocyanin cyanidin-3-o-glucoside, a compound which is also present in Montmorency tart cherry extract (Keane *et al.*, 2016). Montmorency tart cherry products have been shown in a recent meta-analysis of 10 RCTs to enhance endurance exercise performance when consumed for between 7-days and 1.5 hours before exercise (Gao and Chilibeck, 2020). Anthocyanins have also shown to be effective in the form of New Zealand blackcurrant extract across a number of RCTs (Cook *et al.*, 2015, 2017; Murphy, Cook and Willems, 2017). However, the influence of polyphenol subclass may be more complex than just the predominant subclass present as subgrouping within a meta-analysis of food sources of polyphenols on endurance exercise performance showed significant benefits associated with Montmorency tart cherry consumption but not New Zealand blackcurrant or blueberries despite both being anthocyanin rich (d'Unienville *et al.*, 2021). Further investigations are required to elucidate the precise anthocyanin combinations and sources and their potential ergogenic effects. The PHPM used in this study also contained a large proportion of the ellagitannins Lambertianin C and Sanguiin H-6.

Trinity and colleagues in their placebo controlled RCT indicated no ergogenic benefit associated with 1800mg per day of pomegranate extract which was reported to contain ~95% ellagitannins for 7 days ahead of a cycling time trial (Trinity *et al.*, 2014). Similarly, acute pomegranate supplementation of 1000mg did not result in performance enhancement in high intensity cycling at altitude (Crum *et al.*, 2017) nor in repeated high intensity cycling sprints either (Roelofs *et al.*, 2017) suggesting perhaps ellagitannins may be inferior for supporting exercise performance compared with anthocyanins. However, a study investigating the effect of 1000mg of pomegranate extract capsules 30 minutes before running improved time to exhaustion (Trexler *et al.*, 2014). As such the role of ellagitannin rich polyphenol extracts require further investigation to determine whether they possess ergogenic benefits.

4.4 Timing of Polyphenol Ingestion

A narrative assessment of RCTs investigating the acute effects of polyphenol supplementation on endurance exercise performance suggests that the bolus should be consumed within 1 hours prior to the exercise bout (Bowtell and Kelly, 2019). Acute polyphenol ingestion 2 or more hours before exercise has been shown to be non-ergogenic in some RCTs (Crum *et al.*, 2017; Decroix *et al.*, 2017). However, the effect of timing of last polyphenol ingestion is poorly reported and under researched when it comes to chronic polyphenol treatments similar to that used in the current RCT (Somerville, Bringans and Braakhuis, 2017; Bowtell and Kelly, 2019). A systematic review and meta-analysis of food-based polyphenol consumption did not observe any difference in relation to the ergogenic effect during chronic multi-day interventions associated with the timing of last polyphenol ingestion (d'Unienville *et al.*, 2021). Pharmacokinetic studies into acute polyphenol ingestion show peaks in plasma polyphenols 1-2 hours following ingestion with a return to baseline by 12 hours following ingestion (Seymour *et al.*, 2014). However, following chronic strawberry polyphenol intake, plasma anthocyanins have been shown to remain elevated between 12 and 15 hours following last ingestion (Sandhu *et al.*, 2018). Anthocyanins have also been shown to have a biphasic response as a result of enterohepatic recirculation, with secondary release of polyphenols associated with subsequent meal ingestion due to the temporary storage of anthocyanins in the gall bladder and subsequent secretion upon release of bile due to fat ingestion (de Ferrars *et al.*, 2014; Zhong *et al.*, 2017).

In the present study, the last polyphenol ingestion was approximately 10-14 hours prior to exercise. Exercise did however follow breakfast which in theory could have contributed to a biphasic response similar to that observed in previous studies (Martínez-Huélamo *et al.*, 2016; Zhong *et al.*, 2017). However in this study we did not measure plasma polyphenols and as such cannot confirm the presence of this phenomenon. It is possible that the predominant action of polyphenols to support endurance action occurs during the acute 1-2 hours post ingestion peak of polyphenol absorption (Seymour *et al.*, 2014) and as such be a major reason why no ergogenic effect was observed in this RCT.

4.5 Pairing of Polyphenols with Liquid Milk

Numerous studies have investigated the impact of dietary proteins in relation to the bioaccessibility and bioavailability of polyphenols. Positive effects through the binding of polyphenols with protein molecules have been observed and enhance polyphenol stability (Jakobek, 2015; Quan *et al.*, 2019). A recent study indicates that anthocyanins from blackberry puree combined with dairy proteins were protected during in vitro digestion (van de Langerijt *et al.*, 2023). This potential enhanced effect of polyphenol digestion and absorption along with the beneficial effects for milk ingestion surrounding exercise were the rationale for the development of this food prototype.

The effects of combining polyphenols and milk blends are under researched in sports nutrition and this RCT represents the first to assess the blends effects on endurance exercise performance.

Although not in the realm of performance nutrition some research has investigated adding milk to polyphenol rich black tea and saw reduced antioxidant effects in FRAP analysis (Rashidinejad *et al.*, 2017). A number of these studies have shown a blunting of the vascular effects of tea polyphenols, measured via flow mediated dilation, predominantly catechins associated with the addition of milk to tea (Lorenz *et al.*, 2007; Van Der Burg-Koorevaar, Miret and Duchateau, 2011; Rashidinejad *et al.*, 2017). This may in part explain the lack of performance benefit observed with PHPM consumption in this RCT. However, these studies suggest such a blunting effect may result from interactions with milk proteins however this is in contrast with the findings

of two up to date reviews on the topic which conclude that milk can act as a beneficial vehicle for polyphenol delivery to parts of the gastrointestinal tract (Tosif *et al.*, 2021; van de Langerijt, O'Mahony and Crowley, 2023). Further research is required to elucidate the effects of polyphenols and milk combinations on the vascular effect on exercise.

4.6 Participants

It is worth noting that the specific population group can also impact the ergogenic efficacy of a nutritional intervention for endurance performance, particularly the training status of the participants. It is well established that exercise training can improve the ability of the body to resist ROS induced by exercise (Elokda and Nielsen, 2007; Powers *et al.*, 2022). As such it is feasible that the training status of these study participants may play a role in the efficacy of an antioxidant dependent mechanism of improving endurance performance. Other bioactive functional food products that rely on a nitrate dependant vascular action to improve exercise performance, such as beetroot juice have been shown to be less effective when used by well-trained athletes (Conger, Zamzow and Darnell, 2021; d'Unienville *et al.*, 2021; López-Samanes *et al.*, 2023). Although this is the case in beetroot interventions, the ergogenic effects of polyphenol interventions have been shown to be maintained in athletes with a VO_2max of 65ml/kg/min or more, a much higher level of training than was reported in the current RCT (d'Unienville *et al.*, 2021). As a result, the moderate training status of the participants in this RCT is unlikely to have impacted the response to polyphenol ingestion in this case.

There may also be a situation where there are responders and non-responders to ingestion of polyphenol interventions as considerable variation in the response was observed. It has been suggested that the response to pomegranate ellagitannin interventions, differing phenotypes may govern response to ellagitannin metabolism resulting in differing physiological responses (González-Sarrías et al., 2017). Given the rich ellagitannin content of blackberries this may explain the variability in response to the PHPM however, to date this has not been investigated in the context of exercise with much of the research restricted to cardiovascular risk factors (González-Sarrías et al., 2017). Further research is duly required in this regard.

4.7 Limitations & Future Directions

The relatively small sample size of this RCT may have contributed to the limited effect of study treatments on performance outcomes of this study, however the sample size is similar to that which has been used in many other parallel RCTs in this field, many of which have observed a significant effect of a polyphenol intervention on endurance performance outcomes (d'Unienville *et al.*, 2021).

While this study did include a baseline time trial prior to intervention period, this study did not include an extensive familiarisation procedure to ensure there was not a learning effect between TTpre and TTpost. Other similar studies have used multiple repetitions of performance measures such as time trials and time to exhaustion trials prior to the initiation of the intervention period to account for this (Eichenberger *et al.*, 2010; Morgan, Barton and Bowtell, 2019). Being familiar and more comfortable with the laboratory environment may explain the lower heart rate and lactate observed during TTpost however this did not translate into any performance differences due to study treatments.

In this RCT participants arrived at the laboratory in a fasted state and were provided a voucher a breakfast which was repeated identically on both days, specific contents of the breakfasts were not recorded. As such, while there was standardisation across the TTs within participant, there was not a standardised breakfast consumed across intervention groups. As such carbohydrate availability may have differed across groups as well as fat content which is known to impact polyphenol digestibility (Li et al., 2021).

This study required participants to follow a low polyphenol diet, specifically avoiding a selection high polyphenol food (Appendix 19). This acted to reduce the impact of potential confounding polyphenol intake from elsewhere in the diet. While this was deemed the appropriate approach given the proof-of-concept nature of this RCT, it may have reduced the ecological validity of the trial, exacerbating dietary polyphenol differences between groups beyond what would occur in a true free-living scenario.

Although there was extensive characterisation of the polyphenol content of the PHPM used in this study, a potential limitation is that this RCT did not assess plasma polyphenol concentrations or any biomarkers of polyphenol exposure. Research into

the use of dietary biomarkers and 'omics technologies to assess for polyphenol exposure represent an exciting avenue for future research in this field (Maruvada *et al.*, 2020). In particular these approaches may provide particular value to further understanding the in-vivo interactions of polyphenols within food matrices such as milk and the ultimate effect on bioactivity and bioavailability.

This RCT also did not assess any direct outcomes of the vascular response to polyphenol ingestion which has been accounted for in similar studies (Keane *et al.*, 2018; Morgan, Barton and Bowtell, 2019). Future studies should prioritise assessing outcomes related to vascular function such as flow mediated dilation, blood pressure and biomarkers of nitric oxide activity to provide a greater understanding of the action of polyphenols and their potential interaction with milk given the mixed findings of combining milk and polyphenols on their vascular effects (Rashidinejad *et al.*, 2017).

5. Conclusion

In conclusion enriching high protein milk with blackberry polyphenols did not augment exercise performance in a 15km cycling time trial. This is despite previous research indicating that polyphenol rich interventions confer a clear ergogenic benefit in such outcomes (Somerville, Bringans and Braakhuis, 2017; d'Unienville *et al.*, 2021). This is the first RCT to combine polyphenols in a milk matrix and investigate the potential effect on outcomes related to endurance exercise performance. It is also one of the few to conduct a polyphenol and exercise related RCT which fully characterises the polyphenol treatment in terms of total phenolic content and the most prevalent polyphenol subclasses. It has been previously demonstrated that milk may provide a potential protective effect on polyphenol stability and bioavailability through interactions with milk proteins and it was hypothesised that this may result in added benefits to performance (Tosif *et al.*, 2021; van de Langerijt *et al.*, 2023). However, there were no observed benefit to endurance performance in a 15km cycling time trial associated with consumption of a polyphenol enriched high protein milk. The lack of a beneficial effect may have arisen from any one or a combination of a number of factors.

- The prolonged time between the last polyphenol ingestion and the 15km time trial (10-14 hours) may have been a determining factor. Previous effects have

been observed with acute ingestion 1-2 hours prior to exercise (Trexler *et al.*, 2014).

- The high ellagitannin content of this intervention may have impacted on the results as flavonoid and anthocyanin polyphenols may provide greater benefit (Trinity *et al.*, 2014).
- The high polyphenol dosage used in this RCT represents a key difference to many of the previously reported RCTs and further research must be undertaken to elucidate the role of dosage on performance benefits.
- The incorporation of polyphenols into a milk matrix may have resulted in reduced polyphenol bioavailability. This has been observed with tea polyphenols but requires considerable further investigation (Lorenz *et al.*, 2007; Van Der Burg-Koorevaar, Miret and Duchateau, 2011).

Future research should prioritise gaining a greater understanding of the indicative dosage and timing regimens required to elicit ergogenic effects polyphenol interventions and how these may be impacted when polyphenols are incorporated within different food matrices or co-ingested with other nutrients. Based on the results of chapter 5, the polyphenol enriched high protein milk may provide muscular performance benefits in the recovery phase post intense exercise, however this RCT that while not in a recovery phase polyphenol enriched high protein milk shows no benefit to endurance exercise performance during a 15km cycling time trial.

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Chapter 7 – General Discussion

1. Impact of the Research

In the face of mounting challenges posed by environmental concerns, ensuring the sustainability and profitability of the Irish agrifood sector has become imperative. Traditional revenue streams are increasingly under threat, pushing the sector to seek innovative avenues to maintain its vitality. The Department of Agriculture, Food and the Marine have recently released the 'Food Vision 2030' strategy which aims to make Ireland a world leader in sustainable food systems by 2030. Key missions of this strategy discuss the development of value-added products from Irish agricultural outputs and enhance innovation and health outcomes of the food system (Gov.ie - Food Vision 2030 – A World Leader in Sustainable Food Systems). This PhD thesis sits well within this strategy by setting out clear guidelines to shape the future development of innovative value-added in the performance nutrition sector. The strength of Ireland's robust dairy, fruit and vegetable industries is ideal for new product development in this space. The potential is vast due to the considerable growth in the performance nutrition and functional food sectors in recent years and projected growth into the future (Grand View Research, 2022). This PhD thesis has created product development frameworks which address a fundamental need for comprehensive guidance to inform the creation of evidence-based, efficacious performance nutrition products that deliver genuine value to the end user.

In order assess the viability of these frameworks, an exemplar model of an evidence-based nutritional product was developed to support post exercise recovery and endurance exercise performance as part of a multi-disciplinary, multi-institution research project. This prototype took the form of a blackberry polyphenol enriched high protein milk with the aim of supporting recovery post exercise and endurance exercise performance. Through five core research chapters this PhD has envisioned, designed, developed and tested the efficacy of this nutritional product using these product development frameworks. The design of this product has meaningfully incorporated key aspects of nutritional science along with end-user engagement to ensure the product design is rooted in scientific evidence while also addressing key needs of the end-user. The processes outlined throughout this thesis should act as a model for the future design of nutritional products to support athletes and active individuals. Figures 1 & 2 outline how each of the core research elements of this thesis have addressed the steps involved in the product development frameworks.

2. Summary of Key Findings

2.1 Chapter 2

In chapter 2, the scientific literature was assessed for the evidence underpinning the proposed claims that milk protein and polyphenols may be beneficial to athletes. Dairy protein has been repeatedly shown to enhance the repair and regeneration of skeletal muscle in response to exercise through shifting the balance between muscle protein synthesis and breakdown. Evidence suggests that those undertaking regular physical exercise, particularly resistance training, benefit from consuming between 1.2-1.6g/kg/day of high-quality protein (Phillips, 2007, 2012). This should be consumed evenly across ~4 boluses per day and further benefit may be attained through pre-sleep consumption (Stark *et al.*, 2012). The protein in bovine milk provides a highly digestible and leucine rich source of protein which may be near optimal for athletes and active individuals (Roy, 2008; Dupont and Tomé, 2020). It was also ascertained that plant derived polyphenols have been investigated with regards recovery from exercise induced muscle damage (EIMD). Many of the resulting randomised controlled trials (RCTs) have shown promising results in measures of recovery of muscle strength and muscle soreness (Howatson et al., 2010; Haramizu et al., 2013; Bell et al., 2015; Quinlan and Hill, 2020). A key meta-analysis also showed that consumption of polyphenols pre-exercise improves exercise performance (Somerville, Bringans and Braakhuis, 2017). Despite the promising results for both polyphenols and protein in isolation, very limited investigations have investigated these compounds in combination.

2.2 Chapter 3

The goal of chapter 3 was to engage the prospective end-user and to involve them in the new product development process through assessing their current nutrition practices, identifying their key nutritional priorities and evaluating their product preferences. This research enabled key understanding of the needs and wants of athletes and active individuals to inform the formulation of the exemplar new product.

This was the first scientific investigation published in peer-reviewed journal to assess the product preferences and the nutritional priorities of athletic groups within the

context of informing new product development in performance nutrition. The key findings of this study were the identification of muscle recovery as the number one priority of this population and that current practices used to support recovery were often largely lacking an underpinning scientific evidence base. This study also identified a clear desire for food product forms over supplement forms, providing further evidence for a food-first approach to performance nutrition where possible (Burd *et al.*, 2019; Close *et al.*, 2022). This population demonstrated a particular preference for product forms such as smoothies, juices, bars and hot foods. This information provides valuable context for the development of this exemplar model and can be easily applied to future ventures.

2.3 Chapter 4

Chapter 4 aimed to assess key gaps within the scientific literature regarding the efficacy of polyphenols to support recovery from EIMD. While many RCTs had investigated the effect of specific polyphenol rich extracts there was yet to be a systematic review and meta-analysis of polyphenol rich interventions containing flavonoids and their impact on EIMD. Given that many of the RCTs in this field have small sample sizes due to the costly and time-consuming nature of post exercise recovery RCTs, systematic reviews and meta-analyses are essential to discern overall efficacy. This study was the first to quantitatively assess the potential impact of such interventions on functional recovery of muscle strength and post exercise muscle soreness, building on previous meta-analysis which reported positive results in post exercise oxidative stress response (Bloedon *et al.*, 2019). From the 26 RCTs included in the meta-analysis, significant benefits were observed in recovery of muscle strength over the 96 hours post intensive exercise and reduced muscle soreness over the same period. Another notable finding of this systematic review was that many of the included RCTs exhibited very poor characterisation of the polyphenol subclasses and the dosage in the intervention products used in their protocols. Given the considerable variety of polyphenol subclasses that have been identified (Panche et al., 2016), and that different polyphenol subclasses have varying biological activity (Manach *et al.*, 2018), greater characterisation and classification of polyphenol products used in RCTs is required to develop specific recommendations of optimal sources and dosing strategies.

2.4 Chapter 5

Chapter 5 outlines the undertaking of a proof-of-concept RCT to assess the efficacy of consuming the RubusElite beverage prototype for 10 days on post exercise recovery from exercise induced muscle damage. This RCT used EFSA recommended study outcomes such as test-retest muscle strength and perceived soreness in the days following a single intense bout of exercise (Turck *et al.*, 2018). The results of this study indicate that consuming the RubusElite prototype beverage for 10 days, enhanced muscle recovery compared to a high protein milk and a low protein control milk. It significantly enhanced recovery of muscle strength greater than a control low protein milk and significantly reduced muscle soreness compared to both a high protein milk and low protein milk in recreationally active male participants. However, there was no significant improvement in oxidative stress associated with consumption of the RubusElite prototype beverage. A strength of this study was the precise characterisation and quantification of the polyphenols present in the RubusElite prototype beverage, led by key collaborators from the UCC Food Science department and Teagasc Food Research Centre Ashtown. The primary subclasses present in the beverages were ellagitannins and anthocyanins and that the polyphenol content of the beverage was ~1400mg per daily serving. This information combined with key outcomes related to post exercise recovery provide valuable information to inform future recommendations and allow for greater comparison with further RCTs conducted. This RCT was also the first of its kind to show that the benefits of polyphenols to support post exercise recovery are at minimum maintained when provided within a dairy food matrix.

2.5 Chapter 6

Finally, chapter 6 discusses the findings of the RCT which investigated the ability of the RubusElite prototype beverage to enhance endurance performance during a 15km cycling time trial in recreationally active males. Like chapter 5, this RCT adheres to the EFSA recommendation for study outcomes to assess muscle performance claims by using a task dependant performance measure (Turck *et al.*, 2018). This was a secondary outcome of the overall RubusElite RCT. This study observed no significant impact of the RubusElite beverage on total time to complete 15km or on physiological

measures of exertion such as lactate and heart rate during exercise. There was also no effect observed of consuming the beverage on perceived exertion during the exercise test. The precise characterisation of the polyphenols as discussed above has facilitated greater understanding of the possible reasons for the limited effect observed and would allow for more targeted reformulation of the product.

Role of Sports Nutrition in Evidence-Based Sports Food Product Development

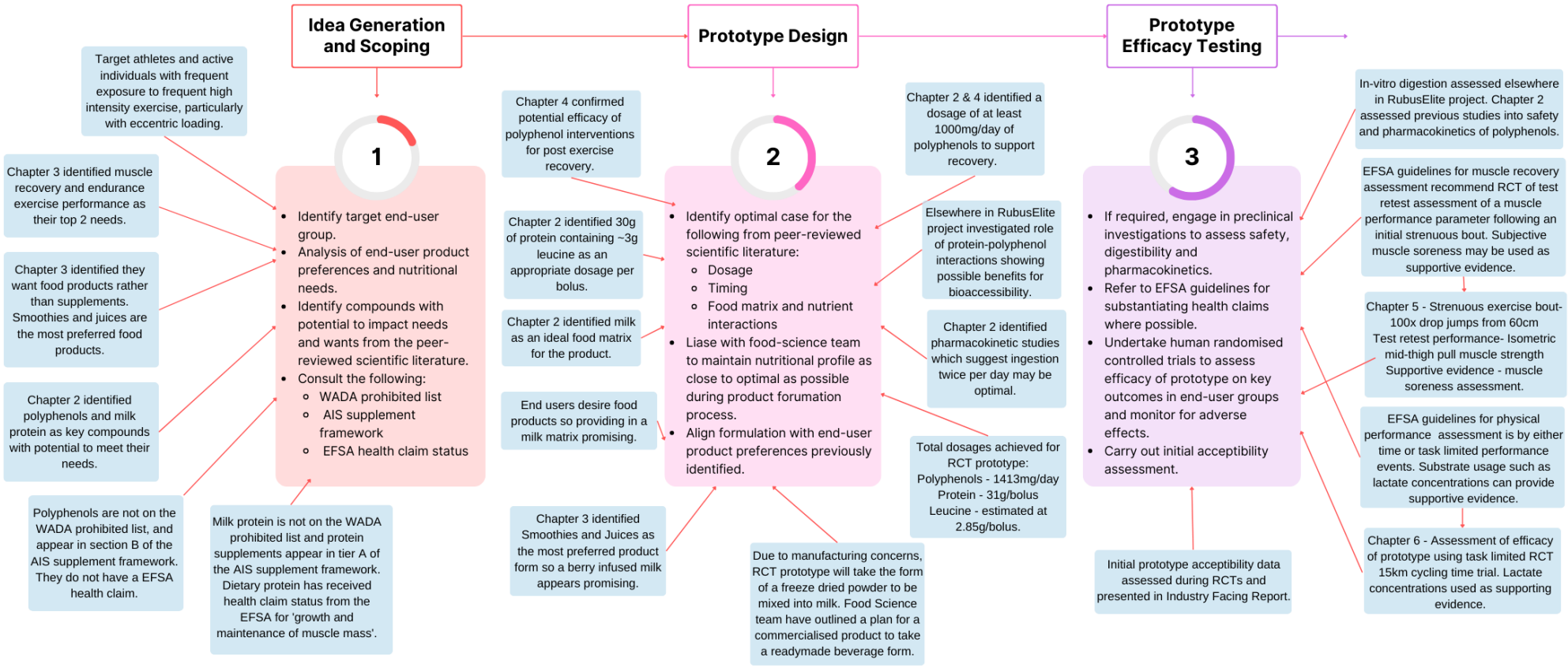


Figure 1. Schematic of how this PhD thesis has addressed each of the key new product development considerations set out at the start of the thesis.

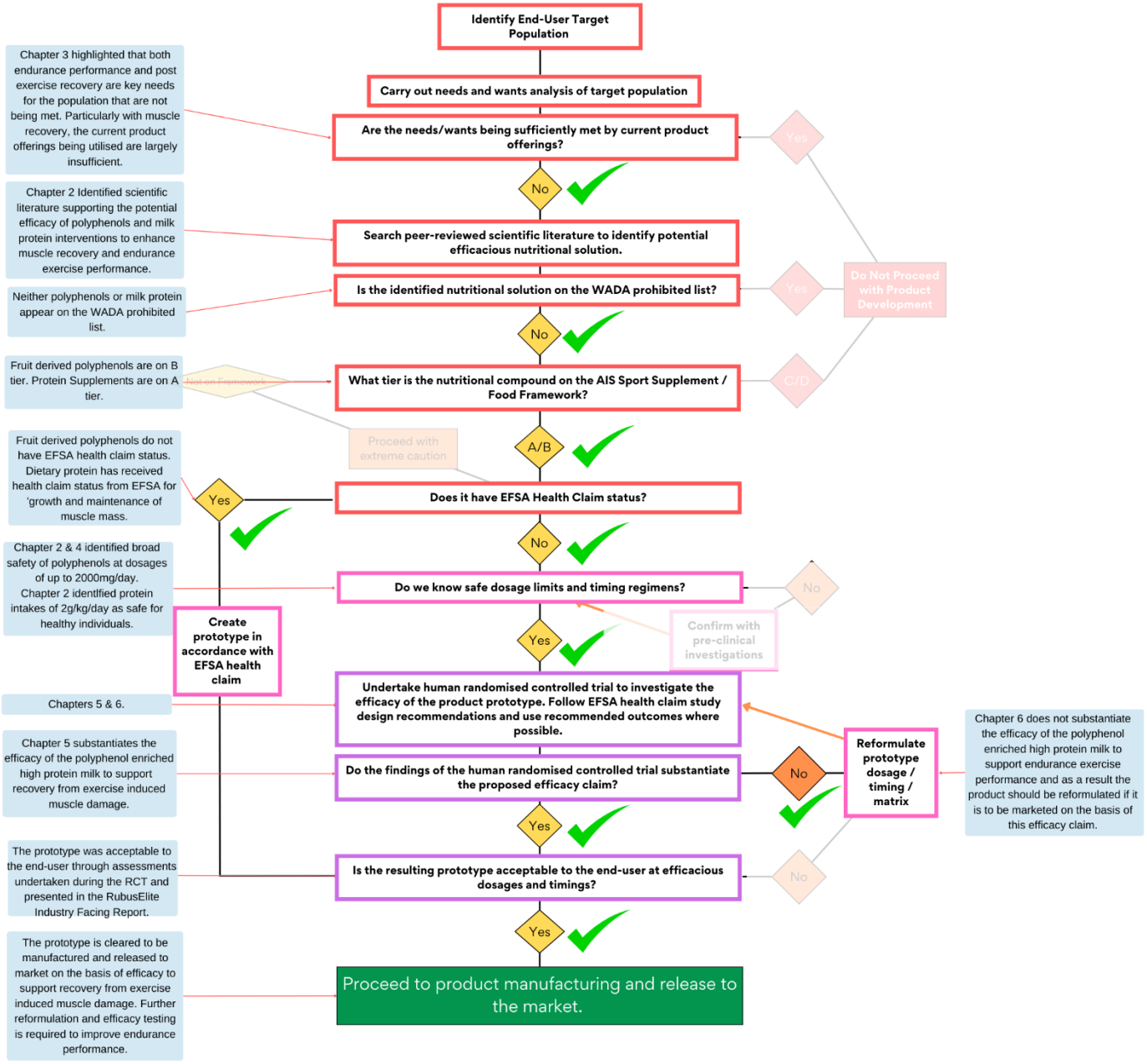


Figure 2. Schematic of how each decision was made through the work undertaken throughout this PhD thesis.

3. Research Dissemination

Alongside the core scientific research tasks in this project there was a concerted effort to disseminate the research findings and principles of the project to major stakeholders outside of research and academia. The three main goals of the dissemination plan of this PhD project:

1. Promote evidence-based insights and the importance of scientific evidence to the end-user of performance nutrition products.
2. Provide evidence-based insights into performance nutrition new product development for the food and agriculture industry.
3. Create opportunities for engagement and communication between food industry, end-user groups, practitioners and academics to foster future innovation and value.

It was deemed essential to promote evidence-based practice in sports nutrition to the end-user and lay individual to promote the importance of nutritional science in the practice of the end-user. As a result, alongside the research, key findings and hot topics were disseminated via custom designed infographics on a dedicated social media page for the RubusElite project (examples in Appendix 2).

It is also important that the findings of this research are communicated to members of the food industry and agri-food sector in an engaging manner. To achieve this, an 'industry facing report' of key findings was created to disseminate to this highly specified community (Appendix 1). This industry facing report was sent to a large database of industry representatives across Ireland through the Food Industry Training Unit at UCC. To promote further innovation in the performance nutrition landscape by the Irish agrifood sector.

Consistent engagement between end-users, nutrition practitioners, industry and academics is a critical determinant for success. We organised the RubusElite Performance Nutrition Forum (June 16th, 2023) which brought together members of the food industry, academics and sport and exercise personnel from across the country (Appendix 3.). Hard copies of the RubusElite Industry Facing report were also distributed to attendees of this event.

4. Future Perspectives

4.1 Performance Nutrition Regulation and New Product Development

The new product development framework delineated in this thesis, while not perfect, optimally utilises the tools, supports, and resources presently available. While more centralised regulation and legislation may offer enhanced guidance in the future, the current framework represents a pragmatic and informed approach, while providing the potential for refinement as the landscape evolves.

Possible first steps for regulation in this space include greater legal oversight of manufacturing standards. To date this responsibility is solely with the company themselves to sign up to manufacturing standards certificates beyond general food safety regulations. Minimum standards for product manufacturing, batch testing and raw material sourcing will lead to safer, more trustworthy and efficacious products to support performance nutrition practices.

Labelling regulation can also be created for performance nutrition efficacy claims. While EFSA health claims provide a high standard for assessing the efficacy of claims, given the fast-moving pace of performance nutrition and the constant desire of athletes to gain an advantage over the competition, a tiered system such as the AIS framework may prove a more appropriate system in this space. Hypothetically, this type of tiered system could be added to labels in a traffic light type system along with a short note on the type of athlete/exercise activity it may support and a centralised, evidence-based recommendation for an effective and safe dosage.

There is considerable scope for consumer investigations in the field of performance nutrition, at least in the publicly available scientific literature. It is well established that the field of food choice and food behaviour change has an added layer of complexity in athletic populations due to sporting culture and norms being added to the wider demands of the modern food space (Birkenhead and Slater, 2015). While chapter 3 of this thesis represents a step forward in this regard there is still much to be ascertained. This was a cross-sectional survey; however it is entirely plausible that some athletes may respond to such a survey in one way but behave differently at the point of purchase. Investigations which directly assess the purchasing practices of sporting individuals are duly warranted. The RubusElite survey clearly showed that

athletes and active individuals have a strong preference for food-based products which aligns well with global recommendations for food first approaches.

4.2 Polyphenols and Exercise Performance and Recovery

The scientific evidence is clear at this stage that polyphenols have strong potential to improve post exercise recovery and endurance exercise performance. Along with chapter 4 of this thesis, there have since been further systematic reviews and meta-analyses which also support the recovery enhancing effects of polyphenol rich foods, extracts and supplements (Bloedon *et al.*, 2019; Doma, Gahreman and Connor, 2020; Hill *et al.*, 2021; Rickards *et al.*, 2021; Martín-Castellanos *et al.*, 2022). There are also multiple systematic reviews and meta-analyses which support the use of polyphenols to support exercise performance (Somerville, Bringans and Braakhuis, 2017; Blake *et al.*, 2021; d'Unienville *et al.*, 2021). Despite this, there are still considerable difficulties in providing dietary recommendations for athletes and for providing clear direction for product development due to the poor characterisation of polyphenol subclasses and dosages. There is also poor understanding of the mechanisms of action of polyphenols in supporting exercise performance and recovery. While polyphenols have been strongly suggested to influence the gut microbiome (Sorrenti *et al.*, 2020) and as a result transcription factors governing endogenous antioxidant enzymes (Zhou *et al.*, 2019; Kanner, 2020), this has yet to be demonstrated in human exercise RCTs. Future investigations harnessing 'omics technologies to understand the mechanisms of action of polyphenols during and after exercise are needed.

While the ubiquitous presence of polyphenols in commonly consumed foods places them as strong candidates for new product development in this space, particularly given the recommendations for food-based approaches, future research should prioritise greater understanding of appropriate dosages and subclasses for specific physiological actions and mechanisms of action rather than just prioritising assessing efficacy. Greater knowledge underpinning these factors will allow for more targeted and efficacious product development ventures.

D'Unienville et al., (2021), as part of their systematic review and meta-analysis has conducted extensive subgroup analysis to determine whether exercise mode, duration and participant groups result in different ergogenic effects in endurance performance

events. They reported that performance enhancement occurs in exercise lasting between 10 and 60 minutes and is most pronounced in graded exercise tests. They also demonstrated significant benefits in males but not females, however further investigations with female participants are warranted. Further research is required that assesses the potential role of these population and exercise related factors on the efficacy of polyphenols for recovery from exercise induced muscle damage. However, to date the poor reporting of study dosages and compositions have considerably hampered any attempted investigations due to the inability to discern whether any observed difference is due to supplementation regimen or other factors related to exercise or the population being studied.

4.3 Protein and Performance Nutrition

The benefits of protein for muscle along with exercise training and competition is well established and has been verified with an EFSA health claim for muscle growth and maintenance (Agostoni *et al.*, 2010). There is a well understood mechanism of action and clear guidelines for dosage and timing regimens (Tipton and Witard, 2007; Tang and Phillips, 2009; Deutz *et al.*, 2014; Kanda *et al.*, 2016). Protein supplements are a clear leader in the space however with the growth in the functional foods sector (Knapik *et al.*, 2016; Garthe and Maughan, 2018), there is further space for innovation with protein rich foods for the performance nutrition market. Dairy protein is currently the most efficacious protein source due to amino acid composition and digestibility however further innovation is warranted in sustainability of protein sources (Vliet, Burd and van Loon, 2015; Gorissen and Witard, 2018). Future research should prioritise alternative sustainable proteins with close to optimal physiological function (Gorissen *et al.*, 2018). These can come from alternative sources such as plant protein blends or potentially dairy or animal food waste products.

4.4 Polyphenols and Protein

Incorporating polyphenols within differing food matrices is a promising avenue for future innovation. Given the recent evidence showing the potential role of protein in polyphenol digestion (Tosif *et al.*, 2021; van de Langerijt, O'Mahony and Crowley, 2023), protein rich food matrices appear to be a strong candidate for polyphenol rich

performance foods. This thesis adds valuable knowledge to a largely underexplored area by showing that a polyphenol enriched high protein milk improved post exercise recovery from exercise induced muscle damage. The previous research investigating polyphenol protein blends is sparse, particularly dairy protein, despite the potential synergistic physiological efficacy (Nieman *et al.*, 2013; Ahmed *et al.*, 2014; Pavis *et al.*, 2022). Future research is required to expand on the potential benefits of combining polyphenols and protein and polyphenols within other food matrices.

Additionally, further investigations are required to understand the specific reason by which this polyphenol dairy protein beverage did not support endurance exercise performance despite the evidence supporting the use of isolated polyphenol interventions for endurance performance. Reformulation of the product prototype and further RCTs are required to identify whether this was a result of the timing of ingestion, interaction with milk or the dosage used influenced the results of this RCT.

Further research is also required to understand the impact of other food matrices on the ergogenic effects of polyphenols. Fat rich matrices may provide an interesting avenue for future investigation as they have also been shown to potentially benefit to digestibility (Ortega *et al.*, 2009; Martínez-Huélamo *et al.*, 2016). Conversely however co-ingestion with certain foods such as bananas may degrade polyphenols due to the presence of polyphenol oxidase (Ottaviani *et al.*, 2023). This is an area ripe for investigation.

4.5 Real-World Applications of the RubusElite Beverage Prototype

The results of the proof-of-concept RCT presented in chapter 5 support the use of the RubusElite product prototype to enhance recovery from EIMD. This suggests that the blackberry polyphenol enriched high protein milk could be a valuable nutritional tool for athletes and active individuals, particularly those undertaking intensive exercise which contains eccentric loading of skeletal muscle (Clarkson et al., 1986). This is particularly important in sports containing large volumes of decelerations, landing loads and repetitive lowering of a weight (Proske & Morgan, 2001). It will also be particularly important for athletes in sports that have intensive periods of multiple events in short periods of time. One example of this could be field hockey, a sport which contains considerable deceleration loading during competition and major

competition structures require athletes to compete in multiple matches often on consecutive days (Chesher et al., 2019). The use of this polyphenol enriched high protein milk could result in greater muscle strength and reduced muscle soreness for subsequent competition bouts.

However, while this prototype can be effectively applied to periods of intensive competition, it should be cautioned that future research is required prior to use during intensive training periods in which adaptation to the exercise stimulus is the primary goal. Previous research has shown that high dose antioxidant supplementation blunts exercise adaptation (Bjørnsen et al., 2016; Gomez-Cabrera et al., 2015). While it has been suggested that the different mechanism of action of polyphenols compared to antioxidant vitamins may nullify these concerns, until further research categorically rules this out, high dose polyphenol supplementation should be avoided when adaptation is the primary goal.

5. Conclusions

This thesis has contributed significantly to the field of performance nutrition and food innovation. The findings of this thesis provide valuable knowledge for a wide range of stakeholders including members of the agrifood sector, performance nutrition practitioners, end-users of performance nutrition products and academics in the fields of nutrition, food science and exercise physiology. In conclusion the key outcomes of this thesis include:

- It has designed and assessed the viability of a new framework to inform evidence-based design and development of future performance nutrition products.
- It also presents a new phase-gate decision making model which will enable the efficient screening of product concepts and identification of those concepts with evidence of efficacy versus those lacking a scientific evidence base.
- It has also used these frameworks to develop an exemplar model of a bioactive functional food, specifically a blackberry polyphenol enriched high protein milk which has shown efficacy in supporting post exercise recovery from exercise-induced muscle damage.

- It has developed key consumer knowledge to direct future product innovation, in particular the clear consumer desire for food products to support post exercise recovery.
- It has confirmed and quantified the potential for polyphenol interventions to support post exercise recovery from exercise induced muscle damage through the publication of a key systematic review and meta-analysis.
- It has added further context to the efficacy of polyphenols in performance nutrition by combining recommended RCT procedures with extensive characterisation and quantification of polyphenol subclasses.

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Appendices

Dissemination Appendices

Appendix 1 RubusElite Industry Facing Report

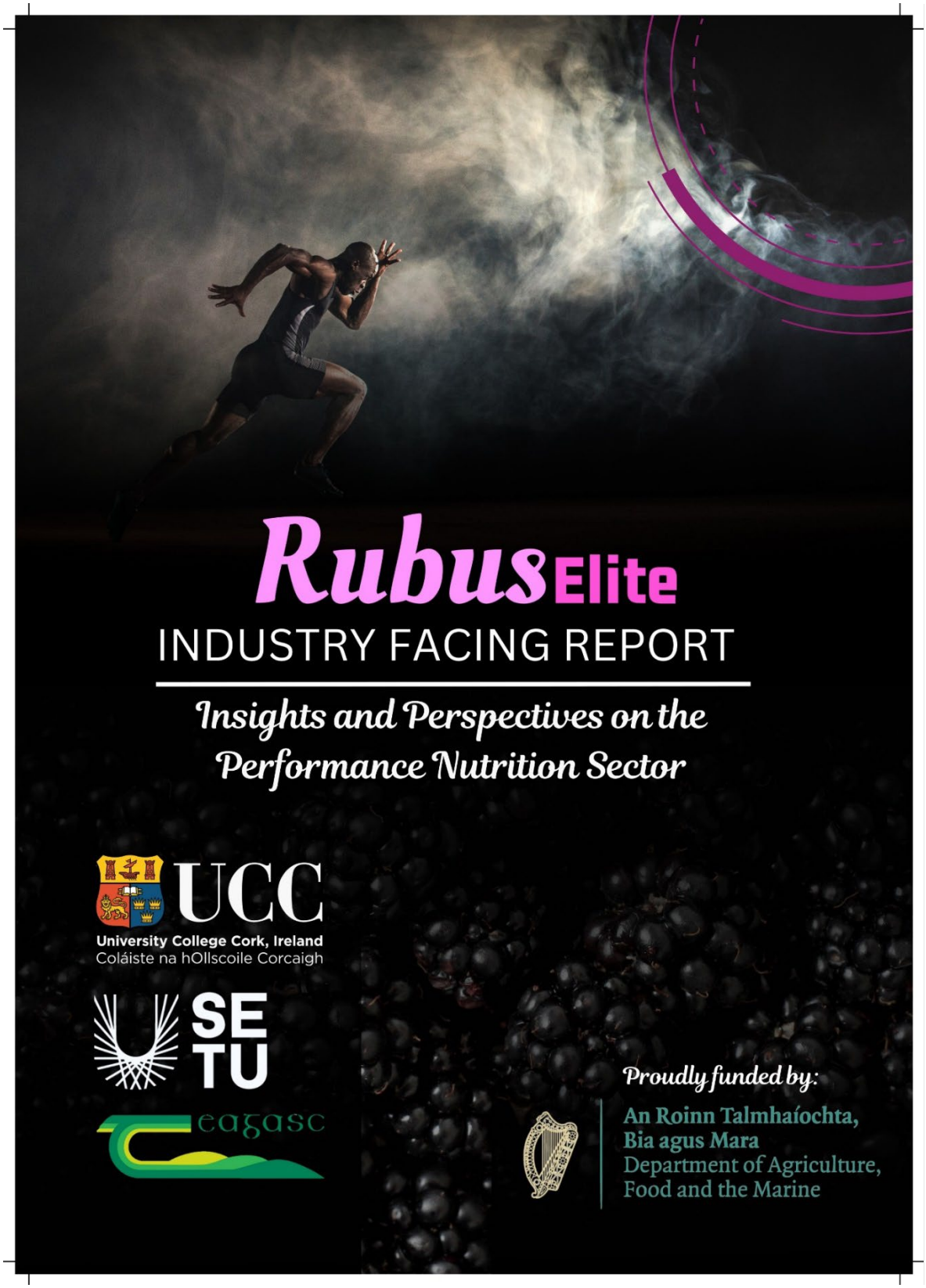


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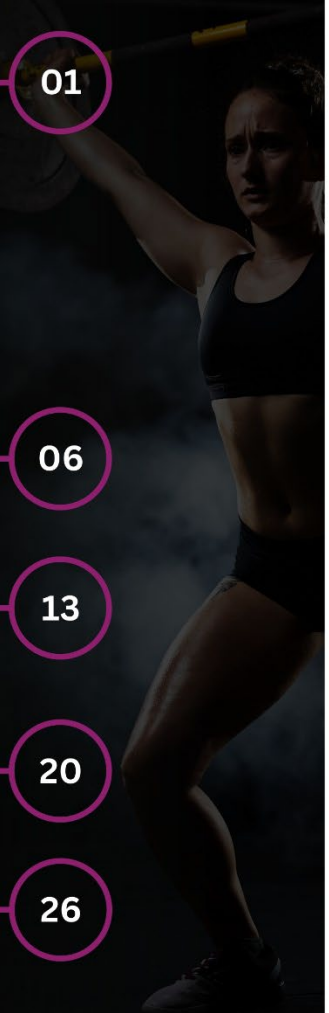


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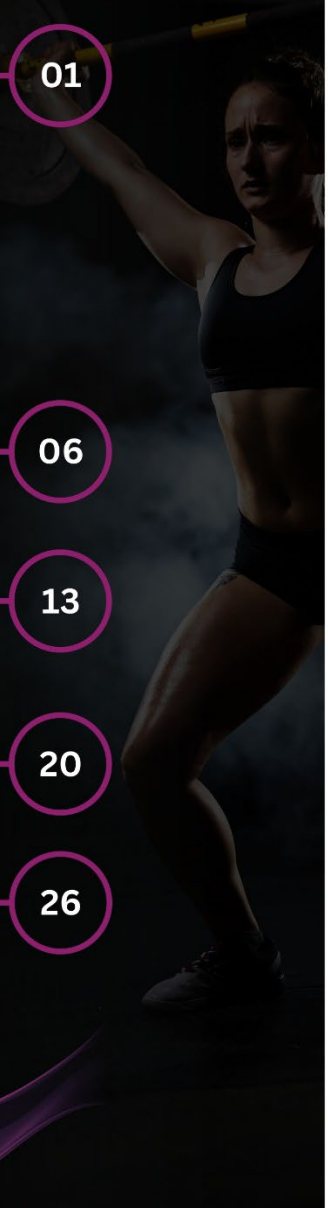
Functional Foods 05

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RubusElite
INDUSTRY REPORT

This industry-facing report serves as a comprehensive conduit for conveying the wealth of scientific insights derived from the RubusElite project to relevant stakeholders in the performance nutrition sector. Its primary goal is to foster an enriched understanding of the interplay between cutting-edge scientific research and food product innovation, thereby promoting the creation of nutrition solutions that are both scientifically robust and consumer-preferred.

Drawing from a range of research methodologies, the report provides a roadmap for aligning the needs of athletes with scientific research, allowing for the translation of these insights into real-world food product innovation. It translates the learnings from a systematic review, end-user engagement survey, and randomised controlled trial into actionable insights that can guide future food product development.

In essence, this report aims to bridge the gap between science and industry, facilitating the evolution of performance nutrition to not only meet the demands of the market but to redefine them based on evidence-based nutrition science.

02



Rubus Elite
THE TEAM

UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

School of Food and Nutritional Sciences

APC Micribiome Ireland

teagasc

Teagasc Food Research Centre Ashtown

SETU
Ollscoil Teicneolaíochta an Oirdheiscirt
South East Technological University

Department of Sport and Exercise Science

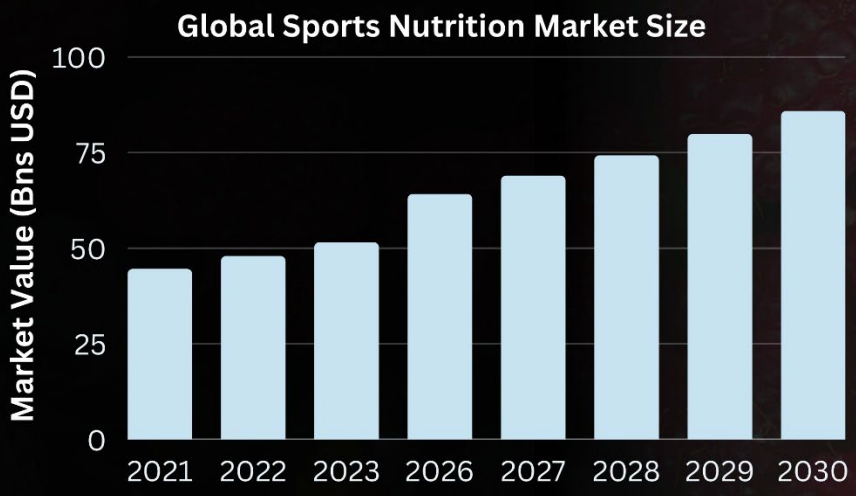
An Roinn Talmhaíochta, Bia agus Mara
Department of Agriculture, Food and the Marine

Funded by Department of Agriculture Food and the Marine, Food Institutional Research Measure



03

PERFORMANCE NUTRITION
STATE OF PLAY



A report from Grand View Reseach has projected a compound annual growth rate of 7.4% between now and 2030

Supplements make up the largest proportion of the market share at 42.3%

Grand View Research 2022

04

FUNCTIONAL FOODS
THE FUTURE?

The functional foods market size was estimated at 280bn USD in 2021 and has a projected CAGR of 8.5% between now and 2030.
(Grand View Research, 2022)

This benefit of a food-first approach arises from the interdependence of food constituents to derive the greatest benefit to human physiology, especially with regards to nutrient digestion and absorption.

Many sports nutrition practitioners recommend supplementation when it is the only option, due to the risk of inadvertent supplement contamination and risk of doping.

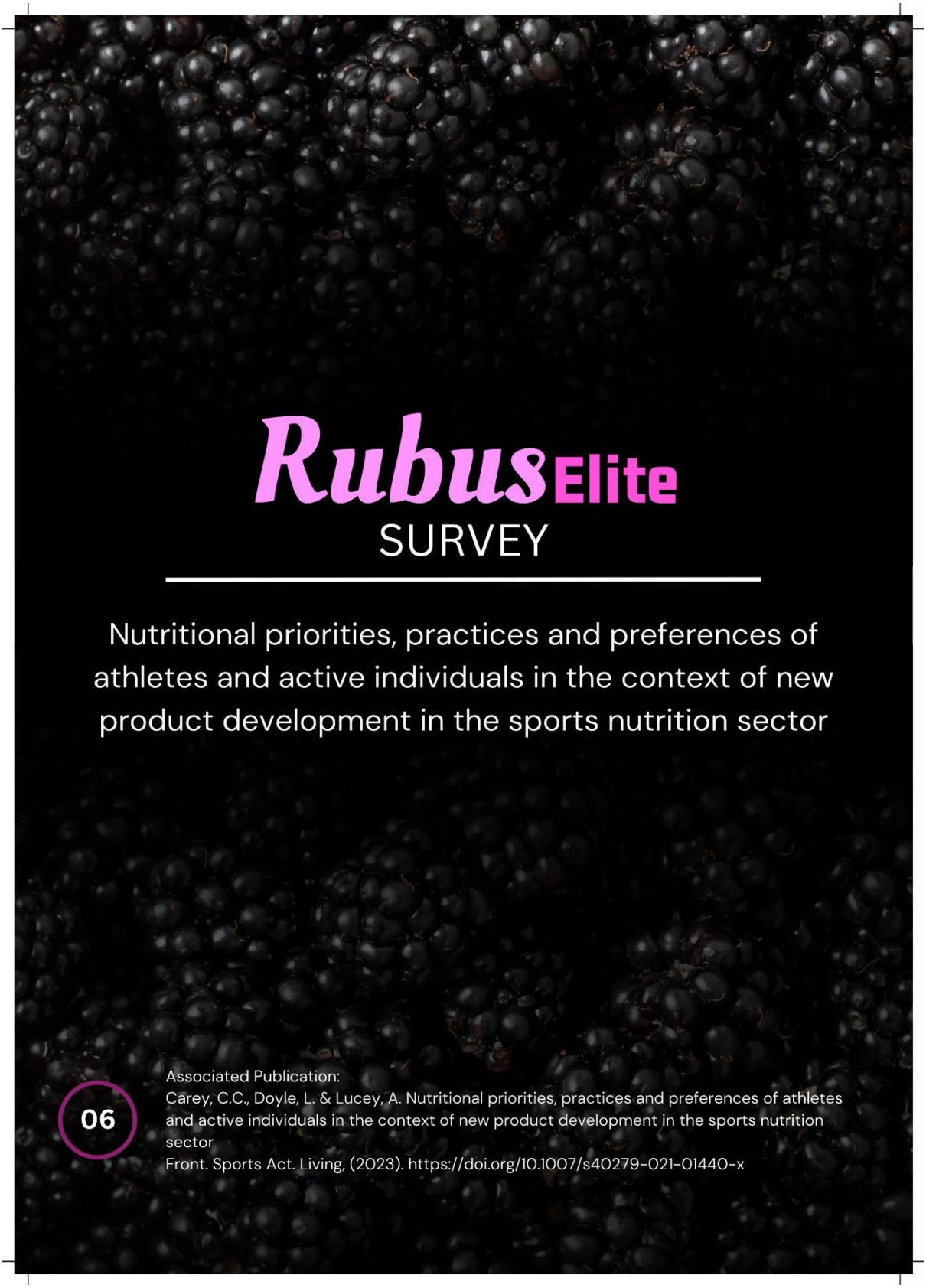
SPORT IRELAND ANTI-DOPING

"Consideration of using a supplement should not be based on product marketing claims. Adopt a food first philosophy, not supplement first, to maximise health, safety and performance"

05

Sports Nutrition Market Size Worth $82.3 Billion By 2030. (Grand View Research 2022)
https://www. grandviewresearch.com/press-release/global-sports-nutrition-market

Functional Foods Market Size Worth $586.1 Billion By 2030. (Grand View Research 2022)
https://www. grandviewresearch.com/press-release/global-functional-foods-market


Rubus Elite
SURVEY
Nutritional priorities, practices and preferences of athletes and active individuals in the context of new product development in the sports nutrition sector
06
Associated Publication:
Carey, C.C., Doyle, L. & Lucey, A. Nutritional priorities, practices and preferences of athletes and active individuals in the context of new product development in the sports nutrition sector
Front. Sports Act. Living, (2023). https://doi.org/10.1007/s40279-021-01440-x



Rubus Elite SURVEY

frontiers in Sports and Active Living

ORIGINAL RESEARCH article

Front. Sports Act. Living, 07 February 2023
Sec. Sport and Exercise Nutrition
Volume 5 - 2023 | https://doi.org/10.3389/fspor.2023.1088979

This article is part of the Research Topic
Women in Sport and Exercise Nutrition: 2022
View all Articles ›

Nutritional priorities, practices and preferences of athletes and active individuals in the context of new product development in the sports nutrition sector

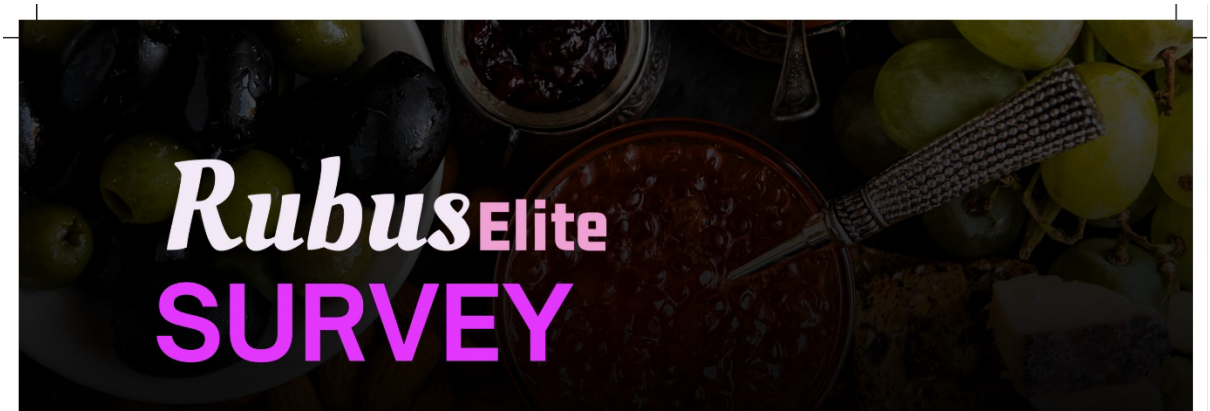
405 athletes and active individuals on the island of Ireland completed an online survey between March and May 2021

Goals of the survey

To gain an evidence-based understanding of the following:

- The current nutritional practices of this cohort of individuals
- Their perceived nutritional priorities
- To identify key preferences and desired attributes for a sports nutrition food product

07



Rubus Elite SURVEY

WHAT IS THE SURVEY?

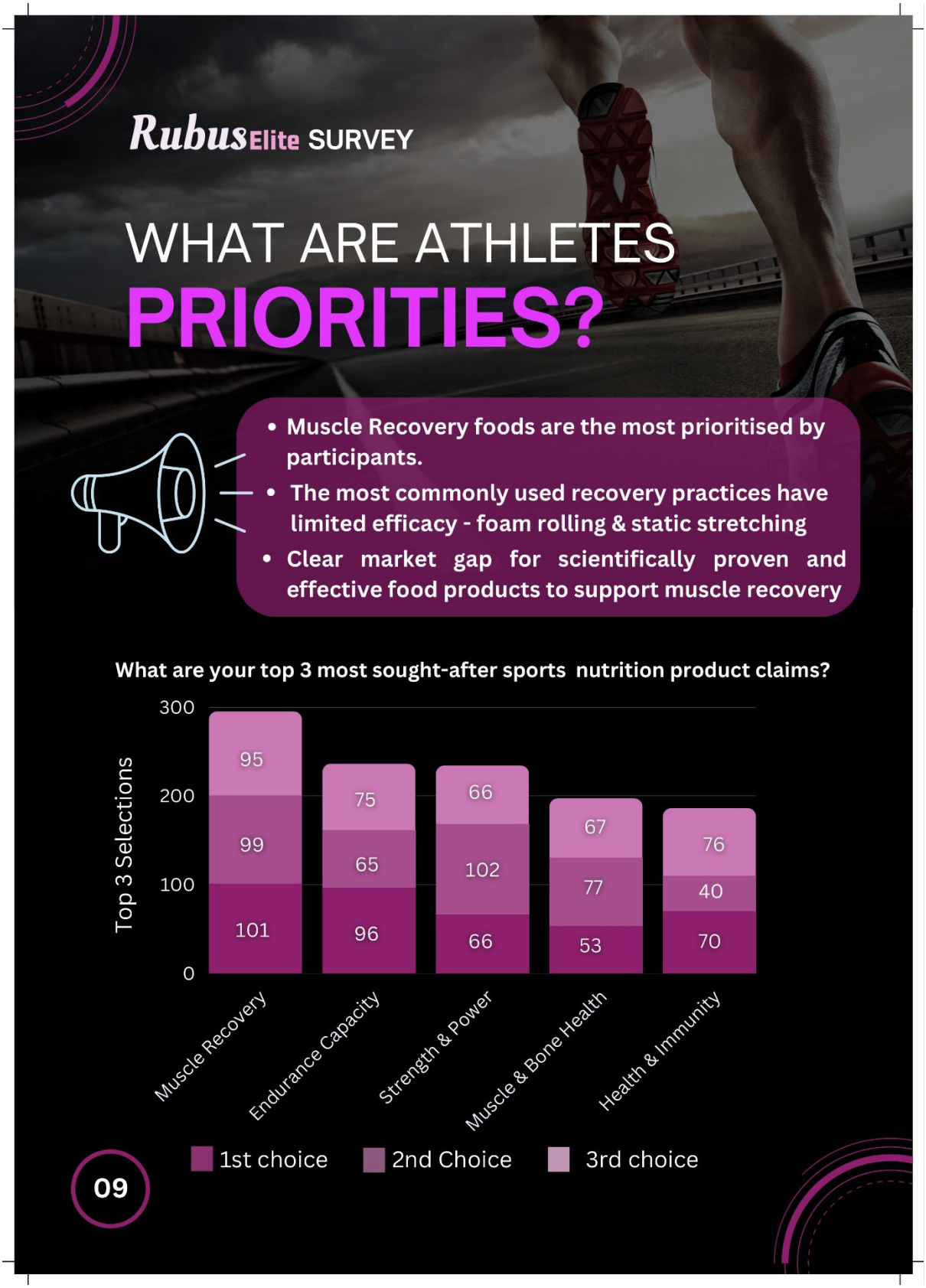
- This comprehensive purpose built survey was designed to collect qualitative and quantitative data on current practices, nutritional priorities and food product preferences in performance nutrition.
- 35 questions were derived on the basis of information gathered from two exploratory focus groups

HOW WAS IT CONDUCTED?

- Participants were obtained through various sources including public advertisement, social media and university wide email lists between March and May 2021.

Who were the Participants? (n=405)	
Sex	Male - 60% Female - 40%
Average Age (Range)	29 years (18 to 64)
Average Total Activity (hours per week)	10.3
Level of competition	International - 8% National - 14% Regional - 18% Local - 33% Not Competitive - 27%

08


Rubus Elite SURVEY
WHAT ARE ATHLETES
PRIORITIES?
• Muscle Recovery foods are the most prioritised by participants.
• The most commonly used recovery practices have limited efficacy - foam rolling & static stretching
• Clear market gap for scientifically proven and effective food products to support muscle recovery
What are your top 3 most sought-after sports nutrition product claims?
Top 3 Selections
300
200
100
0
95
99
101
75
65
96
66
102
66
67
77
53
76
40
70
Muscle Recovery
Endurance Capacity
Strength & Power
Muscle & Bone Health
Health & Immunity
1st choice
2nd Choice
3rd choice
09

Rubus Elite SURVEY

WHAT ARE ATHLETES CURRENT PRACTICES?

52% of participants use nutritional supplements.

31% of participants use **protein** supplements. These are the most commonly used sport and exercise related supplements.

28% and *23%* of participants use **Multivitamins** and **vitamin C** respectively**.** These are the most commonly used health related supplements.

Other than **caffeine** containing functional foods (coffee), other scientifically proven and effective functional foods are poorly utilised by participants.

- **Polyphenols** (tart cherry, green tea etc.) - *6%*
- **Nitrates** (beetroot juice) - *4%*



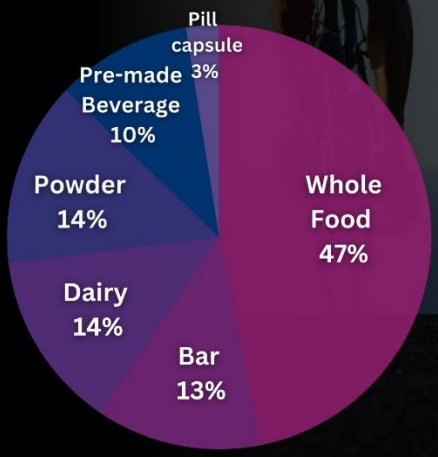
10

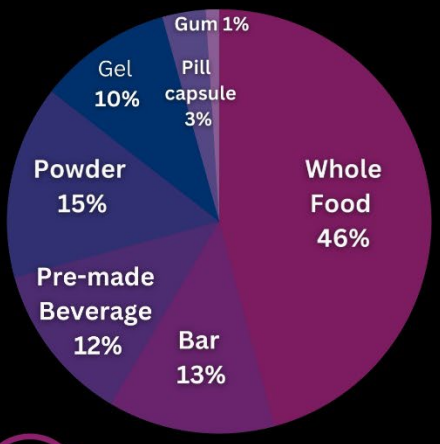
Rubus Elite SURVEY

WHAT ARE ATHLETES PRODUCT PREFERENCES?

Athletes overwhelmingly report 'Whole Food' products as their most preferred performance and recovery enhancing products

Athletes most preferred form of sports nutrition **recovery** product





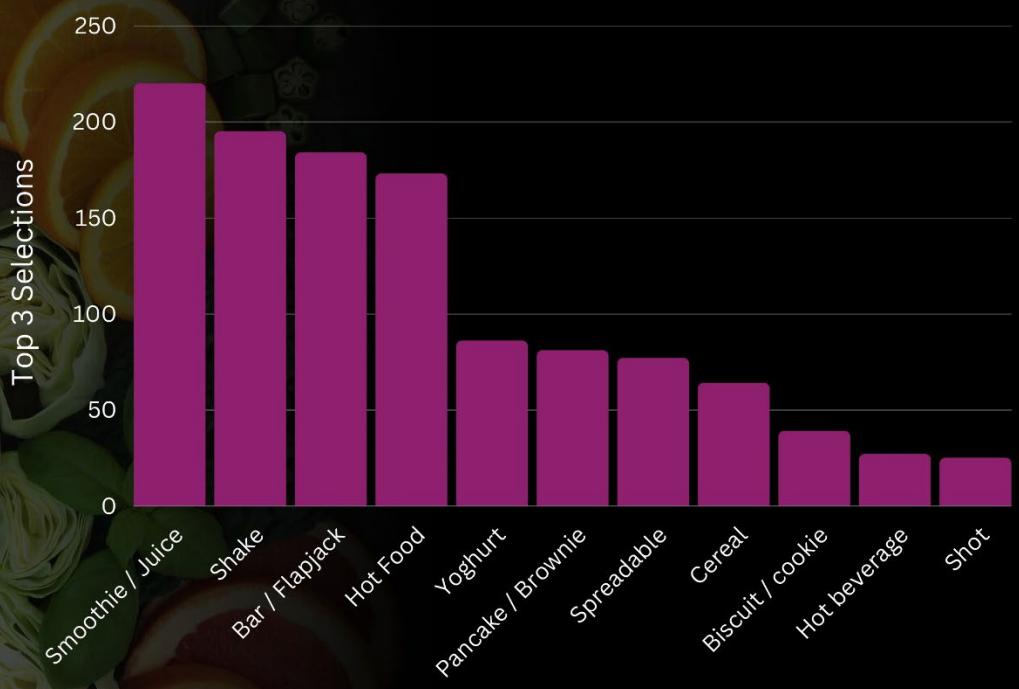
Athletes most preferred form of **performance** enhancing sports nutrition product

11

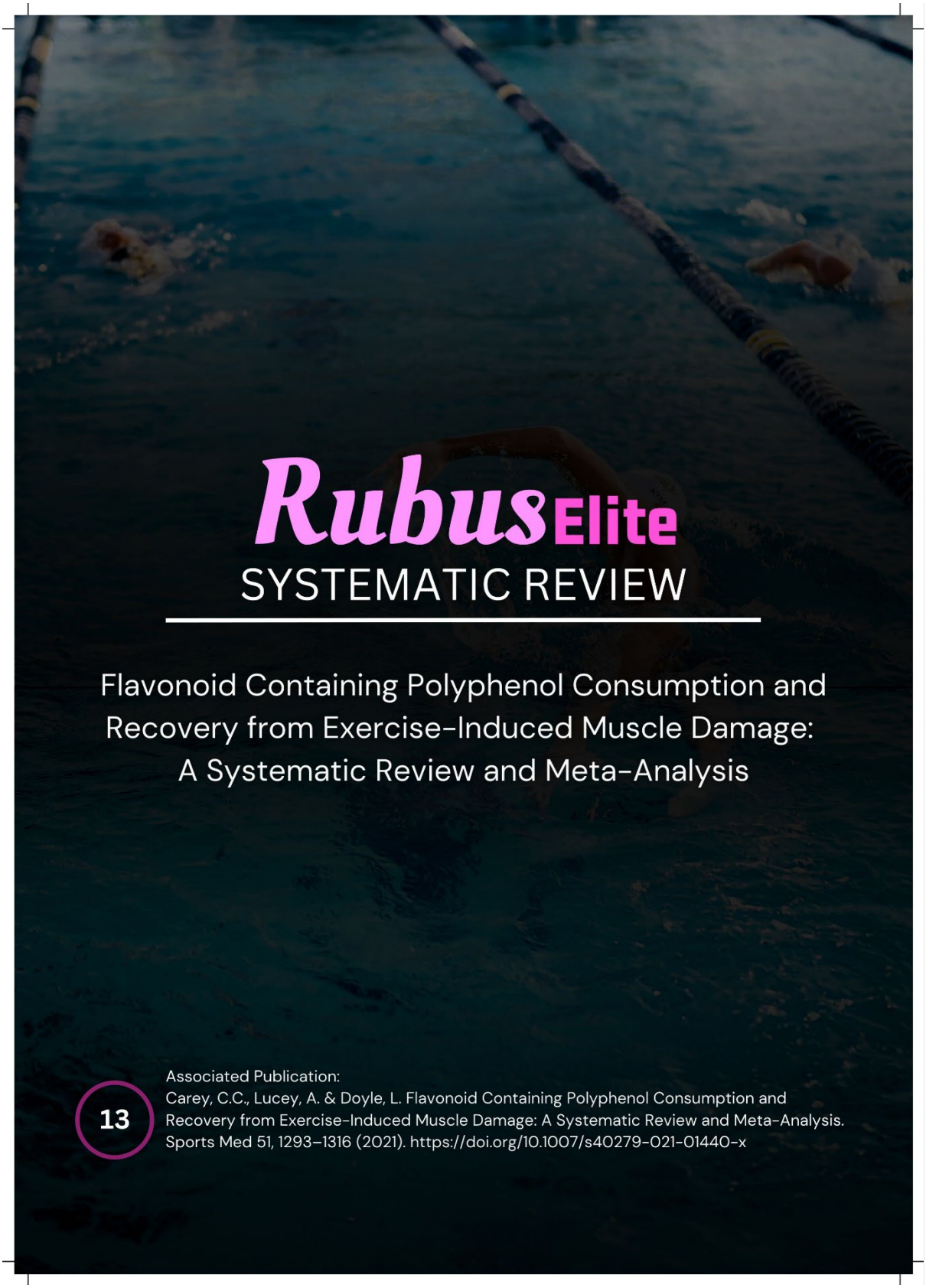
Rubus Elite SURVEY

WHAT ARE ATHLETES PRODUCT PREFERENCES?

Athletes report smoothies, shakes, bars and hot foods are their most preferred sports nutrition food product types.



12



Rubus Elite

SYSTEMATIC REVIEW

Flavonoid Containing Polyphenol Consumption and Recovery from Exercise-Induced Muscle Damage: A Systematic Review and Meta-Analysis

13

Associated Publication:
Carey, C.C., Lucey, A. & Doyle, L. Flavonoid Containing Polyphenol Consumption and Recovery from Exercise-Induced Muscle Damage: A Systematic Review and Meta-Analysis. Sports Med 51, 1293–1316 (2021). https://doi.org/10.1007/s40279-021-01440-x

WHAT ARE **POLYPHENOLS?**

Polyphenols are non-nutritive bioactive phytochemicals which are found in abundance in:

- Berries
- Other Fruits
- Teas
- Dark chocolate



Polyphenols are well known for their antioxidant and anti-inflammatory effects.

Cardiometabolic health benefits have been associated with consumption of polyphenol rich whole foods such as berries. (Heneghan et al 2018)

Polyphenol interventions have also been shown to enhance endurance exercise performance. (Somerville et al. 2017)

Heneghan, C., Kiely M, Lyons J. Lucey, A. The Effect of Berry-Based Food Interventions on Markers of Cardiovascular and Metabolic Health: A Systematic Review of Randomized Controlled Trials. Mol. Nutr. Food Res. (2018). DOI: 10.1002/mnfr.201700645

14

Somerville, V., Bringans, C. Braakhuis, A. Polyphenols and Performance: A Systematic Review and Meta-Analysis. Sports Med. (2017). DOI: 10.1007/s40279-017-0675-5



WHAT IS **RECOVERY?**

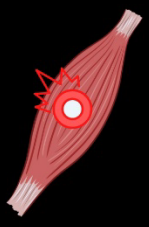
Exercise-induced muscle damage is the transient period of stiffness, soreness and reduced muscle function after intensive exercise.

What does good recovery after intensive exercise look like?

- Faster recovery of muscle strength.
- Reduced muscle pain.
- Lower levels of biomarkers of muscle damage.







How do we measure recovery?

- Maximal voluntary muscle contraction (MVC) - Strength
- Visual Analogue Pain Scales - Soreness
- Creatine Kinase - Muscle damage biomarker

EFSA have stated that functional performance measurements such as MVCs following a strenuous exercise bout is essential to assess recovery. Subjective measures of muscle soreness and muscle damage can provide further support.

Guidance on the scientific requirements for health claims related to muscle function and physical performance. EFSA Journal, 16(10), e05434. https://doi.org/10.2903/J.EFSA.2018.5434

15

Rubus Elite SYSTEMATIC REVIEW

SYSTEMATIC REVIEW AND META-ANALYSIS

Meta-Analysis > Sports Med. 2021 Jun;51(6):1293-1316. doi: 10.1007/s40279-021-01440-x. Epub 2021 Mar 9.

Flavonoid Containing Polyphenol Consumption and Recovery from Exercise-Induced Muscle Damage: A Systematic Review and Meta-Analysis

Conor C Carey [1], Alice Lucey [1], Lorna Doyle [2]

Multiple randomised controlled trials have investigated the ability of polyphenol interventions to impact post exercise recovery.

Due to the cost, burden and time associated with these RCTs, many are under-powered. A systematic review and meta analysis was required to determine a consensus.

Study Goal: To pool the results of previous randomised controlled trials to assess the efficacy of polyphenol products on recovery from exercise-induced muscle damage

16

Rubus Elite SYSTEMATIC REVIEW

SELECTION OF PUBLISHED EVIDENCE

All RCTs had the following characteristics:
Randomised placebo-controlled trial design
Healthy human participants
Participants aged between 18-60
Minimum 6 days of polyphenol or placebo treatment
Completion of a single bout of high intensity or eccentric exercise
Measurements of muscle function, muscle pain or creatine kinase

26 **Randomised Controlled Trials**

568 **Total Participants**

468 MALES

100 FEMALES

Training status of participants ranged from untrained to semi-professional athletes

17

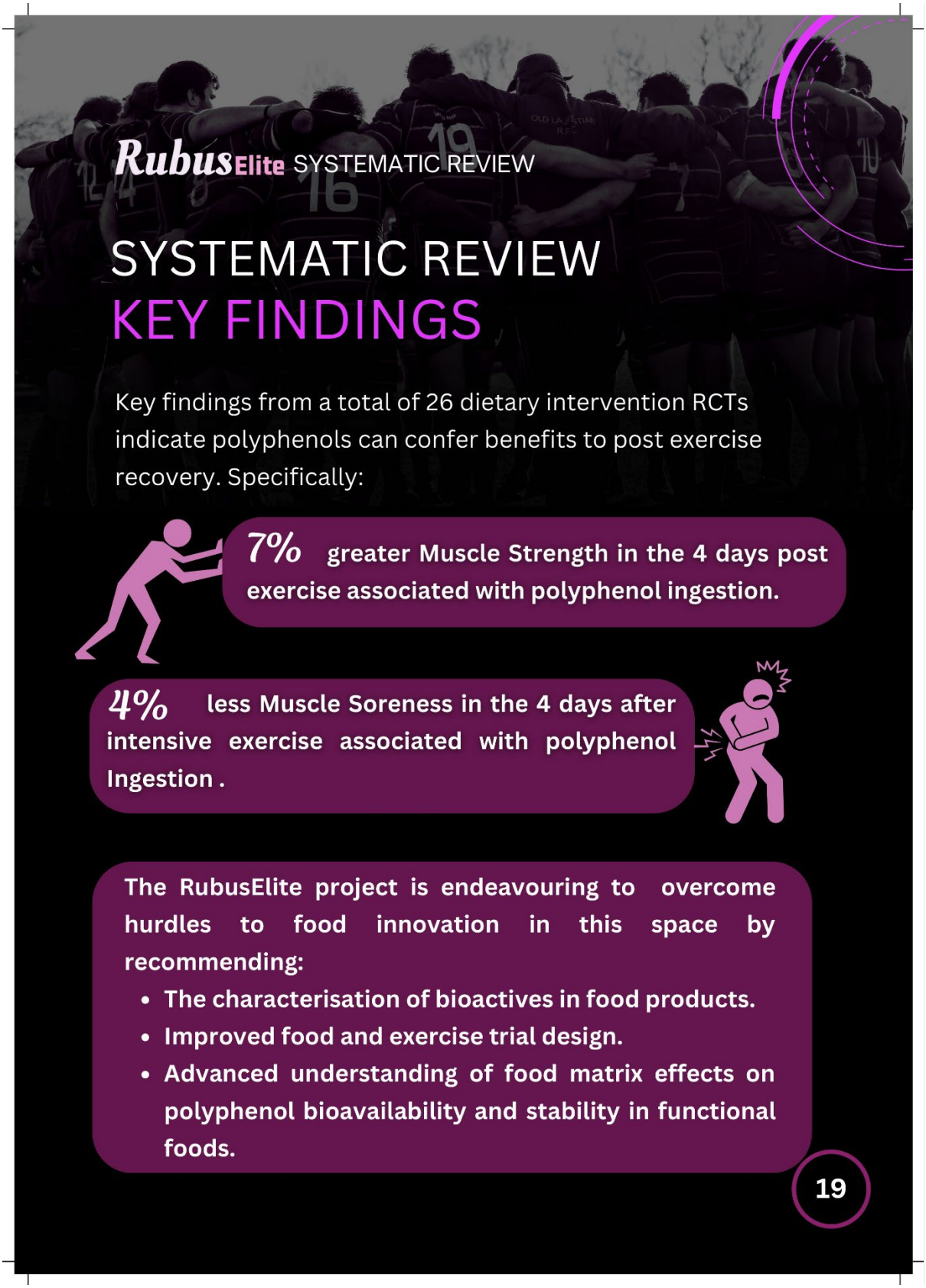
SYSTEMATIC REVIEW
INCLUDED STUDIES

As polyphenols are found abundantly in multiple food sources, studies included in the systematic review utilised various food sources to develop their polyphenol treatments.

The food sources used in these studies are outlined below:

Polyphenol Source	No. of Studies
Tart Cherry 	9
 Tea Extracts	7
Pomegranate 	3
 Quercetin	2
Blackcurrant 	2
 Cacao	1
Lemon Verbena 	1
 Mixed fruit juice	1

18



RubusElite SYSTEMATIC REVIEW

SYSTEMATIC REVIEW
KEY FINDINGS

Key findings from a total of 26 dietary intervention RCTs indicate polyphenols can confer benefits to post exercise recovery. Specifically:

7% greater Muscle Strength in the 4 days post exercise associated with polyphenol ingestion.

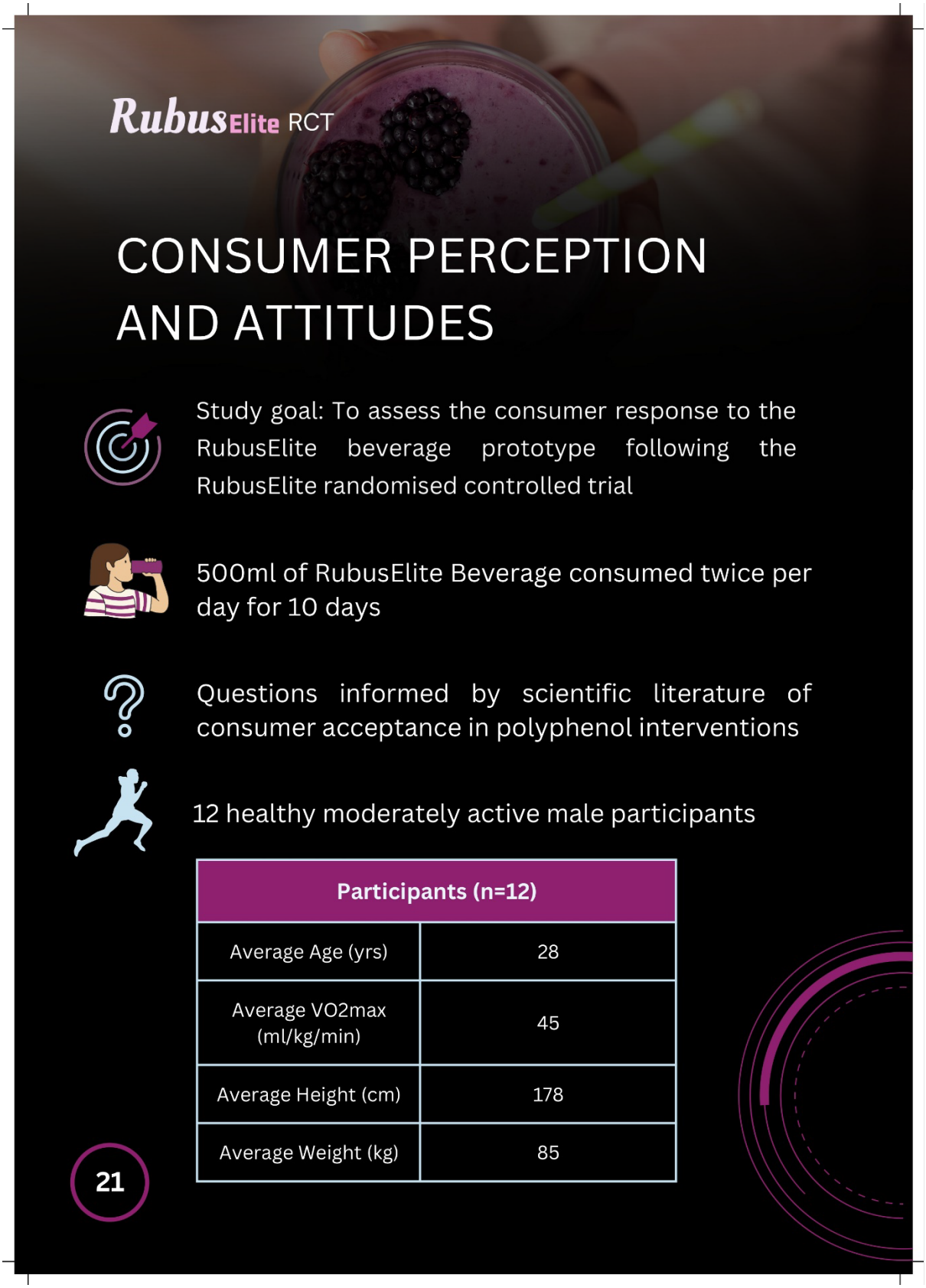
4% less Muscle Soreness in the 4 days after intensive exercise associated with polyphenol Ingestion .

The RubusElite project is endeavouring to overcome hurdles to food innovation in this space by recommending:

- The characterisation of bioactives in food products.
- Improved food and exercise trial design.
- Advanced understanding of food matrix effects on polyphenol bioavailability and stability in functional foods.

19


Rubus Elite
RANDOMISED CONTROLLED TRIAL
Consumer Perception and Attitudes towards a Dairy, High Protein, Blackberry Polyphenol Enriched Beverage
SETU
20


RubusElite RCT
CONSUMER PERCEPTION AND ATTITUDES
Study goal: To assess the consumer response to the RubusElite beverage prototype following the RubusElite randomised controlled trial
500ml of RubusElite Beverage consumed twice per day for 10 days
Questions informed by scientific literature of consumer acceptance in polyphenol interventions
12 healthy moderately active male participants
Participants (n=12)
Average Age (yrs) 28
Average VO2max (ml/kg/min) 45
Average Height (cm) 178
Average Weight (kg) 85
21

RubusElite RCT

THE BEVERAGE



The RubusElite beverage is a high protein dairy beverage enriched with blackberry polyphenols.

It has been designed to contain appropriate amounts of protein and polyphenols to support muscular performance and recovery

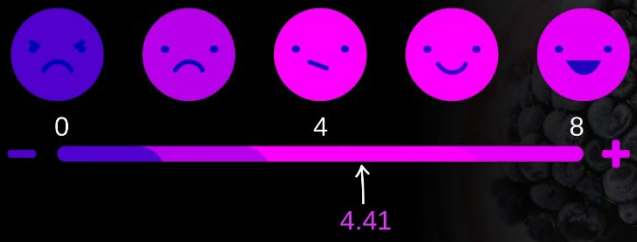
The RubusElite Beverage	
Dose	500ml
Timing	Twice per day, morning and evening
Protein	30g
Polyphenols	750mg
Leucine	Approx 2.5g

22

Rubus Elite RCT

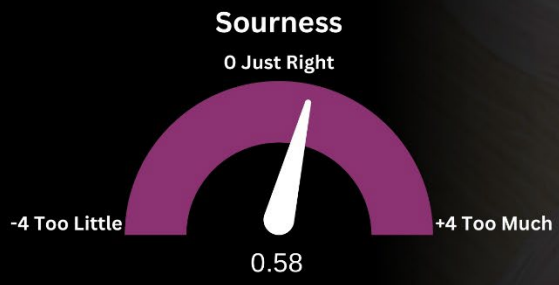
CONSUMER PERCEPTION AND ATTITUDES

How would you rate your experience of drinking the RubusElite beverage?



Average participant ranking

How would you rate each of the following attributes in relation to the RubusElite beverage?





23

RubusElite RCT

CONSUMER PERCEPTION AND ATTITUDES

How would you rate each of the following attributes in relation to the RubusElite beverage?





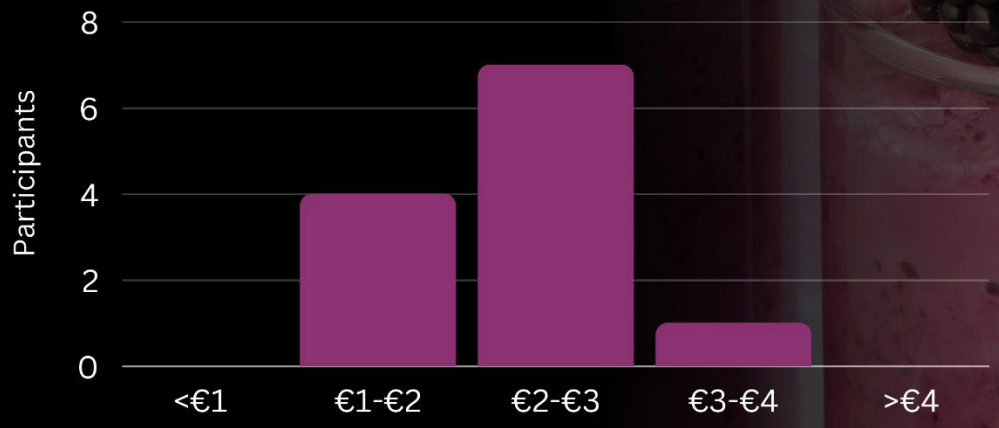


24

Rubus Elite RCT

CONSUMER PERCEPTION AND ATTITUDES

How much would you be willing to pay for a single serving (500ml) of the RubusElite beverage?



How likely would you be to purchase the RubusElite beverage?



25



Proudly funded by:

An Roinn Talmhaíochta, Bia agus Mara
Department of Agriculture, Food and the Marine

THANK YOU!

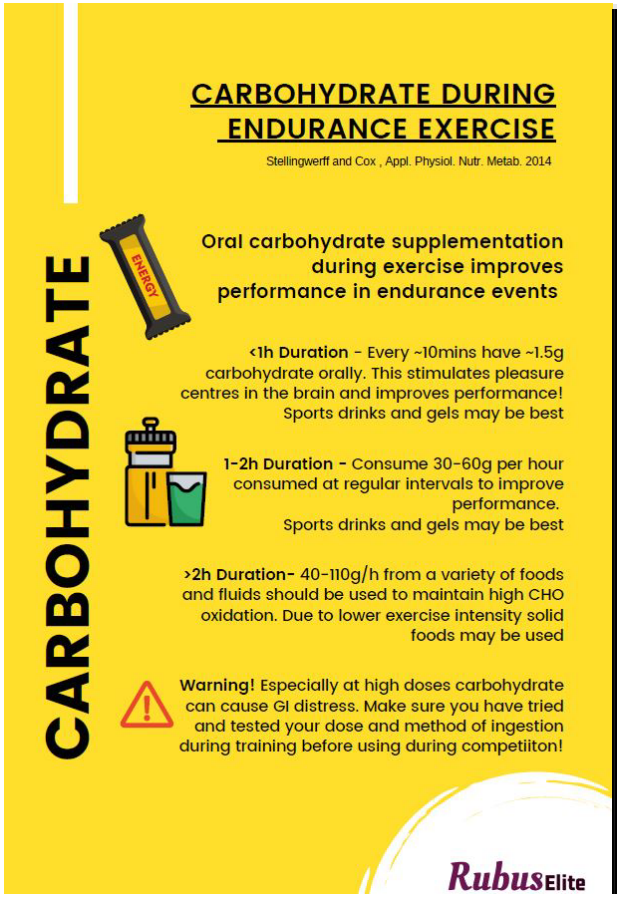
The RubusElite project was funded by the Irish Department of Agriculture Food and the Marine's Food Institutional Research Measure (2018 - 2023)

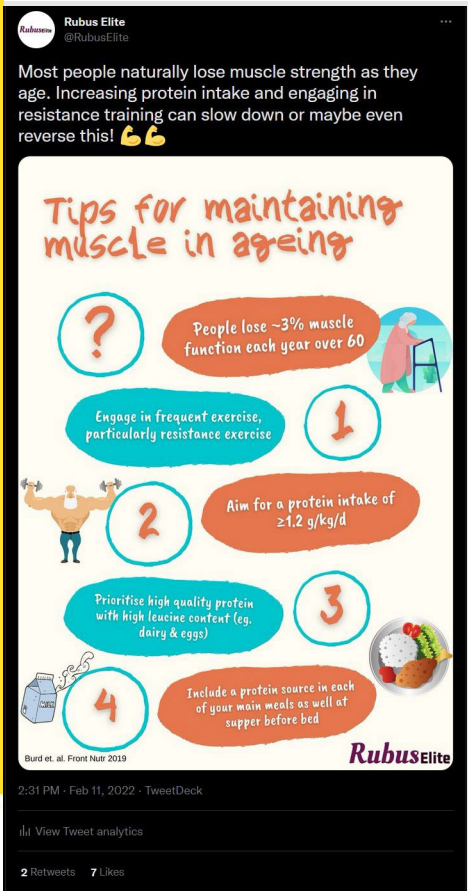
UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

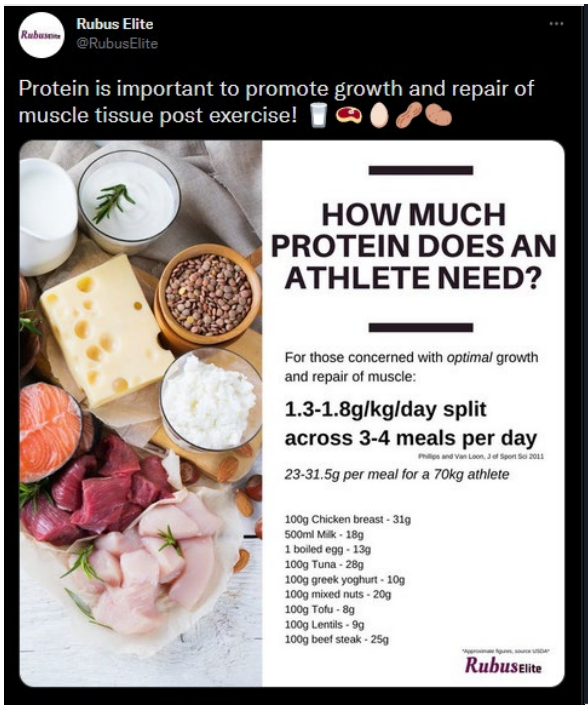
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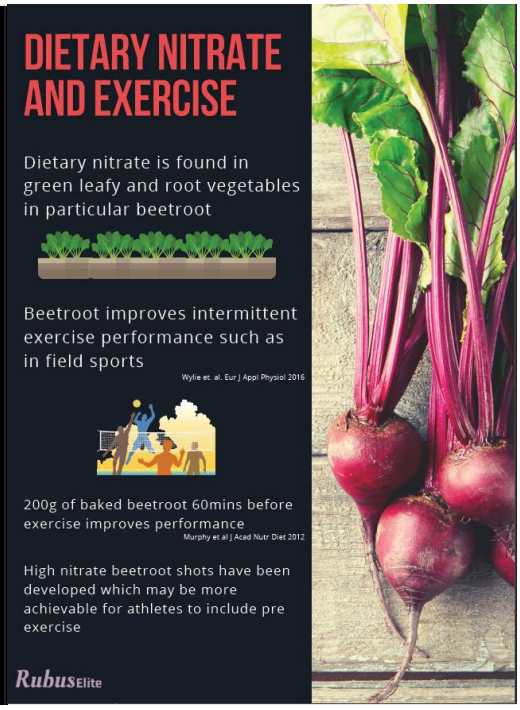
SETU

Appendix 2. Social Media Infographic Examples









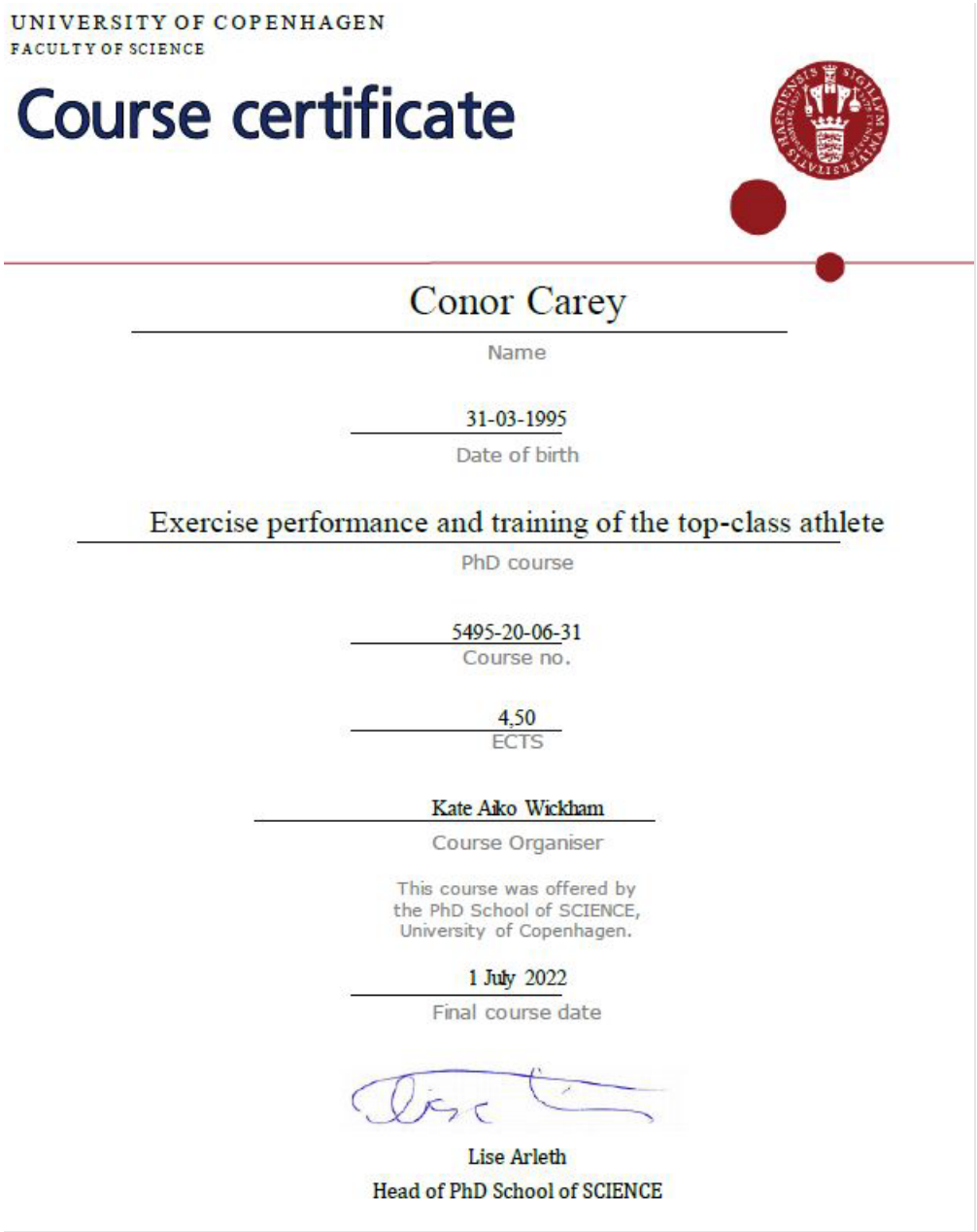
Appendix 3. RubusElite Performance Nutrition Forum





Training Appendices

Appendix 4. University of Copenhagen PhD Summer School



UNIVERSITY OF COPENHAGEN
FACULTY OF SCIENCE

Course certificate

Conor Carey
Name

31-03-1995
Date of birth

Exercise performance and training of the top-class athlete
PhD course

5495-20-06-31
Course no.

4,50
ECTS

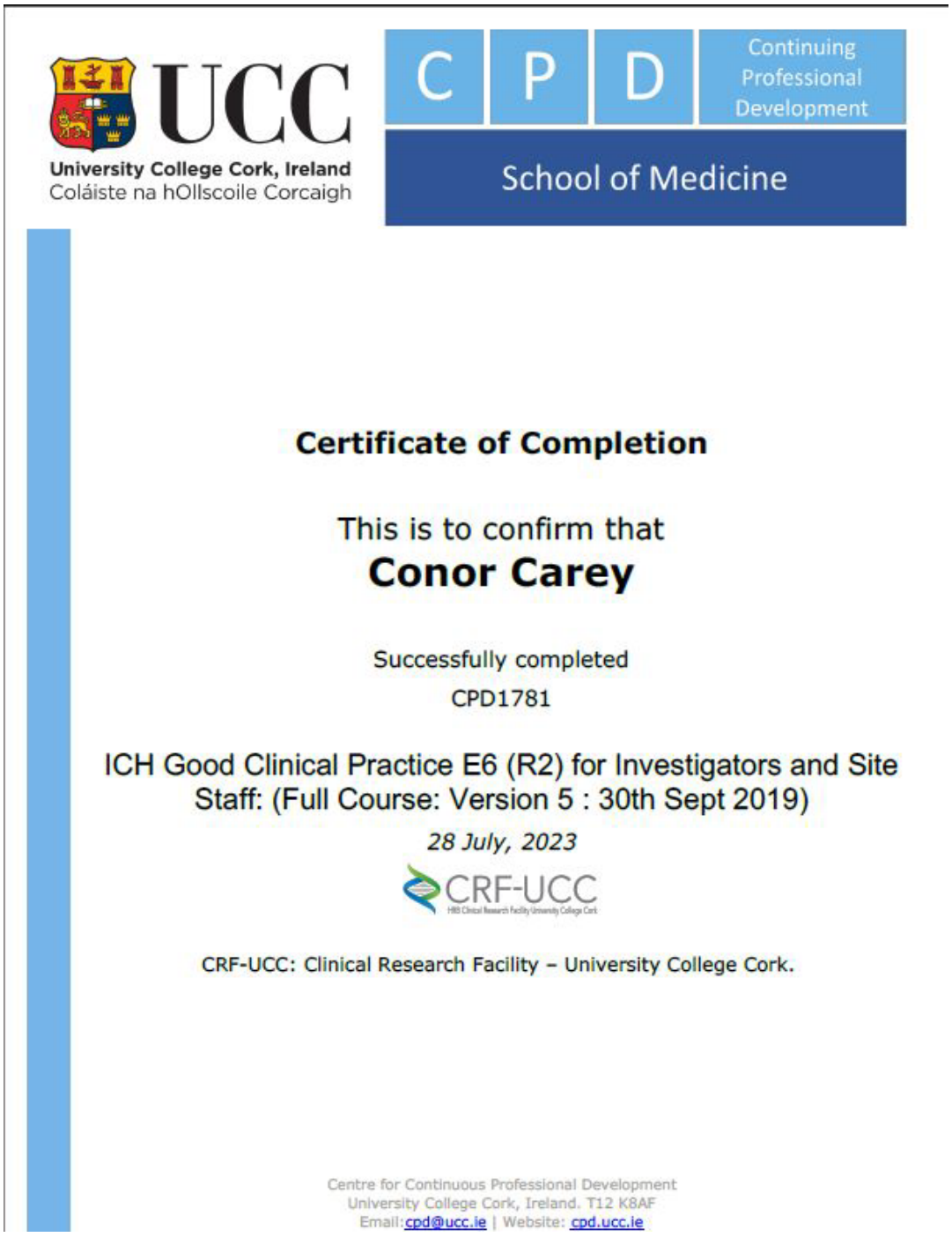
Kate Aiko Wickham
Course Organiser

This course was offered by
the PhD School of SCIENCE,
University of Copenhagen.

1 July 2022
Final course date

Lise Arleth
Head of PhD School of SCIENCE

Appendix 5. ICH GCP Training



UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

C P D Continuing Professional Development

School of Medicine

Certificate of Completion

This is to confirm that

Conor Carey

Successfully completed

CPD1781

ICH Good Clinical Practice E6 (R2) for Investigators and Site Staff: (Full Course: Version 5 : 30th Sept 2019)

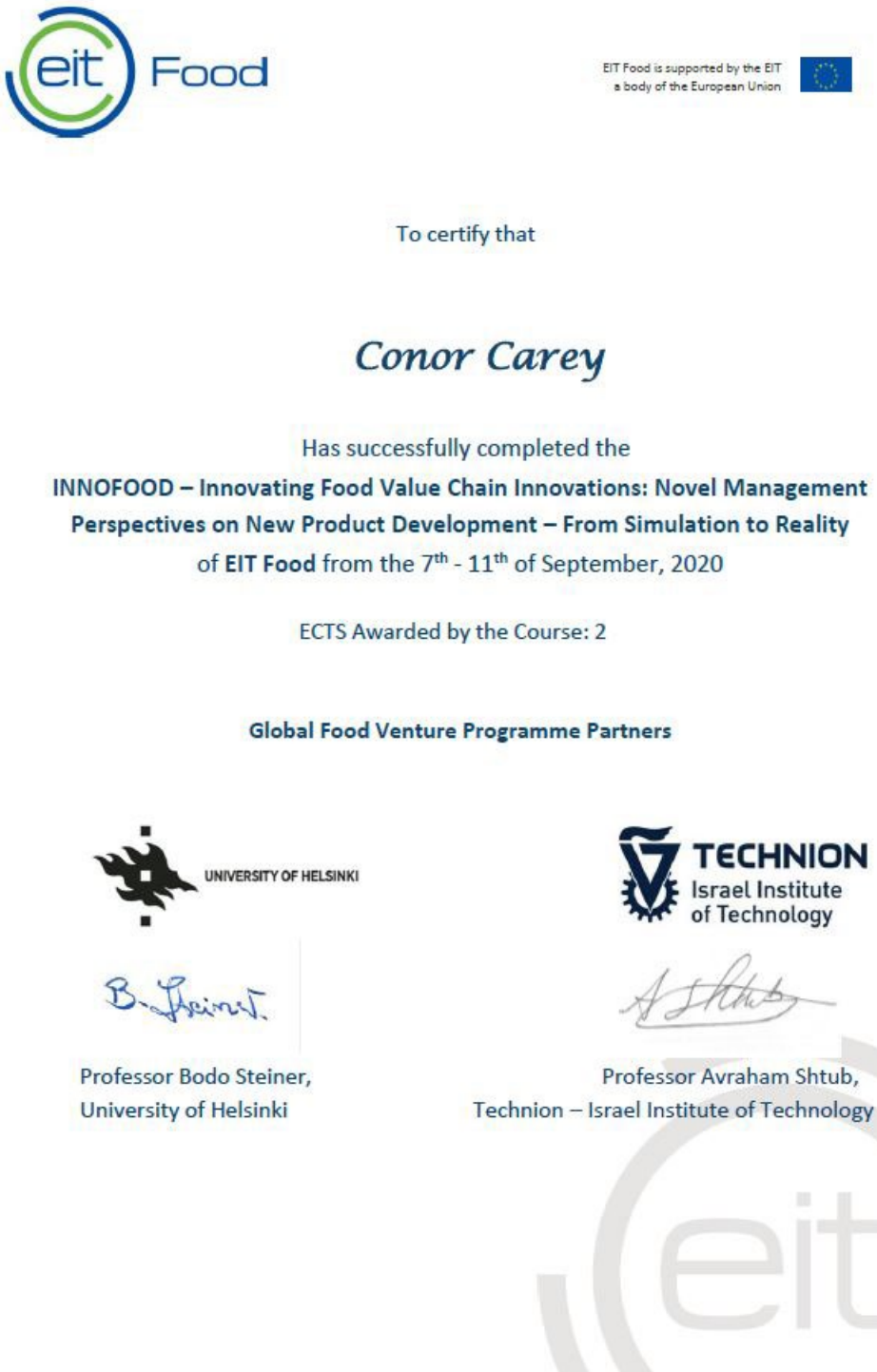
28 July, 2023

CRF-UCC

CRF-UCC: Clinical Research Facility – University College Cork.

Centre for Continuous Professional Development
University College Cork, Ireland. T12 K8AF
Email:cpd@ucc.ie | Website: cpd.ucc.ie

Appendix 6. EITfood Innofood Training



EIT Food is supported by the EIT
a body of the European Union

To certify that

Conor Carey

Has successfully completed the
INNOFOOD – Innovating Food Value Chain Innovations: Novel Management Perspectives on New Product Development – From Simulation to Reality
of **EIT Food** from the 7th - 11th of September, 2020

ECTS Awarded by the Course: 2

Global Food Venture Programme Partners

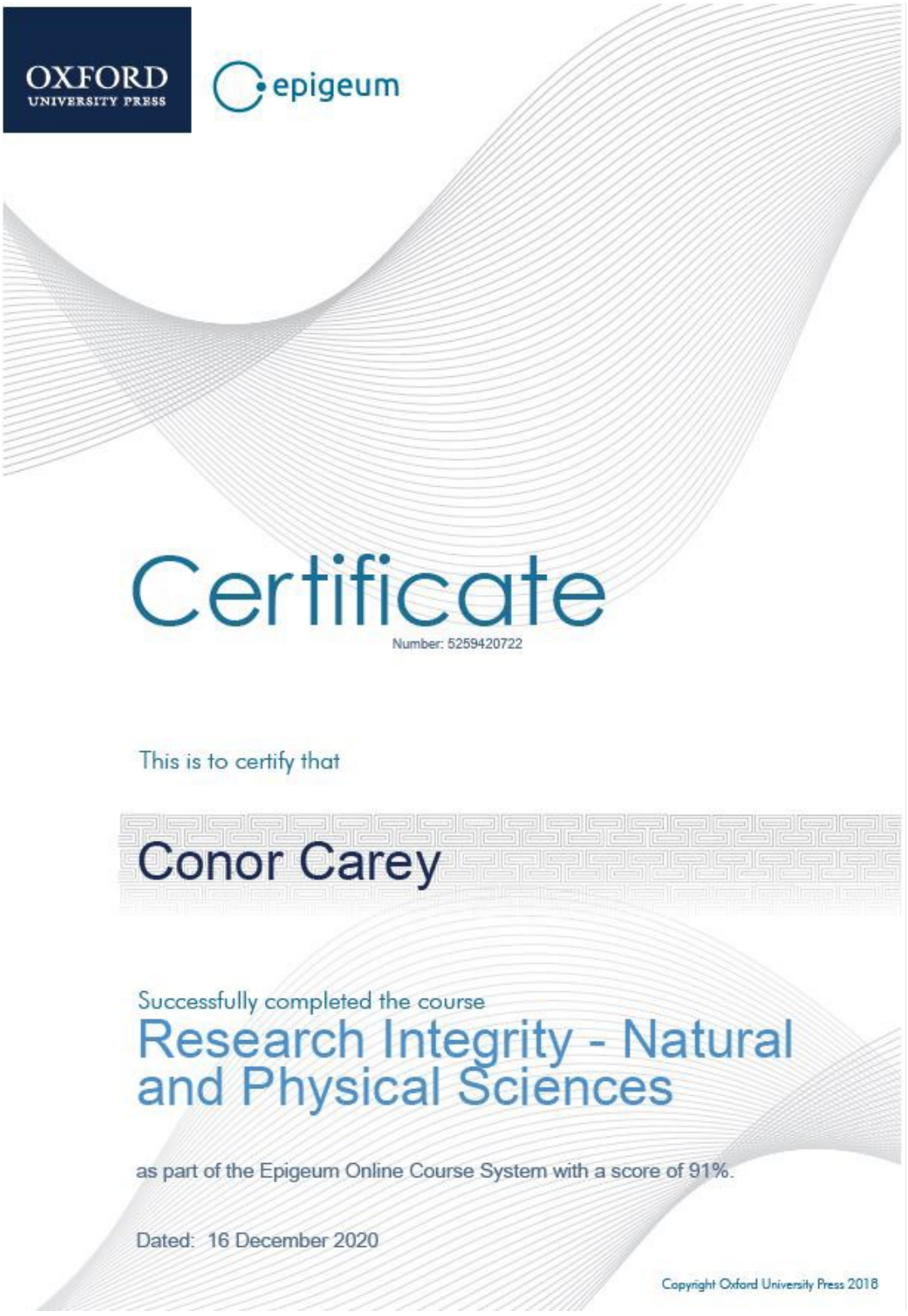
UNIVERSITY OF HELSINKI

Professor Bodo Steiner,
University of Helsinki

TECHNION
Israel Institute
of Technology

Professor Avraham Shtub,
Technion – Israel Institute of Technology

Appendix 7. Epigeum Research Integrity Training



RubusElite Survey Appendices

Appendix 8. RubusElite Consumer Acceptability Survey

Start of Block: Muscle soreness

Q1 Please rate your current level of muscle soreness

	No Pain 0	1	2	3	4	5	6	7	8	9	Extreme Pain 10
Muscle soreness	○	○	○	○	○	○	○	○	○	○	○

End of Block: Muscle soreness

Start of Block: RubusElite beverage acceptability and preferences

Q2 How would you rate your experience of drinking your assigned RubusElite beverage?

	Disliked very much 0	1	2	3	Neither liked nor disliked 4	5	6	7	Liked very much 8
.	○	○	○	○	○	○	○	○	○

Q3 How difficult was it to prepare each serving of the RubusElite beverage?

- ○ Extremely difficult
- ○ Somewhat difficult
- ○ Neither easy nor difficult
- ○ Somewhat easy
- ○ Extremely easy

Q4 How would you rate each of the following attributes in relation to the RubusElite beverage.

	Too little - 4	-3	-2	-1	Just about right	+1	+2	+3	Too much +4
Sourness	○	○	○	○	○	○	○	○	○
Sweetness	○	○	○	○	○	○	○	○	○
Astringency	○	○	○	○	○	○	○	○	○
Aroma	○	○	○	○	○	○	○	○	○
Viscosity	○	○	○	○	○	○	○	○	○

Q5 How likely would you be to purchase the RubusElite beverage?

	Extremely unlikely	Somewhat unlikely	Neither likely nor unlikely	Somewhat likely	Extremely likely
.	○	○	○	○	○

Q6 How much would you be willing to pay for a single serving (500ml) of the RubusElite beverage?

- ○ Under €1
- ○ €1 - €2
- ○ €2 - €3
- ○ €3 - €4
- ○ Over €4

Q7 If you were to change one thing about the RubusElite beverage what would it be?

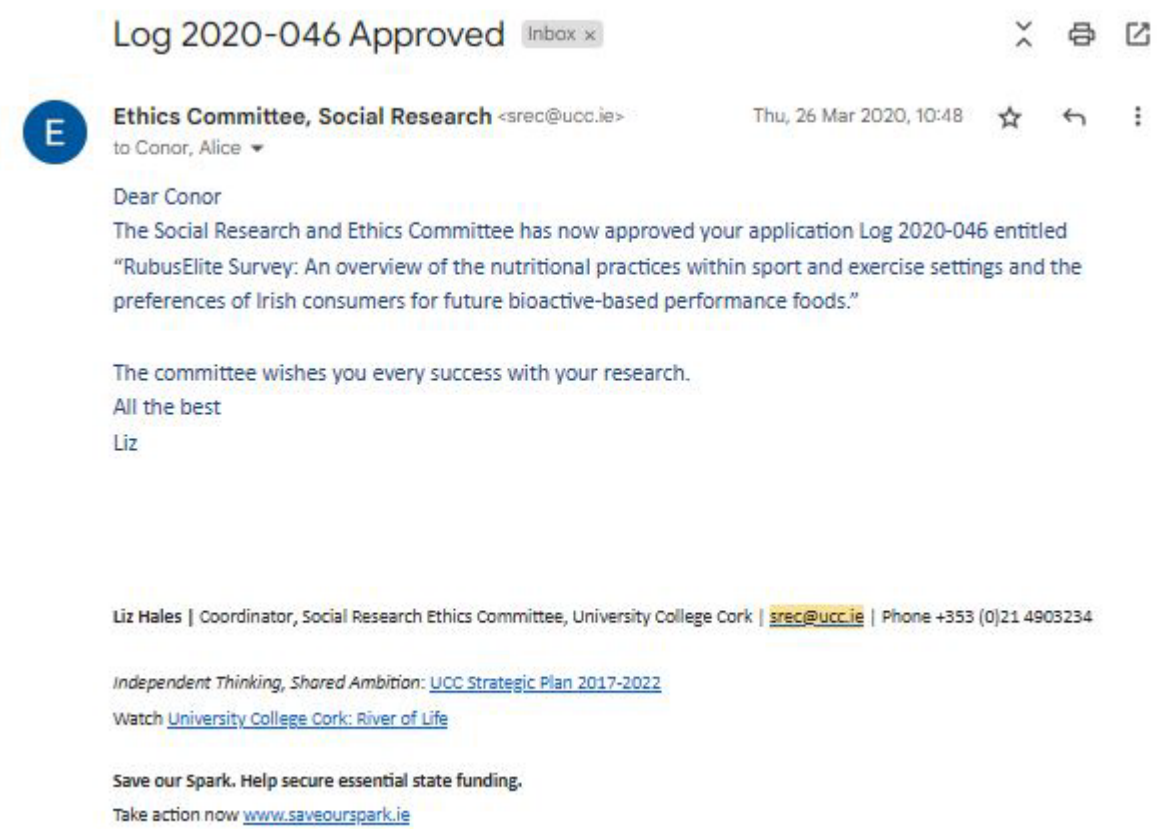
_____

Q8 Rank in order, your top 5 preferred forms of sports nutrition products? 1=most preferred

_____ Powder / shake
_____ Spreadable (nut butter etc)
_____ Pancake / brownie (or similar)
_____ Bar / flapjack
_____ Yoghurt
_____ Shot (
_____ Biscuit / cookie
_____ Hot beverage
_____ Cereal
_____ Gel
_____ Gum
_____ Pre-made Beverage
_____ Pill / capsule
_____ Other (Please Specify)

End of Block: RubusElite beverage acceptability and preferences

Appendix 9. RubusElite Survey Social Research Ethics Approval



Log 2020-046 Approved Inbox ×

Ethics Committee, Social Research <srec@ucc.ie> Thu, 26 Mar 2020, 10:48

to Conor, Alice

Dear Conor

The Social Research and Ethics Committee has now approved your application Log 2020-046 entitled "RubusElite Survey: An overview of the nutritional practices within sport and exercise settings and the preferences of Irish consumers for future bioactive-based performance foods."

The committee wishes you every success with your research.

All the best

Liz

Liz Hales | Coordinator, Social Research Ethics Committee, University College Cork | srec@ucc.ie | Phone +353 (0)21 4903234

Independent Thinking, Shared Ambition: UCC Strategic Plan 2017-2022

Watch University College Cork: River of Life

Save our Spark. Help secure essential state funding.

Take action now www.saveourspark.ie

Appendix 10 Focus Group Conversation Guidance Template

SREC Application: Focus Group Discussion Template

A qualitative overview of the nutritional practices within sport and exercise settings and the preferences of Irish consumers for future bioactive-based performance foods

Introduction

- Welcome and introduce all participants
- Distribution of name tags
- Overview of focus group format
- Tea, coffee, light refreshments

Section 1. – Current Nutritional Practice

Aim: To establish the differing perceived nutritional needs of the different target market segments who may consider using products to improve musculoskeletal function.

- What are your priorities for your current exercise nutritional practices? e.g. Performance, recovery, immune function, healthy ageing.
- What are your motivations and or barriers to using an exercise nutrition product or functional food?
- What nutritional foods/supplements are you currently taking that meet both these priorities and motivations?
- Where/from whom (if any) do you currently get your nutritional information?
- Roughly how much would you spend per week on your exercise nutritional practice beyond what you would consume on a regular diet?

Section 2. – Issues with dissemination of sports nutritional knowledge and practice

Aim: To identify hidden needs and current misunderstandings with exercise and nutritional practice which can be addressed through future scientific research, dissemination and marketing.

- When planning your nutritional practice, can you confidently identify the scientifically based nutritional claims from marketing claims?
- Do you understand the current athletic nutritional recommendations such as protein/carbohydrate intake etc.?
- If not, what are the main issues underpinning this difficulty?
- If so what is your approach to understanding and these claims?

Section 3. – Product Preferences

Aim: To create a profile of ideal characteristics of nutritional products to maximise adherence for future science-based sport and exercise nutritional practices.

- What is your form preference for a nutritional aid? Food, beverage, powder, yoghurt, bar, pill etc.?
- What characteristics would the above have? Flavour, colour, viscosity
- What price range would you be willing to pay for a nutritional product that met these characteristics?

Section 4. – Rubus Elite Specific Investigation

Aim: To specifically investigate the current knowledge and needs for a polyphenol enhanced dairy based musculoskeletal enhancement product.

- What is your current knowledge of polyphenols? Refer back if any mentioned in Section 1.
- Do you currently use dairy based recovery aids e.g. whey, high protein milk, high protein yoghurt
- What flavours do you prefer in dairy recovery aids or products?
- Naming discussion, identification of potential naming options

Section 5. – Debrief

- Thank participants
- Brief outline of the future aims and objectives of Rubus*Elite* Project
- Time provided for informal sport and exercise nutritional discussion with researchers should the need arise.
- Reminder that data can be withdrawn for 2 weeks from the date of the focus group

Appendix 11 Focus Group Participant Outline

	Total	**Focus Group 1**	**Focus Group 2**
No. Participants	10	5	5
Sex	M – 7 F – 3	M – 3 F – 2	M – 4 F – 1
Average Age (years)	25.9	28.2	23.6
Average training volume (hours per week)	8.7	7.3	10
Sports/ Activities	Gaelic Football – 3 Soccer – 3 Hurling – 1 Golf – 1 Tennis – 1 Bodybuilding – 1	Gaelic Football – 2 Bodybuilding – 1 Soccer – 1 Golf – 1	Soccer – 2 Gaelic Football – 1 Hurling – 1 Tennis – 1
Competition Level	International – 2 National – 3 Regional – 4 Local – 1	International – 1 National – 1 Regional – 2 Local – 1	International – 1 National – 2 Regional – 2

Appendix 12. RubusElite Survey Questions

RubusElite Sports Nutrition Survey

Q1 What gender do you most identify with?

- ○ Male
- ○ Female
- ○ Prefer not to say
- ○ Other (Please Specify) _____

Q2 What age are you?

_____

Q3 Do you currently partake in competitive sport?

- ○ Yes
- ○ No

Skip To: Q7 If Do you currently partake in competitive sport? = No

---

Q4 What sport(s) do you compete in?

_____

_____

_____

Q5 How many hours per week on average would you spend training/exercising in preparation for or in competition in these sports?

_____

Q6 What is the highest level you currently compete at?

- ○ Local
- ○ Regional
- ○ National
- ○ International
- ○ Other (Please Specify) _____

Q7 Do you regularly partake in non-competitive structured physical activity?

- ○ Yes
- ○ No

Skip To: Q10 If Do you regularly partake in non-competitive structured physical activity? = No

Q8 What non-competitive activity do you partake in mostly?

_____

Q9 How many hours per week on average would you spend taking part in this activity?

_____

Q10 Does your engagement in physical activity frequently cause a stiffness and/or pain in your muscles in the hours and/or days following exercise?

- ○ Yes
- ○ No

Skip To: Q12 If Does your engagement in physical activity frequently cause a stiffness and/or pain in your muscle... = No

Q11 How frequently do you experience this stiffness or pain?

- ○ Most days
- ○ A few times per week
- ○ About once per week
- ○ A few times per month
- ○ Less than once a month

Q12 Do you alter your routine after training/exercise/competition to improve recovery?

- ○ Yes
- ○ No

Skip To: Q14 If Do you alter your routine after training/exercise/competition to improve recovery? = No

Q13 How do you alter your routine? (Tick all which apply)

- ☐ Increase Sleep
- ☐ Changes to daily nutrition (Meal content / Meal timing / Number of meals / snacking)
- ☐ Nutritional Supplements
- ☐ Stretching
- ☐ Foam Rolling
- ☐ Massage
- ☐ Cold Water Immersion (Ice bath)
- ☐ Compression Clothing
- ☐ Active Recovery
- ☐ Meditation
- ☐ Other (Please Specify) _____

Q14 On a scale of 1-10 how would you rate your knowledge of exercise nutrition?

	Minimal								Excellent
	1	2	3	4	5	6	7	8	9	10
Knowledge of Exercise Nutrition										

Q15 Do you currently take any nutritional supplements for health and wellbeing purposes?

- ○ Yes
- ○ No

Skip To: Q17 If Do you currently take any nutritional supplements for health and wellbeing purposes? = No

Q16 What supplements are you currently taking for health and wellbeing purposes? (Tick all which apply)

- ☐ Multivitamin
- ☐ Omega-3 PUFA (Fish oils)
- ☐ Calcium
- ☐ Vitamin C
- ☐ Vitamin D
- ☐ Iron
- ☐ Folic Acid
- ☐ Calcium
- ☐ B Vitamins
- ☐ Other (Please Specify) _____

Q17 Are you currently taking any supplements to improve exercise performance or recovery?

- ○ Yes
- ○ No

Skip To: Q19 If Are you currently taking any supplements to improve exercise performance or recovery? = No

Q18 What nutritional supplements do you currently take for exercise performance or recovery? (tick all which apply)

- ☐ Protein (whey, casein, pea, soy etc)
- ☐ Creatine Monohydrate
- ☐ Caffeine
- ☐ Branched Chain Amino Acids (BCAA's)
- ☐ Carbohydrate (Gel, powder etc)
- ☐ Other (Please Specify) _____

Q19 Do you use any 'functional food products', specifically for exercise performance or recovery?

- [ ] No
- [ ] Caffeine (Coffee)
- [ ] Polyphenols (Tart cherry juice, green tea, dark chocolate etc.)
- [ ] Dietary Nitrate (Beetroot juice)
- [ ] Other (Please Specify) _____

Q20 Do you have a typical post-exercise meal, food or beverage?

- ○ Yes
- ○ No

Skip To: Q22 If Do you have a typical post-exercise meal, food or beverage? = No

Q21 What is your typical post-exercise meal, food or beverage? Briefly state food, beverage or meal or combination you consume

_____

Q22 Do you frequently check the label for the nutritional content of the foods you purchase?

- ○ Always
- ○ Most of the time
- ○ Sometimes
- ○ Never

Q23 On a scale of 1-4, how important is it that the effects of a sports nutrition product have been scientifically proven

	Not important 1	2	3	Crucial 4
How important?				

Q24 Would you be willing to pay extra for a product which had been scientifically proven to improve performance / recovery?

- ○ Yes
- ○ No

Q25 From where do you source your nutrition information from? Tick all which apply

- [ ] Social Media
- [ ] Blogs / websites
- [ ] Qualified nutritionist
- [ ] Dietician
- [ ] Doctor / GP
- [ ] Coach
- [ ] Other Athletes
- [ ] Online programme / meal plan
- [ ] Books
- [ ] Scientific literature
- [ ] I do not source nutrition information as it is difficult to locate / understand
- [ ] I am not interested in sourcing nutrition information
- [ ] Other source (Please Specify)

Q26 On a scale of 1-4 how likely would you be to take on board and implement nutrition advice from each of the following?

	1	2	3	4
Doctor / GP	○	○	○	○
Coach	○	○	○	○
Nutritionist / Dietician	○	○	○	○
A high profile athlete	○	○	○	○
Online Website / Blog	○	○	○	○
Social Media influencer	○	○	○	○
Close friend	○	○	○	○

Q27 On a scale of 1-4 how important would the following features be in your decision to purchase a food for exercise performance / recovery?

	1	2	3	4
Taste of the product	○	○	○	○
Price of the product	○	○	○	○
Nutritional profile	○	○	○	○
Ease of preparation	○	○	○	○
Accessibility	○	○	○	○
Sustainability (Environmental impact)	○	○	○	○

*

Q28 Rank in order, your top 3 preferred types of recovery product. 1=most preferred

_____ Pre-made Beverage
_____ Powder
_____ Dairy (yoghurt, milk)
_____ Bar (protein bar, flapjack)
_____ Pill / Capsule
_____ Whole food
_____ Other (Please Specify)

*

Q29 Rank in order, your top 3 preferred types of performance enhancing nutrition product. 1=most preferred

_____ Pre-made Beverage
_____ Powder
_____ Bar (energy bar)
_____ Gel (caffeine, carbohydrate etc)
_____ Gum
_____ Pill / capsule
_____ Whole food
_____ Other (Please Specify)



Q30 Rank in order, your top 3 preferred form of sports nutrition food product? 1=most preferred

_____ Shake
_____ Smoothie / juice
_____ Spreadable (nut butter etc)
_____ Pancake / brownie (or similar)
_____ Bar / flapjack
_____ Yoghurt
_____ Shot (
_____ Biscuit / cookie
_____ Hot beverage
_____ Cereal
_____ Hot food
_____ Other (Please Specify)

Q31 If the same sports nutrition product was available in dairy or plant-based variety, which would you choose?

○ Dairy-based

○ Plant-based

○ No Preference

Skip To: Q33 If the same sports nutrition product was available in dairy or plant-based variety, which would y... = No Preference

Q32 What is your primary reason for the above choice in the previous question?

- ○ Taste
- ○ Sustainability (Environmental impact)
- ○ Ethical or welfare concerns
- ○ Intolerance
- ○ Potential of greater health / performance benefit
- ○ Other (Please Specify) _____

Q33 Rank your top 3 features when choosing whether to use a performance food or product?

1= most important

_____ Improved endurance performance
_____ Improved strength / power performance
_____ Enhanced muscle recovery
_____ Benefits for muscle and bone health
_____ Supports immunity / overall health and wellbeing
_____ Low risk of supplement contamination

Q34 Rank in order of likelihood, if you were to purchase a sports nutrition product, from where would you be most likely to purchase it? 1=most likely

_____ Supermarket
_____ Pharmacy
_____ Online
_____ Health Food Shop
_____ Cafe / Restaurant
_____ Other (Please Specify)

Randomised Controlled Trial Appendices

Appendix 13. RCT Ethics Approval

Institúid Teicneolaíochta Phort Láirge Waterford Institute *of* Technology

Port Láirge, Éire
T: +353-51-302000
info@wit.ie

Waterford, Ireland
T: +353-51-302000
www.wit.ie

Ref: WIT2021REC044

20th January 2022

Mr Conor Casey
PhD Student
Department of Sports & Exercise

Dear Conor,

Thank you for bringing your project *'RubusElite randomised controlled trial; efficacy testing of a novel high protein, berry polyphenol enriched beverage for athletes and physically active individuals"* to the attention of the WIT Research Ethics Committee.

I am pleased to inform you that we fully approve WIT's participation in this project and we will convey this to Academic Council.

We wish you well in the work ahead.

Yours sincerely,

Prof John Wells,
Chairperson,
Research Ethics Committee

cc. Dr Lorna Doyle

Appendix 14. – Eligibility Screening Questionnaire

1. Are you between 18 and 45 years of age?	Yes / No
2. Are you biologically male/female/prefer not to say?	M / F/ Other/ prefer not to say
3. Are you willing to consume a dairy product during your participation in this study?	Yes / No
4. Have you ever been diagnosed with a psychiatric disorder or are taking anti-psychotic, anxiolytic or anti-depressant medication? E.g. anxiety disorders, depression, bipolar disorder	Yes / No
5. Do you have a current diagnosis of chronic coexisting illness [cardiovascular, gastrointestinal (GI) [to include functional GI disorders, inflammatory bowel disease, coeliac disease, lactose intolerance]	Yes / No
6. Have you used any antibiotic medication in the last 2 months or are you currently taking any prebiotics or probiotics?	Yes / No
7. Do you class yourself as physically active?	Yes / No
8. Are you willing to discontinue use of all non-medically prescribed nutritional supplements and nutraceutical that may impact exercise performance for the duration of your involvement in this study? E.g. whey protein, creatine, branched chain amino acids' (BCAA's), beta-alanine, caffeine	Yes / No
9. Are you willing to discontinue any personal exercise training programme you are undertaking in the 48h prior to and following your test exercise day during this study?	Yes / No
10. Do you have a history of any landing related lower limb injuries? E.g. Anterior cruciate ligament (ACL), Posterior Cruciate Ligament (PCL) or Lateral Collateral Ligament (LCL) rupture, Achilles rupture etc	Yes / No
11. Are you currently undertaking a plyometric based training programme (jump training) or have a recent history of international sporting competition?	Yes/No

Appendix 15. – Participant Information Sheet

PARTICIPANT INFORMATION SHEET

Project Title: RubusElite Randomised Controlled Trial

Conor Carey (PhD Researcher): conor.carey@umail.ucc.ie

Dr. Lorna Doyle, RNutr (Principal Investigator): lmdoyle@wit.ie

INFORMATION TO POTENTIAL PARTICIPANTS

1. What is the purpose of the project?

Reduced muscle function and muscle soreness in the days after intensive exercise is a considerable issue for both athletes with intense training loads as well as those undertaking new and unaccustomed exercise. This soreness, stiffness and inflammation can significantly hamper performance in sport and also discourage continued physical activity in those new to intense exercise. Adapting nutritional practices has been shown to be one of the primary methods of reducing the negative impact on muscle in the days immediately after intense or unaccustomed exercise. This study aims to test the ability of 3 different beverages to reduce the negative impact on muscle strength, pain and inflammation in the 72-hour period following a bout of unaccustomed exercise. Active properties in these beverages have also been suggested to positively impact on both exercise performance and psychological stress and anxiety via the gut microbiome. Further analysis into these outcomes will be measured during this study to assess the impact of each beverage on exercise performance, stress and anxiety and microbial composition of the gut.

2. Why have I been selected to take part?

We are looking for physically active males aged between the ages of 18 and 45 to take part in this study.

3. What will I have to do?

Overview

During your involvement in this study you will be required to visit the laboratory at Waterford Institute of Technology on 5 occasions which will comprise of a screening visit,

baseline visit, a test exercise day and two follow up visits. Across these study visits you will be asked to provide a series of biological samples that will consist of the collection of venous blood samples during 4 visits, four urine samples, two stool samples and a series of saliva mouth swabs during two visits. During four of these study visits you will be required to arrive at the laboratory in a fasted state to facilitate blood sampling, on each of these days you will then be provided breakfast at WIT free of charge. As part of this study, you will be asked to ingest your assigned study beverage twice per day for 10 consecutive days, we will provide all beverages to you. Throughout your involvement in this study you will be provided with a list of certain foods that you will be asked to refrain from consuming for the duration of your involvement in the study, these mostly include certain berries, fruits and other related products. Alongside this, on key days related to the study you will be asked to refrain from consuming caffeinated products prior to your study visit and avoid strenuous training for 48 hours prior to the visit.

Screening Visit (Day 0)

Initially you will have to fill out a pre-screening questionnaire which you will be asked to answer a list of yes/no questions which may impact on your eligibility to take part in this study. Once you return this pre-screening questionnaire and you meet the relevant criteria, you will be invited to Waterford Institute of Technology for a screening visit during which you will undertake a baseline VO_2max fitness test to assess your current level of aerobic fitness. This test will involve cycling to exhaustion for between 8 and 15 minutes, while wearing a mask that will monitor your breathing. If you meet our inclusion criteria you will be asked to return to Waterford Institute of Technology on a separate day for a baseline visit. It is estimated that this screening visit will take roughly 45 minutes in total.

Baseline Visit (Day 1)

During the baseline visit you will be required to arrive to the laboratory in a fasted state in order to provide some baseline biological samples including saliva mouth swabs, urine, blood and stool. Following this you will be provided with complimentary standardised breakfast. After breakfast you will return to the laboratory to undertake a series of questionnaires related to your physical and psychological health and wellbeing, take part in a short interview related to the food you have eaten in the previous 24 hours and a 15km cycling time trial on a cycling ergometer. You will then be provided with a 6-day supply of

one of the study beverages. You will be randomly assigned one of 3 beverages, two of which are active interventions while one is a control arm. Over the following 6-days you will consume your assigned study beverage morning and evening and confirm this on a compliance sheet. On the seventh day of the study you will return to WIT for your "test exercise day". It is estimated that the baseline visit will take roughly 1 hour, and a half split across 2x 45 minute sessions in the laboratory separated by breakfast.

Study Beverages

You will be randomly assigned to one of three possible study beverages. All beverages are dairy-based and will be ingested in two doses of 500ml per day for a total of 10 days. Each dose will be ingested morning and evening with between 10 and 14 hours between each dose. All beverages have been produced to commercial standard in regulated production facilities compliant with all food safety standards. Two of these beverages are active interventions while one is a control arm, as such you will have a one in three chance of being in a control group.

Test exercise day (Day 7)

On the morning of the seventh day of this study you will take a saliva swabs for cortisol measurement. You will then be required to arrive to the laboratory in a fasted state where you will be required to provide fasted blood, urine and faecal stool sample. Following this you will be provided with breakfast and your morning study beverage. You will then rest for 2 hours to facilitate digestion. After two hours have passed you will commence exercise which will comprise of a strength test, repeat cycling time trial on a cycling ergometer followed by a series of maximal drop jumps under the supervision of the researchers on the project. After exercising you will be asked to provide a urine sample as well as some saliva swabs. You will then also repeat the strength test you undertook earlier in the day. This will conclude your test exercise day. It is estimated that this study visit will take approximately 2 and a half hours not including a break where you will get breakfast.

24h & 48h Follow-Up (Day 8 & 9)

At 24 and 48h after your test exercise trial you will be asked to return to WIT for follow-up testing. You will be required to arrive in a fasted state to undertake some follow-up measurements including blood samples, urine samples, questionnaires and strength

measurement in a similar manner to as was undertaken earlier in the study. It is estimated each of these visits will take less than one hour.

72h Follow Up (Day 10)

72 hours post exercise you will receive a phone call as a final follow up to rank your level of muscle soreness at this stage. This will mark the end of your involvement in the study

4. What are the exclusion criterion (i.e. are there any reasons why I should not take part)?

- Inability or unwillingness to discontinue use of any nutritional supplement or nutraceutical that may impact performance or recovery
- Have an ongoing injury which will impact their ability to exercise, or a history of landing related lower limb injury
- Being unwilling to consume a dairy product
- Any diagnosis of a psychiatric disorder or taking any anti-psychotic, anxiolytic or anti-depressant medication
- Any use of antibiotic medication within the previous two months or current use of prebiotic or probiotics.
- Any diagnosis of chronic coexisting illness [cardiovascular, gastrointestinal (GI) [to include functional GI disorders, inflammatory bowel disease, coeliac disease, lactose intolerance]
- Have a history of international sporting competition, or recent plyometric training

5. Will my participation involve any physical discomfort?

VO_{2max} testing, which occurs during the screening visit, is challenging even to the fittest individuals and therefore carries exertional discomfort during participation. Delayed onset muscle soreness is commonly experienced in the days following high intensity exercise. A key outcome of this study is to assess your recovery from this soreness and as such it is expected that you will experience this. This soreness is not serious and normally subsides after 48-72 hours varying from individual to individual. Furthermore, for the purpose of measuring blood biomarkers we will require a qualified phlebotomist to take venous blood samples before the exercise protocol. This procedure is considered extremely safe and

carries minimal risk when performed by appropriately trained and certified individuals however may cause some mild discomfort.

6. Will my participation involve any psychological discomfort or embarrassment?

No

7. Will I have to provide any bodily samples (i.e. blood, saliva)?

Yes this study will require you to provide a number of bodily samples including blood samples, saliva samples, urine and stool samples.

Biological Sample	**Timepoint Collected**
Venepuncture Blood Sample	Baseline, Test Exercise Day, 24h Post, 48h Post
Point of Care blood lactate sample (pin prick)	During Cycling Time Trial at baseline & Test Exercise Day
Urine	Baseline, Test Exercise Day (x2), 24h Post, 48h Post
Cortisol Awakening Response	Baseline, Test Exercise Day
Faecal Stool Sample	Baseline, Test Exercise Day

8. How will confidentially be assured?

A number of procedures to protect the confidentially of participants have been put in place. You will be allocated a participant code that will be used to identify any data that you provide. Your name or other personal details will not be associated with your data, for example the consent form you sign will be kept separate from your data.

All paper records will be stored in a locked filing cabinet, accessible only to the research team, and all electronic information will be stored on an encrypted, password protected computer. In general all of the information you provide will be treated in accordance with the General Data Protection Regulation (GDPR).

You will not be identifiable from any academic publications that may arise from this study.

9. Who will have access to the information that I provide?

Any information and data gathered during this research study will only be available to the research team identified. Should the research be presented or published in any form, then that information will be generalised (i.e. your personal information or data will not be identifiable).

10. Has this investigation received appropriate ethical clearance?

Yes, the study and its protocol have received full ethical approval from the Waterford Institute of Technology Research Ethics Committee. If you require confirmation of this please contact the Secretary to the Ethics Committee stating the title of the research project and the name of the principal investigator.

11. Will I receive any benefits for taking part?

For your participation you will receive a €35 "one for all" voucher as a thank you for the time you have taken to be a participant in this study. Alongside this, the VO_2max exercise test results may be of benefit to you as the results of which are commonly used for helping to programme exercise training in elite sport. To carry out this test in a commercial setting often costs between €100 and €150. You will also undergo a dietary assessment during this study which may provide some interesting insight into your habitual nutritional practices and feedback on which may benefit your sporting performance in the future. You will also be provided with breakfast on four occasions free of charge.

12. How can I withdraw from the project?

The research you will take part in will be most valuable if few people withdraw from it, so please discuss any concerns you might have with the investigators. During the study itself, if you decide that you do not wish to take any further part then please inform one of the research team as soon as possible, and they will facilitate your withdrawal. Your involvement in this study is entirely voluntary and you have the right to withdraw at any point without repercussions of any kind. Any personal information or data that you have provided (be it in paper or electronic form) will be destroyed/deleted as soon as possible.

13. If I require further information who should I contact and how?

Conor Carey, PhD Student, conor.carey@umail.ucc.ie, 0877621617

Lorna Doyle, Lecturer WIT, lmdoyle@wit.ie

Appendix 16. – Informed Consent Form

INFORMED CONSENT FORM

TITLE OF PROJECT: RubusElite Nutritional Randomised Controlled Trial

PARTICIPAN ID NUMBER: ☐

Please tick if applicable

I have read and understand the Participant Information Sheet.	☐
I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.	☐
I understand I am free to withdraw from the study at any time, without having to give a reason and without prejudice.	☐
I agree to take part in this study.	☐
I confirm that I have disclosed relevant medical information before this study.	☐
I know that the results may be published, but they will not be linked to me.	☐
I am aware of any possible risks and discomfort.	☐
I agree to inform the researcher immediately if I am in pain, or if I feel uncomfortable.	☐
I would like to receive feedback on the overall results of the study at the email address given below.	☐

Email address……

Signature of participant…… Date…… (NAME IN BLOCK LETTERS)…….
Signature of researcher…… Date…… (NAME IN BLOCK LETTERS)……

This consent is specific to the particular test described in the information sheet attached and shall not be taken to imply my consent to participate in any subsequent experiment or deviation from that detailed here.

Appendix 17. – Compliance Monitoring sheet

Instructions: For the duration of this study, all participants' will be required to consume a beverage twice a day, morning and evening, with an approximate 10–14-hour time period between each dosage. In order to monitor consumption, all participants are required to fill out this sheet and insert a ✅ in the appropriate box, once the drink has been consumed. As well as this, please state the approximate time for both morning and evening that the drink is consumed, which can be recorded in the same box. If in rare circumstances, an issue occurs where the drink is not consumed as instructed, please note any necessary details in the boxes provided so this can be allocated for.

Day of Trial	**Morning**	**Evening**	**Additional Notes**
Day 1	☐ Time:_____	☐ Time:_____	
Day 2	☐ Time:_____	☐ Time:_____	
Day 3	☐ Time:_____	☐ Time:_____	
Day 4	☐ Time:_____	☐ Time:_____	
Day 5	☐ Time:_____	☐ Time:_____	
Day 6	☐ Time:_____	☐ Time:_____	
Day 7	**TEST EXERCISE DAY**	**TEST EXERCISE DAY**	
Day 8	☐ Time:_____	☐ Time:_____	
Day 9	☐ Time:_____	☐ Time:_____	
Day 10	☐ Time:_____	☐ Time:_____	

Appendix 18. – Muscle Soreness Scale

No Pain										Extreme Pain
0	1	2	3	4	5	6	7	8	9	10

Appendix 19. RCT Polyphenol Rich Foods to Avoid List

While taking part in this study, participants are kindly asked to avoid the inclusion of the various foods and drinks listed below, in their diet over the course of 10 days. Participants are also required to avoid alcohol at least 48 hours prior to the test exercise day and avoid caffeine on the days of exercise testing.

Fruits

- Plums
- Apples
- Peaches
- Prunes
- Sweet cherry
- Pomegranate
- Black grapes

Berries

- Blackcurrants
- Blueberries
- Blackberries
- Strawberries
- Red Raspberries
- Black chokeberry
- Black elderberry

NB - *Please be mindful of the possible inclusion of these fruits in various smoothies and juices and please avoid.*

Juices

- Non- concentrated apple juice
- Non- concentrated pomegranate juice
- Non- concentrated Juice
- Tart cherry juice

Jam

- Strawberry Jam
- Plum Jam
- Blackberry Jam
- Raspberry Jam
- Cherry Jam

Beverages

- Green tea
- Black tea (Without milk)
- Red wine
- Filter Coffee
- Star Anise tea

- Blackberry Tea
- Raspberry Tea
- Blueberry Tea

Vegetables

- Black Olives
- Green Olives
- Red chicory
- Green chicory
- Spinach
- Shallot
- Artichoke heads

Seeds

- Chestnuts
- Flaxseed meal
- Soybeans

Nuts

- Hazelnuts
- Pecan Nuts
- Chestnuts
- Almonds

Cereals

- Soy flour
- Wholegrain rye flour
- Wholegrain rye bread
- Maize flour

Powders and Supplements

- Cocoa powder
- Cacao powder
- All protein Supplements
- Creatine
- Branched Chain Amino Acid Supplements (BCAA's)

Other

- Dark chocolate

Appendix 20. Unadjusted RCT data presented as mean ± SD

	Polyphenol & High Protein Milk (n=12)				High Protein Milk (n=10)				Low Protein Milk (n=10)			
	Baseline	Test Day	24h post exercise	48h post exercise	Baseline	Test Day	24h post exercise	48h post exercise	Baseline	Test Day	24h post exercise	48h post exercise
FRAP (microM FeSO4/L serum)	1129±212	1036±157	1118±92	1074±122	1322±204	1234±273	1268±344	1249±224	1296±253	1294±233	1425±319	1257±280
MDA (nmols/mL serum)	0.77±0.12	0.70±0.13	0.73±0.16	0.81±0.21	0.74±0.20	0.72±0.20	0.76±0.15	0.69±0.14	0.83±0.21	0.75±0.14	0.76±0.16	0.78±0.35
F_2 8-Isoprostane (ng/mL plasma)	0.46±0.33	0.56±0.36	0.59±0.38	0.61±0.35	0.37±0.36	0.34±0.32	0.38±0.34	0.43±0.33	0.60±0.52	0.64±0.49	0.61±0.41	0.65±0.50
Protein Carbonyls (nmol/mg prot)	0.47±0.07	0.50±0.08	0.48±0.07	0.48±0.07	0.50±0.03	0.49±0.04	0.45±0.06	0.44±0.04	0.44±0.05	0.46±0.06	0.47±0.05	0.45±0.06

	Polyphenol & High Protein Milk (n=12)				High Protein Milk (n=10)				Low Protein Milk (n=10)			
	Pre-exercise	0h post exercise	24h post exercise	48h post exercise	Pre-exercise	0h post exercise	24h post exercise	48h post exercise	Pre-exercise	0h post exercise	24h post exercise	48h post exercise
IMTP Muscle Strength (N)	2241±509	2117±417	2036±443	2069±468	2694±346	2408±452	2458±542	2259±362	2238±612	2103±524	1959±497	2057±526

	0h post exercise	24h post exercise	48h post exercise	72h post exercise	0h post exercise	24h post exercise	48h post exercise	72h post exercise	0h post exercise	24h post exercise	48h post exercise	72h post exercise
Muscle Soreness	2.3±1.6	4.9±2.0	4.0±1.5	1.25±1.0	4.0±2.2	5.8±2.3	5.2±2.7	3.5±3.0	4.0±1.8	5.9±1.4	5.1±2.1	2.3±2.1