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The effectiveness of dairy milk as an exercise recovery beverage: Assessment on overall recovery markers.

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Lion Dairy and Drinks.

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Abstract

Prolonged, strenuous exercise is known to reduce endogenous muscle and liver glycogen stores, induce skeletal muscle damage, and body water and electrolyte losses, transiently depress innate immune function, and disrupt gastrointestinal integrity and function. It is common practice for athletes to implement recovery nutrition practices to promote recovery from exercise stress. Current recovery nutrition guidelines offer generalised recommendations for replacement of energy substrate, restoration of body water losses, and repair of damaged tissues (e.g., skeletal muscle). However, these guidelines have failed to consider the restoration of immunocompetency, and the impact of exercise-induced gastrointestinal syndrome (EIGS) on regulation of nutrient intake, digestion and (or) absorption.

Over recent years there has been increased awareness and consumption of dairy products for exercise recovery, including dairy 'whole foods' (e.g., flavoured dairy milk and dairy-based drinking yoghurts) and specially formulated dairy-based sports beverages. Flavoured dairy milk beverages contain naturally occurring (lactose) and added (sucrose, fructose and (or) glucose) carbohydrate, protein (whey and casein), and sodium, in close alignment with current recovery nutrition guidelines.

The purpose of this thesis was therefore to firstly conduct a systemic review of the current body of literature to determine the ideal nutritional intake to support specific aspects of recovery (i.e., skeletal muscle glycogen resynthesis, skeletal muscle protein synthesis, rehydration, immune function and gastrointestinal integrity and function). A second review was conducted to determine the effectiveness of dairy milk products in the context of recovery optimisation. A normative methodological approach to exercise recovery research was established and applied to three counterbalanced randomised cross-over trials to investigate the effectiveness of a flavoured dairy milk beverage (i.e., chocolate milk (CM)) on markers of recovery optimisation, and subsequent exercise performance, compared to a) a non-nitrogenous carbohydrate-electrolyte beverage (CEB), b) a high nutrient density reconstituted milk-

based sports beverage (MBSB) and c) an iso-nitrogenous low carbohydrate dairy milk-based sports beverage (L-CHO).

Findings from this thesis indicate that a 2 h high intensity interval exercise (HIIT) exercise resulted in mild intestinal epithelial injury, depressed innate immune functional responses, post-exercise skeletal muscle glycogen stores of <300 mmol/kg dry weight, and mild body water losses (i.e., ~2% body mass loss). During the recovery period, clinically relevant malabsorption occurred with all recovery beverages (i.e., peak breath hydrogen >10ppm). Significantly greater malabsorption and associated gastrointestinal discomfort was observed after consumption of a high nutrient density reconstituted milk-based sports recovery beverage, compared to the flavoured dairy milk beverage. *E.coli* lipopolysaccharide stimulated neutrophil function reduced on all beverages (20-49%), most likely associated with the delay in feeding. The flavoured dairy milk beverage containing the recommended 1.2 g carbohydrate/kg body mass for resynthesis of skeletal muscle glycogen stores, failed to increase muscle glycogen stores within 90 min of consumption, possibly associated with impaired insulin signalling and (or) GLUT-4 translocation due to plyometric induced muscle damage. All recovery beverages successfully restored hydration status to pre-exercise levels. Subtle differences in fluid dynamics were observed, as it appears that a greater nutrient density mediates greater fluid retention. All dairy nitrogenous beverages increased intramuscular anabolic signalling of mTOR, but the non-nitrogenous CEB did not. Physiological and performance outcomes on the following day did not differ between any of the trials.

Based on the findings within this thesis, athletes are advised to consume small and frequent doses of nutritional composition equivalent to 1.2 g carbohydrate and 0.4 g protein/kg BM over the 1-2 h acute recovery time period, and return to habitual dietary patterns thereafter. While nutritional composition of the recovery beverage does not influence subsequent performance 24 h after the initial exercise bout, these practices are likely to have acute clinical (i.e., minimising gastrointestinal burden,

stimulating neutrophil function towards clearance of tissue debris, endotoxins and luminal-derived pathogenic agents, reduced risk of illness and soft tissue injury) and physiological (i.e., maximised nutrient absorption and hydration, substrate provision for repletion of skeletal muscle glycogen, and substrate and anabolic stimulus for skeletal muscle repair and adaptation) benefits. Given the large individual variation in susceptibility to EIGS, food and fluid tolerance, and absorptive capacity, practitioners are encouraged to conduct individual tolerance and (or) EIGS clinical assessment.

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes one original paper submitted as an industry report, two published in peer reviewed journals and two submitted publications. One additional publication is included within the Appendices. The core themes of the thesis are sports nutrition and applied exercise physiology. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the Department of Nutrition and Dietetics under the supervision of Dr Ricardo Costa and external supervisors Associate Professor Judi Porter, School of Exercise and Nutrition Sciences, Deakin University, and Professor Louise Burke, Mary MacKillop Institute for Health Research, Australian Catholic University.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapters 2-5 my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student Y/N*
2 Part I	Systematic literature review: The ideal recovery nutrition intake to support recovery optimisation.	Accepted (Industry report)	60%. Concept, collecting data and writing first draft.	1) Vera Camões-Costa assisted with study screening, data extraction and input into manuscript (2%) 2) Stephanie K. Gaskell assisted with risk of bias assessment and input into manuscript (2%) 3) Judi Porter assisted with search strategy and input into manuscript (3%) 4) Louise M. Burke concept and input into manuscript (3%) 5) Ricardo J. S. Costa assisted with concept, data analysis and input into manuscript (30%)	1) No 2) Yes 3) No 4) No 5) No
2 Part II	Systematic Literature Review: The Effect of Dairy Milk on Markers of Recovery Optimisation in Response to Endurance Exercise	Accepted	60%. Concept, collecting data and writing first draft.	1) Vera Camões-Costa assisted with study screening, data extraction and input into manuscript (2%) 2) Stephanie K. Gaskell assisted with risk of bias assessment and input into manuscript (2%) 3) Judi Porter assisted with search strategy and input into manuscript (3%) 4) Louise M. Burke concept and input into manuscript (3%) 5) Ricardo J. S. Costa assisted with concept, data analysis and input into manuscript (30%)	1) No 2) Yes 3) No 4) No 5) No

3	Assessing overall exercise recovery processes using carbohydrate and carbohydrate-protein containing recovery beverages.	Submitted	60% Concept, data and sample collection, data and sample analysis, drafting manuscript	1) Paul A. Della Gatta performed muscle sample analysis and input into manuscript (2%) 2) Andrew Garnham performed muscle biopsy procedures and input into manuscript (2%) 3) Judi Porter assisted with concept and input into manuscript (3%) 4) Louise M. Burke assisted with concept and input into manuscript (3%) 5) Ricardo J. S. Costa assisted with concept, data collection and analysis and input into manuscript (30%)	1) No 2) No 3) No 4) No 5) No
4	Does the nutritional composition of dairy milk based recovery beverages influence post-exercise gastrointestinal and immune status, and subsequent markers of recovery optimisation in response to high intensity interval exercise?	Accepted	As above	As above	As above
5	The effect of an acute 'train-low' nutritional protocol on markers of recovery optimisation in endurance trained male athletes.	Submitted	As above	As above	As above

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: Isabella Russo **Date:** 08/01/2021

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: Ricardo Da Costa **Date:** 08/01/2021

Publications

The following publications are included as **Chapter 2 (Part I and II), Chapter 3, Chapter 4, Chapter 5 and Appendix 1** within this thesis with the contribution to publications previously outlined in the 'thesis including published works declaration'.

Russo, I., Camões-Costa, V., Gaskell, S. K., Porter, J., Burke, L. M., & Costa, R. J. (2018) Systematic literature review: The ideal post-exercise nutrition intake to support recovery optimisation. Industry Report for Lion Dairy and Drinks.

Russo, I., Camões-Costa, V., Gaskell, S. K., Porter, J., Burke, L. M., & Costa, R. J. S. (2019). Systematic literature review: The effect of dairy milk on markers of recovery optimisation in response to endurance exercise. *International Journal of Sports Science*, *9*(4), 69-85.

Russo, I., Della Gatta, P.A., Garnham, A., Porter, J., Burke, L.M., Costa, R.J.S., (2021). Assessing overall exercise recovery processes using carbohydrate and carbohydrate-protein containing recovery beverages. *Frontiers in Physiology*, 12, 50.

Russo, I., Della Gatta, P.A., Garnham, A., Porter, J., Burke, L.M., Costa, R.J.S., (2020). Does the nutritional composition of dairy milk based recovery beverages influence post-exercise gastrointestinal and immune status, and subsequent markers of recovery optimisation in response to high intensity interval exercise?. *Frontiers in Nutrition,* 7, 343.

Russo, I., Della Gatta, P.A., Garnham, A., Porter, J., Burke, L.M., Costa, R.J.S., (2021) The effect of an acute 'train-low' nutritional protocol on markers of recovery optimisation in endurance trained male athletes. *International Journal of Sports Physiology and Performance,* In Press.

Russo, I., Della Gatta, P.A., Garnham, A., Porter, J., Burke, L.M., Costa, R.J.S., (2020) The influence of fitness status and biological sex on markers of recovery optimisation following high intensity interval exercise. *International Journal of Sports Science,* 10(6): 145-163.

The following two co-authored publications were completed as team-based research during the Ph.D. candidature and are not included within this thesis. My contribution to these publications includes assistance with data and sample collection, data and sample analysis and input into each manuscript.

Costa, R. J.S., Camões-Costa, V., Snipe, R. M., Dixon, D., Russo, I., & Huschtscha, Z. (2019). Impact of exercise-induced hypohydration on gastrointestinal integrity, function, symptoms, and systemic endotoxin and inflammatory profile. *Journal of Applied Physiology*, *126*(5), 1281-1291.

Costa, R. J.S., Camões-Costa, V., Snipe, R. M., Dixon, D., Russo, I., & Huschtscha, Z. (2020). The Impact of a Dairy Milk Recovery Beverage on Bacterially Stimulated Neutrophil Function and Gastrointestinal Tolerance in Response to Hypohydration Inducing Exercise Stress. *International Journal of Sport Nutrition and Exercise Metabolism*, *30*(4), 237-248.

Conference Abstracts

The Impact of Post-Exercise Chocolate Flavoured Dairy Milk Beverage Consumption on Hydration Status in Endurance Athletes, September 2019, International Journal of Sports Physiology and Performance 14(S1):2. Presented October 2019, the 6th Annual Congress on Medicine & Science in Ultra-Endurance Sport, Cape Town.

The Impact of Post-Exercise Chocolate Flavoured Dairy Milk Beverage Consumption on Gastrointestinal Symptoms, Breath Hydrogen and Blood Glucose Responses in Endurance Athletes, September 2019, International Journal of Sports Physiology and Performance 14(S1):4. Presented October 2019, Sports Dietitians Australia Conference, Melbourne.

Poster presentation - The impact of recovery nutrition on immune cell trafficking, innate function, and cytokine profile in response to intermittent high intensity endurance exercise- preliminary data, November 2019, International Society of Exercise Immunology Conference, Shanghai.

Poster presentation - The Impact of Post-Exercise Chocolate Flavoured Dairy Milk Beverage Consumption on Hydration Status in Endurance Athletes, October 2019, Sports Dietitians Australia Conference, Melbourne.

Poster presentation - The Impact of Post-Exercise Chocolate Flavoured Dairy Milk Beverage Consumption on Gastrointestinal Symptoms, Breath Hydrogen and Blood Glucose Responses in Endurance Athletes, October 2019, the 6th Annual Congress on Medicine & Science in Ultra-Endurance Sport, Cape Town.

Poster presentation - Exercise-induced dehydration causes malabsorption of carbohydrate rich pre-exercise meal, December 2018, The International Sports and Exercise Nutrition Conference, Newcastle Upon Tyne.

Oral presentation – Sports Recovery Nutrition and Hydration, May 2019, Sports Medicine Australia; Weekend Warrior Symposium, Traralgon.

Oral presentation – Recovery Optimisation for Female Athletes, October 2020, Sports Dietitians Australia Female Athlete Symposium, [virtual] Australia.

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Table of Contents

Thesis Format

The general introduction (**Chapter 1**) and literature reviews (**Chapter 2, Part I and Part II**) provide background, rationale and justification for the aims and research methodology provided in this thesis. This thesis consists of three original research studies which build on the findings and methodology presented in **Chapter 2**. All studies follow the same general experimental protocol within **Chapter 3, 4 and 5**. **Chapter 3** explores the impact of two common post-exercise beverages (a non-nitrogenous carbohydrate-electrolyte beverage and a flavoured dairy milk beverage) on integrative markers of recovery following an exercise stress known to perturb many aspects of physiological and metabolic homeostasis. **Chapter 4** compared the effect of nutrient density on comprehensive markers of recovery optimisation, by comparing a flavoured dairy milk beverage to an isovolumetric intake of high nutrient density dairy milk-based sports beverage. Finally, **Chapter 5** compared acute recovery outcomes following consumption of a low-carbohydrate diary milk-based beverage to an iso-nitrogenic flavoured dairy milk beverage. Each study presents outcomes in response to the exercise stress, and subsequently recovery outcomes related to gastrointestinal function and symptomology, immune function, muscle glycogen resynthesis, intramuscular cellular signaling, hydration, and psychophysiological outcomes the following morning. These experimental studies are followed by a general discussion chapter (**Chapter 6**) that provides a summary and critical analysis of the overarching findings of this thesis, including limitations and directions for future research, and is followed by the thesis conclusions. An additional sub-group analysis by fitness status and biological sex is included in **Appendix 1**. Throughout the thesis, abbreviations are defined at first use, excluding the list of tables and figures. A full list of abbreviations, tables and figures is provided prior to **Chapter 1**. An overlap in content occurs between chapters due to the complementary nature of the studies and existing links between chapters. Bold type is used to reference other sections within this thesis.

List of Tables

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List of Abbreviations

4E-BPI	eukaryotic initiation factor 4E-binding protein
Akt	protein kinase B
AMPK	5’ adenosine monophosphate kinase
ANOVA	analysis of variance
ATP	adenosine triphosphate
AUC	area under the curve
BIA	bioelectric impedance analysis
BM	body mass
bpm	beats per minute
BSA	bovine serum albumin
C MRS	carbon magnetic resonance spectroscopy
CEB	carbohydrate electrolyte beverage
CHO	carbohydrate
Δ	change
CI	confidence interval
CK	creatine kinase
CM	chocolate flavoured dairy milk
CRB	carbohydrate replacement beverage
CV	coefficient of variation
dw	dry weight
EAA	essential amino acid
ECW	extracellular water
EE	endurance exercise
eEF2	eukaryotic elongation factor 2

EIGS	exercise induced gastrointestinal syndrome
ELISA	enzyme-linked immunosorbent assay
≥	equal to or greater than
≤	equal to or less than
F	female
FODMAP	fermentable oligo- di- and monosaccharides and polyols
FoxO-3A	forkhead box OA
FSR	fractional synthetic rate
GIS	gastrointestinal symptoms
GIT	gastrointestinal tract
GLUT-4	glucose transporter 4
GSK-3β	glycogen synthase kinase 3β
h	hour
H_2	hydrogen
HP-MILK	high protein milk
I-FABP	intestinal fatty acid binding protein
ICW	intracellular water
IL	interleukin
IU	international units
kg	kilogram
L	litre
LBP	lipopolysaccharide binding protein
LS-MILK	low sugar milk
M	male
m	metre

MBSB	milk-based sports beverage
MEOTime	main effect of time
µg	microgram
min	minute
MJ	megajoule
ml	millilitre
µL	microlitre
mmol	millimole
mOsmol	milliosmole
MPB	muscle protein breakdown
MPS	muscle protein synthesis
mRNA	messenger ribonucleic acid
mTOR	mammalian target of rapamycin
MuRF1	muscle ring finger protein 1
Neg	negligible
NFB	net fluid balance
NPB	net protein breakdown
p38-MAPK	p38 mitogen-activated protein kinase
p70S6K	ribosomal protein p70 S6 kinase
pg	picogram
PGC-1	peroxisome proliferator-activated receptor-γ-1α
PICOS	participant intervention control outcome study
P_{osmol}	plasma osmolality
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRO	protein

P_v	plasma volume
ra	receptor antagonist
RE	resistance exercise
RH	relative humidity
rpS6	ribosomal protein S6
s-IgA	salivary immunoglobulin A
SD	standard deviation
SEM	standard error from the mean
SIR	systemic inflammatory response
SLR	systematic literature review
T_{amb}	ambient temperature
TBST	tris-buffered saline-tween
TBW	total body water
TNF	tumour necrosis factor
T_{re}	rectal temperature
TT	time trial
TTE	time to exhaustion
vs	versus
W_{max}	maximum wattage
ww	wet weight

Chapter One

General Introduction

Endurance and moderate- to long-duration intermittent sports are amongst the most popular sports worldwide (1). For example, participation in distance running events has risen by 57% over the past decade by attracting mass participation of recreational and sub-elite runners (2), while soccer, tennis and basketball have been consistently identified as the most participated in sports across continents (1). With increasing popularity in recreational and mass-participation endurance events, there has been a concomitant rise in the sports foods and supplement industry (3). These products are largely marketed to the sub-elite and recreationally active masses, but are frequently used by elite athletes alike (4). The most commonly cited reason for use of sports supplements and foods is to aid recovery (5).

It is well established that the extent of physiological and biochemical perturbations that occur during exercise are major determinants of the quantity of nutrients required to optimally recover (6). Prolonged strenuous exercise (i.e., 90-120 min at 70-75% $\dot{V}O_{2max}$) (7,8), and prolonged intermittent exercise (i.e., 90 min Loughborough Intermittent Shuttle Test) (9,10), is known to reduce muscle glycogen and body water content, and induce skeletal muscle damage, respectively. However, less attention has been given to the effect of exercise induced perturbations on the bioaccessibility and bioavailability of these nutrients. Exercise induced gastrointestinal syndrome (EIGS) is a term used to describe the multifaceted physiological responses to exercise stress that impact gastrointestinal function (i.e., reduced gastric motility, gastric emptying and translocation of epithelial transporters), integrity (i.e., ischemic damage and erosion of epithelial cells and physical breaks in the epithelium), and symptoms (i.e., reduced tolerance to food and fluid, and gastrointestinal discomfort) (11). Moreover, prolonged strenuous exercise is also known to induce a systemic leukocytosis, and depress several immune cell functional responses (12). A coordinated and functional response is required for removal of environmental and (or) luminal-derived pathogenic agents, repair gastrointestinal-

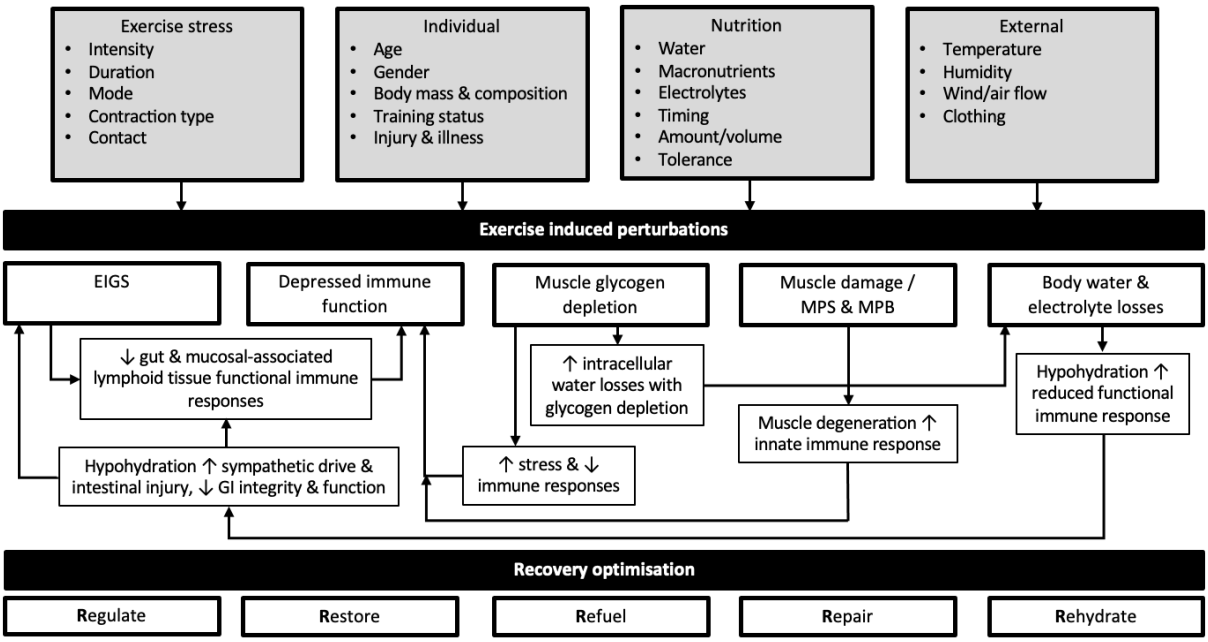
associated lymphoid tissue structure and function, and clear skeletal muscle tissue debris (11,13-16). Therefore EIGS and exercise-induced immunodepression following prolonged strenuous exercise may impair nutrient assimilation and subsequent recovery outcomes.

Recovery from exercise can be characterised by the replacement of fluid and substrate losses, and repair and adaptation of skeletal muscle tissue (6). This is typically referred to as the "*three Rs*" of exercise recovery; refuelling of substrate losses, repair of damaged muscle and rehydration. However, the "*three Rs*" approach does not take into consideration nutritional intake for regulation of the gastrointestinal tract and restoration of immunocompetency (17,18). Exercise recovery outcomes often have intersecting nutritional goals, however there are occasions when nutrition strategies conflict. For example, withholding carbohydrate during recovery with the intention of minimising muscle glycogen repletion, and completing the subsequent training with low carbohydrate availability is a strategy that may support endurance training adaptations (19). '*Recovery optimisation*' provides an additional contribution to the sophistication of our knowledge and practice of recovery nutrition by integrating recovery strategies that maximise desired outcomes while minimising those that cause detrimental outcomes within the complex and interrelated recovery responses to exercise.

Athletes are reportedly consuming a wide variety of forms and doses of recovery nutrition foods and supplements (4,5). Moreover, the variability in nutrient quantity and quality between analogous recovery nutrition products further adds to the likelihood that within professional practice, athletes may be under-meeting or over-consuming nutrients. Over recent years there has been increased awareness and consumption of dairy products for exercise recovery (20). This includes dairy 'whole foods' (e.g., flavoured dairy milk and dairy-based drinking yoghurts) and specially formulated dairy-based sports beverages. Flavoured dairy milk beverages contain naturally occurring (lactose) and added (sucrose, fructose and (or) glucose) carbohydrate, protein (whey and casein), and electrolytes (i.e., sodium and potassium), in close alignment with current recovery nutrition guidelines (6). Compared to

supplements, there is currently a lack of research examining the use of dairy products for overall recovery outcomes, with outcomes specific to gastrointestinal regulation and immune restoration, research is excessively scarce.

Figure 1.1 Schematic representation of inter-connected nature of exercise-induced perturbations.



EIGS: Exercise-induced gastrointestinal syndrome, GI: gastrointestinal, MPS: muscle protein synthesis, MPB: muscle protein breakdown.

There is a large body of research examining the efficacy of recovery nutrition strategies on isolated aspects of recovery, however well-controlled laboratory-based studies examining recovery in its entirety is lacking. Studies are at risk of confounding by usual dietary habits, individual nutritional requirements and variability in exercise-induced perturbations of the work performed due to training status of participants and experimental exercise stress (21). Ultimately, the conferred training and performance benefits of sports supplements are achieved by providing nutrients where nutritional needs are unmet by normal food intake, and through appropriate application of evidenced-based protocols. In order to design evidenced-based protocols for targeted training and performance outcomes, it is important to foremost understand the acute metabolic and mechanistic changes following consumption of supplements or sports foods. Thereon, individual responses to a given exercise stress as well as the bioaccessibility and bioavailability, which is predominantly regulated by

the patency and functionality of the gastrointestinal tract, must also be considered in the application of a recovery beverage within the athlete's competition and training regimen (21).

The purpose of this thesis was therefore to firstly conduct a systemic review of the current body of literature to determine the ideal nutritional intake to support specific aspects of recovery (i.e., skeletal muscle glycogen resynthesis, skeletal muscle protein synthesis, rehydration, immune function and gastrointestinal integrity and function). A second review was conducted to determine the effectiveness of dairy milk products in the context of recovery optimisation. A normative methodological approach to exercise recovery research was established and applied to three counterbalanced randomised cross-over trials to investigate the effectiveness of a flavoured dairy milk beverage on markers of recovery optimisation, and subsequent performance, compared to a) a non-nitrogenous carbohydrate-electrolyte beverage (CEB), b) a high nutrient density reconstituted milk-based sports beverage (MBSB) and c) an iso-nitrogenous low carbohydrate dairy milk-based sports beverage (L-CHO). It was hypothesised that a) the greater energy and nutrient density of a flavoured dairy beverage (CM) would result in greater muscle glycogen resynthesis, muscle protein synthesis, fluid retention, and enhanced immune functional responses, compared with a non-nitrogenous carbohydrate-electrolyte beverage (CEB). In addition, the greater energy and nutrient density would result in greater ratings of feeding intolerance and gastrointestinal symptoms (GIS); b) the greater energy and nutrient density of a dairy based supplement beverage (MBSB) would result in greater muscle glycogen resynthesis, muscle protein synthesis, fluid retention, and enhanced immune functional responses, compared with a dairy milk beverage (CM). In addition, the greater energy and nutrient density would result in greater ratings of feeding intolerance and GIS; and c) the low-carbohydrate nutritional protocol (L-CHO) would result in a diminished rate of glycogen resynthesis, rehydration properties, immune functional responses, and subsequent endurance performance the following day, compared with standard carbohydrate intake (CM); but would not differ in protein synthesis expression markers, and may limit the gastrointestinal burden in response to nutrient intake into an exercise-associated compromised gastrointestinal tract.

The findings from the novel research within this thesis have the potential to add to the current recovery nutrition guidelines thereby increasing the applicability across demographics and enhance nutrition strategies for recovery optimisation. From a practical perspective, nutritional interventions that reduced gastrointestinal burden may result in improved nutritional bioaccessibility and bioavailability, which in turn may reduce illness and infection rates, and improved refuelling of skeletal muscle glycogen stores, skeletal muscle repair, and subsequent day performance. Likewise, possible clinical implications include reduced risk of contracting pathogen-borne infection, reduced risk of soft-tissue injury and reduced gastrointestinal symptoms. Moreover, the established methodological approach may provide guidance for future recovery nutrition research and data interpretation for the investigation of novel nutritional strategies, as well as validation of the acute metabolic and mechanistic changes, and exercise performance outcomes, induced by sports recovery foods and supplements.

Chapter Two

Part I

Systematic literature review: The optimal nutrition intake to support recovery optimisation in response to endurance exercise.

Abstract

The food and fluid provided in the acute post exercise period plays an essential role in recovery and adaptation to strenuous exercise. Strenuous physical exertion places metabolic, thermoregulatory, cardiovascular, and inflammatory strain on the body, resulting in muscle damage, endogenous glycogen depletion, dehydration, and impaired immune function and gut integrity. In order to effectively restore all of these systems and maximally enhance training adaptations, an all-encompassing approach to recovery nutrition is required. The aim of the current review was to systematically review the literature investigating optimal acute recovery nutrition intervention to support i) muscle glycogen resynthesis ii) muscle protein synthesis iii) rehydration iv) immune function and v) gastrointestinal function and integrity after prolonged, strenuous exercise (i.e., 60-120 min at 70-75% $\dot{V}O_{2max}$). Results from this review suggest that an intake of 0.8-1.2 g carbohydrate/kg body mass (BM)/h and 0.2-0.4 g protein/kg BM/h during the acute recovery period (i.e., within 4 h after strenuous exercise) will maximally stimulate muscle glycogen resynthesis and muscle protein synthesis, and support functional immune responses by preventing a decrease in bacterially-stimulated neutrophil degranulation and increase salivary lysosome concentration. Additionally, fluid intake at a rate of 20-35 ml/kg BM/h with 20-40 mmol/L sodium is optimal for rehydration when moderate to mild dehydration (e.g. <3% BM loss) is experienced. The current review retrieved only one study related to gastrointestinal integrity and function, and recovery nutrition, showing mild intestinal injury and carbohydrate malabsorption during the acute recovery period.

Introduction

It is well established that the repeated skeletal muscular contractions performed during exercise cause disruptions to physiological (i.e., cardiorespiratory, neuroendocrine, thermoregulatory, and immune stress) and biochemical (i.e., substrate depletion, increased redox potential and other metabolic by-products) homeostatic systems. Shifts within these systems occur in order to regenerate adenosine tri-phosphate (ATP) molecules at a rate matched to the exercise's energy demands. Sports and exercise can be classified by muscle metabolism (i.e., aerobic, or anaerobic), as well as the mechanical action of muscle (i.e., static or dynamic) (22). Therefore, the physiological and biochemical responses to exercise are influenced by the duration, intensity, and mode of exercise. For example, short duration, powerful sports that require maximal recruitment of motor units from anaerobic energy systems (e.g., lifting, throwing, jumping, and sprinting) will induce an acute elevation of heart and respiratory rates, and blood lactate levels (23). Conversely, prolonged, strenuous exercise, such as distance running and road cycling, is primarily dependent on energy produced through oxidation of fatty acids and glucose. This type of exercise results in a sustained elevation of heart and respiratory rates, production of metabolic heat and reactive oxygen species, electrolyte imbalances across the myofibrillar membrane, and depletion of energy substrates (i.e., muscle glycogen stores) (23). Sports such as soccer, basketball, and tennis, whereby games or matches are interspersed with periods of walking, running, sprinting, and jumping for up to several hours, require athletes to concurrently develop an efficient aerobic energy system, while maintaining the ability to draw upon explosive, anaerobic power (23).

Recovery from exercise can be characterised by two objectives: firstly, the restoration of homeostatic systems and replacement of fluid and substrate losses, and secondly, the initiation of adaptation processes to the exercise stimulus (24). With regards to prolonged, strenuous exercise, homeostatic recovery has historically focused on the *'three Rs'*; refuelling of muscle glycogen stores, repair of damaged muscle and rehydration. However, the importance of restoration of immunocompetency and regulation of the gastrointestinal tract have recently been acknowledged as additional focal points of

exercise recovery (11,25). Adaptation to prolonged, endurance exercise is characterised by an increase in the oxidative capacity of the working muscle through an increase in the size and density of mitochondria, and increased activity of oxidative enzymes (26). Both objectives of recovery may be influenced acutely by nutritional intake.

Muscle glycogen resynthesis

It is widely accepted that the availability of stored skeletal muscle glycogen is a major determinant of prolonged exercise performance (27). During moderate- to high-intensity exercise (65-85% $\dot{V}O_{2max}$), muscle glycogen is the primary substrate utilised for oxidative phosphorylation (28). Failure to adequately replenish muscle glycogen stores between training sessions and competition is a major limiting factor for subsequent endurance performance, particularly when there is limited time for recovery.

Carbohydrate intake is the primary determinant of muscle glycogen resynthesis (29). In the absence of carbohydrate intake, glycogen is resynthesised at a slower rate (~7-12 mmol/kg dry weight (dw)/h) via gluconeogenesis (30). When carbohydrate is consumed, glucose transport across the sarcolemma is facilitated by insulin- and (or) contraction-induced translocation of glucose-tranporter-4 (GLUT-4) (31). Within the muscle, glycogen synthase activity is regulated by muscle contractions, insulin, and low muscle glycogen concentrations (i.e., <150 mmol/kg dw) (32). Maximum rates of glycogen resynthesis have been reported between 40-50 mmol/kg dw/h with carbohydrate intake during the acute recovery period (29). The rate of synthesis then attenuates as glycogen stores become replete (33).

The muscle biopsy technique is commonly used by researchers to observe changes in muscle glycogen content of individual muscle samples in response to exercise stress and (or) nutrition interventions (34). Procedures using magnetic spectroscopy have also been implemented in sports and nutrition research. While these methods provide an equally, or more accurate, measurement of muscle glycogen content,

along with lower participant burden, they are seldom used due to limitations related to accessibility and time required to perform each measurement (35,36). Moreover, biopsy samples may also be used for analysis of metabolites and proteins (i.e., GLUT-4, glycogen synthase activity) within the muscle. Collection of venous and (or) capillary blood samples for analyses of blood glucose, insulin, cortisol and other biomarkers have provided further insight into glucose dynamics and glycogen storage.

Muscle protein synthesis

Damaged or dysfunctional proteins within the muscle are continually being hydrolysed into constituent amino acids (muscle protein breakdown (MPB)), while new proteins are simultaneously being synthesised from the existing amino acid pool (muscle protein synthesis (MPS)). The continuity of these processes maintains the quality and function of proteins, while the relative magnitudes of MPS and MPB underpin the progression of training adaptations. A positive net protein balance (NPB), whereby MPS exceeds MPB, is required for the accretion of proteins (37). The specificity of the contractile stimulus, as well as exogenous protein intake, have independent and cumulative effects on MPS. MPS transiently increases following resistance exercise (RE) (38,39), and endurance exercise (EE) (40,41). Without exogenous protein intake, NPB will remain negative (39,40). Hyperaminoacidemia initiates signalling cascades that stimulate MPS, and adds to the precursor pool to provide a substrate for MPS. RE and EE are known to stimulate intersecting yet distinct signalling pathways towards specific skeletal muscle phenotypes (42). For example, RE will stimulate myofibrillar protein synthesis to a greater extent than EE, resulting in greater cross-sectional area and neural adaptation, towards muscle fibre hypertrophy and increased strength and (or) power (39). Differently, skeletal muscle adaptation of endurance athletes is primarily characterised by an increase in mitochondrial biogenesis and mitochondrial enzyme activity, thereby increasing the oxidative capacity and fatigue resistance of the contracting muscle (26).

Mechanoreception is the mechanism underpinning the morphological, biochemical and physiological alterations to the muscle that occur as a result of coupling mechanical, contractile stimuli to intracellular biochemical signal (43). For example, muscular contractions and the associated cellular disruptions (i.e., reactive oxygen species, lactate, calcium influx, and energy availability) will stimulate a signalling cascade involving thousands of proteins that converge to initiate messenger RNA (mRNA) translation. The mRNA translation phase regulates the progression of MPS towards muscular adaptations (44). Mechanistic target of rapamycin (mTOR) has been implicated as a key regulator of translation initiation and protein synthesis (45). Preceding and subsequent signalling proteins of the mTOR signalling pathway including protein kinase B (Akt) and ribosomal protein S6 (rpS6), respectively, have also been associated with signalling towards increased myofibrillar protein synthesis. Phosphorylation of the mTOR pathway has been shown to increase most notably after RE, and to a lesser extent, EE and concurrent exercise protocols (42,46-48). EE is a more potent stimuli for signalling pathways that increase mitochondrial protein synthesis. This includes peroxisome proliferator-activated receptor-γ-1α (PGC-1α), which in turn is activated by signalling kinases p38 mitogen-activated protein kinase (p38-MAPK) and 5' adenosine monophosphate-activated protein kinase (AMPK). These signalling pathways intersect and respond to multiple stimuli (48). As such, measuring activation of these protein does not quantify the magnitude of MPS. In the laboratory setting, the rate of MPS is quantified by means of stable isotope tracer methodology. The fractional synthetic rate (FSR) of mixed, myofibrillar and mitochondrial proteins can be measured by determining the rate at which the labelled amino acid or deuterium oxide is incorporated into the specific muscle-bound protein, compared to the enrichment of the precursor pool, over a given period of time (49,50).

Hydration status

Replacement of fluid and electrolyte losses incurred during exercise (i.e., sweat, respiratory, and urine losses) in the initial hours following exertional stress is required to prevent decrements in performance during subsequent training or competitive events (6,51,52). Exercising in a hypohydrated state will

increase physiological strain, characterised by an increased core body temperature, heart rate, and perceived exertion, and decreased cognitive capacity and endurance performance (53). The degree of dehydration experienced after exercise is highly variable. Factors influencing fluid losses include environmental conditions (i.e., temperature, humidity and air flow) (54), intensity and duration of exercise (55), fluid replacement during exercise (i.e., fluid type, volume, and timing), and individual factors (i.e., body mass, heat acclimatisation, individual variability in sweat rates) (56). Therefore, current recommendations for post-exercise fluid replacement state that fluid should be consumed relative to losses incurred during the exercise session (52).

Fluid-electrolyte balance is complex and dynamic. Assessment of this balance in the laboratory setting requires the use of multiple techniques, as there is no single method that that can accurately and reliably measure hydration status (57). It has been suggested that the use of plasma osmolality (P_{Osmol}) and total body water (TBW) represent the 'gold standard' for methods of assessing hydration status in laboratory studies (58). Bioelectrical impedance analysis (BIA) with validated equipment may provide a reliable estimate of TBW. Vascular markers of hydration including P_{Osmol} and plasma volume (P_V) change are under homeostatic control, but will vary acutely in response to diet and exercise (59). In the controlled laboratory setting, P_{Osmol} can provide a valid and reliable measure of dehydration, with an approximate increase of 5 mOsmol/kg for every 2% BM loss, when fluid loss exceeds solute loss (59). Use of nude BM changes and (or) net fluid balance (NFB) as an indicator of body fluid change are valuable markers when used in conjunction with other hydration assessment methods. Urinary measures (including urine specific gravity and urine osmolality), rating of thirst and salivary measures do not measure extracellular (ECW) and intracellular (ICW) directly. As such, they do not provide the validity and reliability required for use in the laboratory setting. These methods should be applied as secondary outcomes to TBW, P_{Osmol} and nude BM change.

Immunocompetency

The immune system is comprised of an intricate network of cells and proteins that protect the body from pathogenic invasion. In the context of training and athletic performance, the immune system also plays a role in de- and regeneration of muscle and epithelial tissue, metabolism, thermoregulation, and sleep (60). Regulation of the immune system is typically categorised into innate and acquired immune systems. Innate immune function is the non-specific first line of defence against pathogenic invasion. Defensive mechanisms include physical (e.g., epithelium and mucosal barriers) and chemical barriers (e.g., acidic stomach fluids), and phagocytic and other non-specific killer cells. Leukocytes including neutrophils, dendritic cells, macrophages and natural killer cells, and proteins such as interferon, defensins and complement proteins act to identify and remove pathogen agents before infection occurs, and (or) initiate an adaptive immune response (12).

A single bout of prolonged strenuous exercise has been shown to decrease innate and adaptive immune system functions including acute leukocytosis (e.g., neutrophilia and lymphocytosis followed by lymphopenia), reduced immune cell function (e.g., chemotaxis, phagocytosis, degranulation and oxidative burst), and increased inflammatory cytokine responses (12,61). Athletes competing in endurance events or intensified training appear to be at increased risk of developing upper respiratory tract infections caused by viral pathogens over-riding the body's defences, however the clinical association between acute immunodepression and contraction of illness remains elusive (62,63). More recently, the risk of intestinal derived pathogenic agents overriding immune defences during periods of immunodepression, leading to cytokinemia and endotoxemia has been highlighted (11,13,64). Current guidelines for immuno-nutrition support focus on provision of adequate daily energy intake with minimum requirements for carbohydrate and protein (65). Guidelines specific to acute exercise recovery nutrition for restoration of immune function has been explored, however there is a lack of original research examining the effects of recovery nutrition interventions of different quantities and quality on indices of innate immune function.

Gastrointestinal function and integrity

The gastrointestinal tract (GIT) plays a fundamental role in regulating the passage of substances between the external and internal environments of the body. It does so by creating a physical barrier to prevent the passage of pathogenic substances into systemic circulation, while facilitating the digestion and absorption of nutrients and fluid for energy production, and maintenance of hydration status. *"Exercise-induced gastrointestinal syndrome"* is a term used to describe the multifaceted physiological responses to exercise stress that impact gastrointestinal function, integrity and symptoms (11). During prolonged, strenuous exercise (i.e., >1 h running at 70% $\dot{V}O_{2max}$), blood flow is diverted to the working muscles thereby reducing splanchnic perfusion by up to 80% (66,67). Increased thermoregulatory strain and reduced blood volume will further exacerbate hypoperfusion of the gut (11,14). Ischemic damage and erosion of epithelial cells (i.e., enterocytes, goblet, Paneth and enteroendocrine cells) stimulates a local inflammatory response. Multi-protein complexes (i.e., claudins and occulin) of the tight junction and tight-junction regulatory proteins (i.e., zona-occludens) are damaged as a result of exercise-induced ischemia and the associated cytokine response. Physical breaks in the epithelium permits translocation of bacteria and pathogenic substances into circulation, thereby stimulating a local and systemic inflammatory response. Moreover, increased sympathetic activation during exercise leads to increased circulation of stress hormones (i.e., cortisol and adrenaline) and reduced gastric motility, gastric emptying and translocation of epithelial transporters, thereby reducing overall gastrointestinal functional capacity. Recent studies have provided clear evidence that the digestion and absorption of nutrients is impaired in response to exercise, including reduced passive and active absorption of carbohydrate during prolonged running at 70% $\dot{V}O_{2max}$, carbohydrate and protein malabsorption during prolonged running at 70% $\dot{V}O_{2max}$, and impaired protein absorption after resistance exercise (68-70). It is therefore possible that nutrient digestion and absorption during recovery may also be compromised due to epithelial cell damage, and reduced gastrointestinal motility and gastrointestinal enzyme release, associated with EIGS.

The aim of the current review was to systematically review the literature investigating optimal acute recovery nutrition intervention to support i) muscle glycogen resynthesis ii) muscle protein synthesis iii) rehydration iv) immune function and v) gastrointestinal function and integrity after prolonged, strenuous exercise.

Methods

This review was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (71). The review protocol was prospectively registered with PROSPERO (http://www.crd.york.ac.uk/PROSPERO), registration number CRD42017083594.

Eligibility criteria

In order to obtain the level of methodological detail required, only laboratory-controlled studies and field studies were considered for review. The Participant-Intervention-Comparator-Outcomes-Study design format (PICOS) was used to determine whether studies were eligible for inclusion Table 2.1. Each recovery outcome has been individually reviewed. Each subsection of the review as follows; 1) muscle glycogen resynthesis, 2) muscle protein synthesis, 3) rehydration, 4) immune function, and 5) gastrointestinal integrity. Elite athletes and habitually trained adolescents or adults with no known diseases were the focus of each arm of this review. Interventions considered for inclusion were those that required participants to undergo prolonged, endurance, interval or mixed-model (circuit, crossfit and (or) cardiovascular or resistance) exercise, followed by the provision of a quantified post-exercise nutrition regime during an acute recovery period (<6 h). Excluded interventions include resistance type exercise interventions, pharmacological interventions, chronic dietary supplementation or dietary manipulation interventions, or interventions that offered pre- or mid-exercise supplementation. However, if the control arms of studies fit all other inclusion criteria, data from these trials were included for review. Studies with incomplete information regarding the dose, timing and nutritional composition of the post-exercise food and fluid provided were excluded.

Search Strategy

A three-step search strategy was developed with the assistance of an academic liaison librarian. A summary of the search strategy is outlined in Table 2.2. This search of published English-language studies was implemented across six electronic databases in September 2018: Ovid MEDLINE, EMBASE, Cinahl, SportsDISCUS, Web of Science and Scopus. The reference lists of all identified studies were searched to identify any additional studies for inclusion. This search was rerun in August 2020.

Study Selection

The database search was imported into Endnote and duplicates were manually removed. The searches were then imported into Covidence. Two reviewers (IR and VC) worked independently and in duplicate to assess papers against the PICOS. A third reviewer (RC) resolved conflicts where these arose. Full texts were also assessed against the PICOS statement by reviewers (IR and VC) independently and in duplicate, with consensus reached by a third reviewer (RC).

Data Extraction

Data were independently extracted in duplicate (IR and VC) from each included study into a standardised table developed for reporting this review. Data of interest related to study design and population, exercise stress, nutrition intervention and primary and secondary outcomes.

Synthesis and Analysis of Data

The post-exercise nutrition interventions and effects on relevant outcomes have been analysed individually for the muscle protein synthesis, muscle glycogen resynthesis, hydration, gut integrity, and immune function arms of this review. Measurements have been converted for consistency when necessary. Where numerical data was not given, values have been estimated from figures. Data have been analysed by plotting dose relative to BM (i.e. g/kg BM/h or ml/kg BM/h) against the relevant recovery outcome.

Risk of Bias Assessment

Risk of bias assessment was performed using the Cochrane “risk of bias” assessment tool. Two reviewers independently (IR and SG), and in duplicate conducted the assessments by referring to the criteria for judging risk of bias in the ‘Risk of bias’ assessment tool (72). Conflicts were resolved by discussion and mutual agreement.

Table 2.1 Inclusion and exclusion criteria for recovery nutrition systematic literature review.

	Inclusion	Exclusion
Population	Habitually trained and elite level active individuals (including masters, adolescents (13+ years), and adults).	Infants and children, sedentary individuals (i.e. no adherence to exercise or structured physical activity programs), diagnosed disease state, and (or) untrained participants.
Intervention	Acute (immediately to 4 h) post-exercise (prolonged or interval-based endurance, and mixed model (i.e., circuit, crossfit and (or) cardiovascular-resistance programs)) nutritive or non-nutritive food or fluid intake.	Pre- or mid-exercise nutrition intervention only, chronic supplementation, pharmaceutical interventions, short duration high intensity (e.g., maximal effort), resistance exercise, alcoholic intervention beverages, or incomplete information regarding dose, timing, and nutritional composition of intervention.
Comparator	Comparators and controls not assessed.	
Outcome (Primary)	1. Muscle glycogen concentration and (or) rate of muscle glycogen resynthesis 2. Myofibrillar, mitochondrial and (or) mixed protein fractional synthetic rate 3. Plasma osmolality and 4. Leukocyte function and (or) trafficking 5. Intestinal fatty acid binding protein	Failure to meet at least one primary and one secondary outcome inclusion criteria.
Outcome (secondary)	1. Blood glucose and insulin response 2. Muscle intracellular signalling proteins concentration 3. Bioelectrical impedance analysis, plasma volume change, and (or) net fluid balance 4. Salivary defences and (or) circulating stress hormones 5. Systemic endotoxins, gastrointestinal symptoms, breath hydrogen	
Study design	Laboratory controlled trials and field studies.	All other study designs.

Table 2.2 Search strategy for 1) muscle glycogen resynthesis 2) muscle protein synthesis 3) rehydration 4) immune function and 5) gastrointestinal integrity and function nutrition interventions

	Intervention concept 1	Intervention concept 2	Outcome	Limits
1	Postexercis* OR Post exercis* OR Recover*	Feed* OR Supplement* OR Intake OR Ingest* OR Coingest* OR co ingest* OR nutri*	Refuel* OR Glycogen	Medline, Ovid, WoS and Scopus- Humans WoS and Scopus – NOT (rat* OR mice OR mouse OR sheep OR cat OR cattle OR "chick embryo" OR dog* OR goat* OR horse* OR plant* OR bacteria OR mollusc* OR insect* OR beetle*)
2	As above	As above	Muscle protein OR Muscle damage OR creatine kinase OR protein synthesis OR mitochondrial OR myofibrillar OR fractional synthe*	
3	As above	As above + drink*	Hydrat* OR Dehydrat* OR rehydrate* Balance adj1 (fluid OR electrolyte OR water electrolyte)	
4	Exercise induce* OR Postexercis* OR Post exercise* OR Exercis*	Feed* OR Supplement* OR Intake OR Ingest* OR Coingest* OR co ingest* OR nutri*	(Gastrointestinal OR gastro intestinal OR intestinal OR gut) AND (injury OR permeability OR malabsorption OR symptom*)	
5	Exercise induce* OR Postexercis* OR Post exercis*	As above	Immun* OR neutrophil OR cytokine*	

Results

Muscle Glycogen Resynthesis

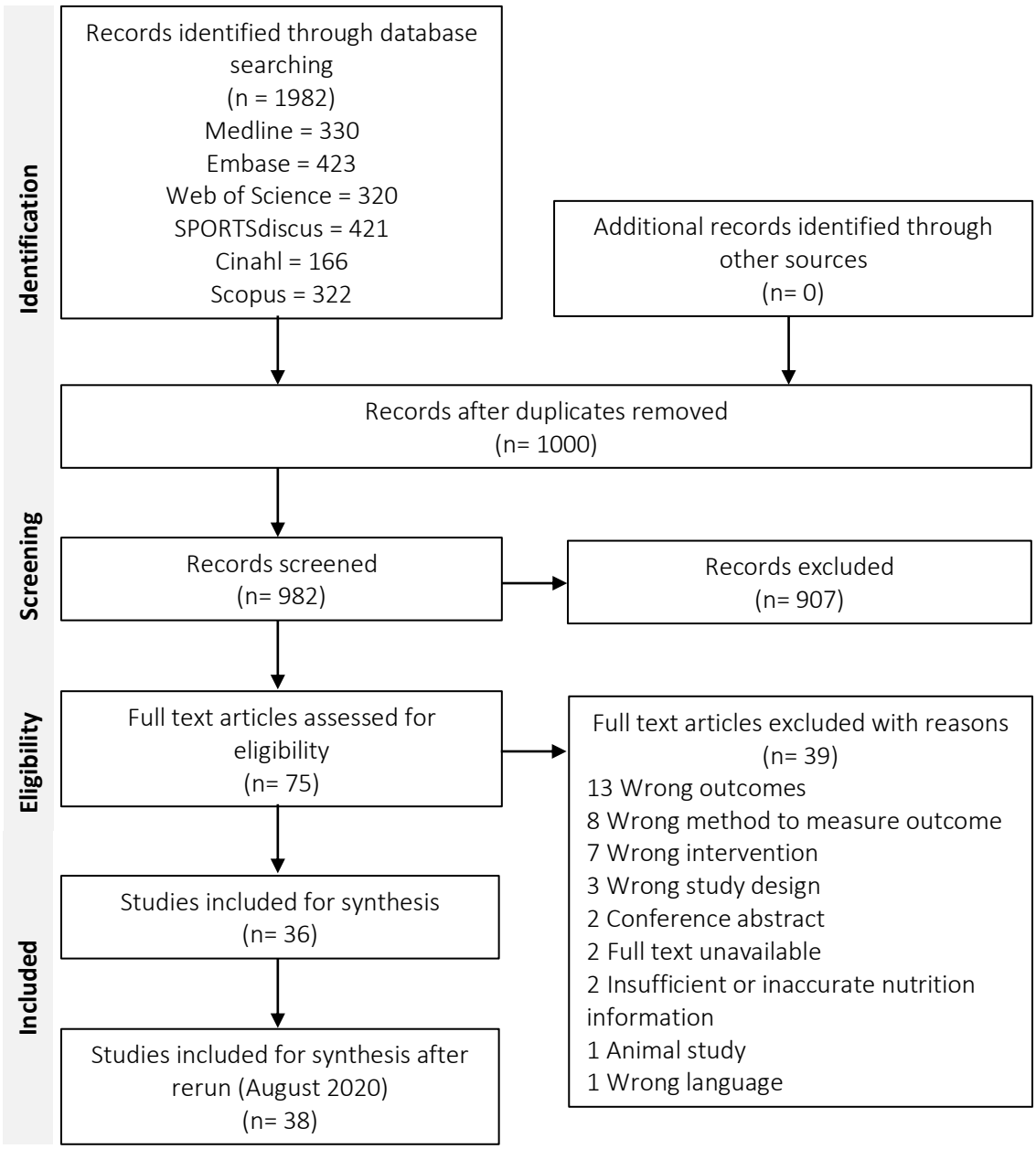
The search strategy yielded 38 studies, for a total of 91 trials that met the inclusion criteria **Figure 2.1A**. The study designs and results from these studies are summarised in **Table 2.3**. Of the 38 studies that were reviewed, 32 were conducted amongst male participants (7,73-103) and six studies included both males and females (104-109). The exercise protocols varied in duration, intensity and modality, resulting in wide-ranging values for post-exercise muscle glycogen concentration (20-296 mmol/kg dw).

Figure 2.2A depicts the relationship between carbohydrate intake and the rate of glycogen resynthesis when multiple doses of carbohydrate were given during the recovery period. These results suggest that a dose of 0.8-1.2 g carbohydrate/kg BM/h will result in a rate of muscle glycogen resynthesis of 20-40 mmol/kg dw/h. Carbohydrate intake greater than 1.2 g /kg BM/h does not appear to further increase the rate of muscle glycogen resynthesis. Three studies observed very high rates of glycogen resynthesis $\geq$55 mmol/kg dw/h (73,93,104). These high rates of resynthesis are observed during the first, second and third hours of the recovery period, with carbohydrates intakes between 1.0 and 1.2 g/kg BM/h. **Figure 2.2B** depicts the relationship between carbohydrate intake and rate of glycogen resynthesis where a single bolus of the recovery nutrition was provided immediately or within 30 min of completion of exercise. The rate of resynthesis appears to decline over time (i.e., $\leq$25 mmol/kg dw/h by the fourth hour of recovery) when a single bolus of carbohydrate (i.e., 0.8-1.1 g/kg BM/h) is provided.

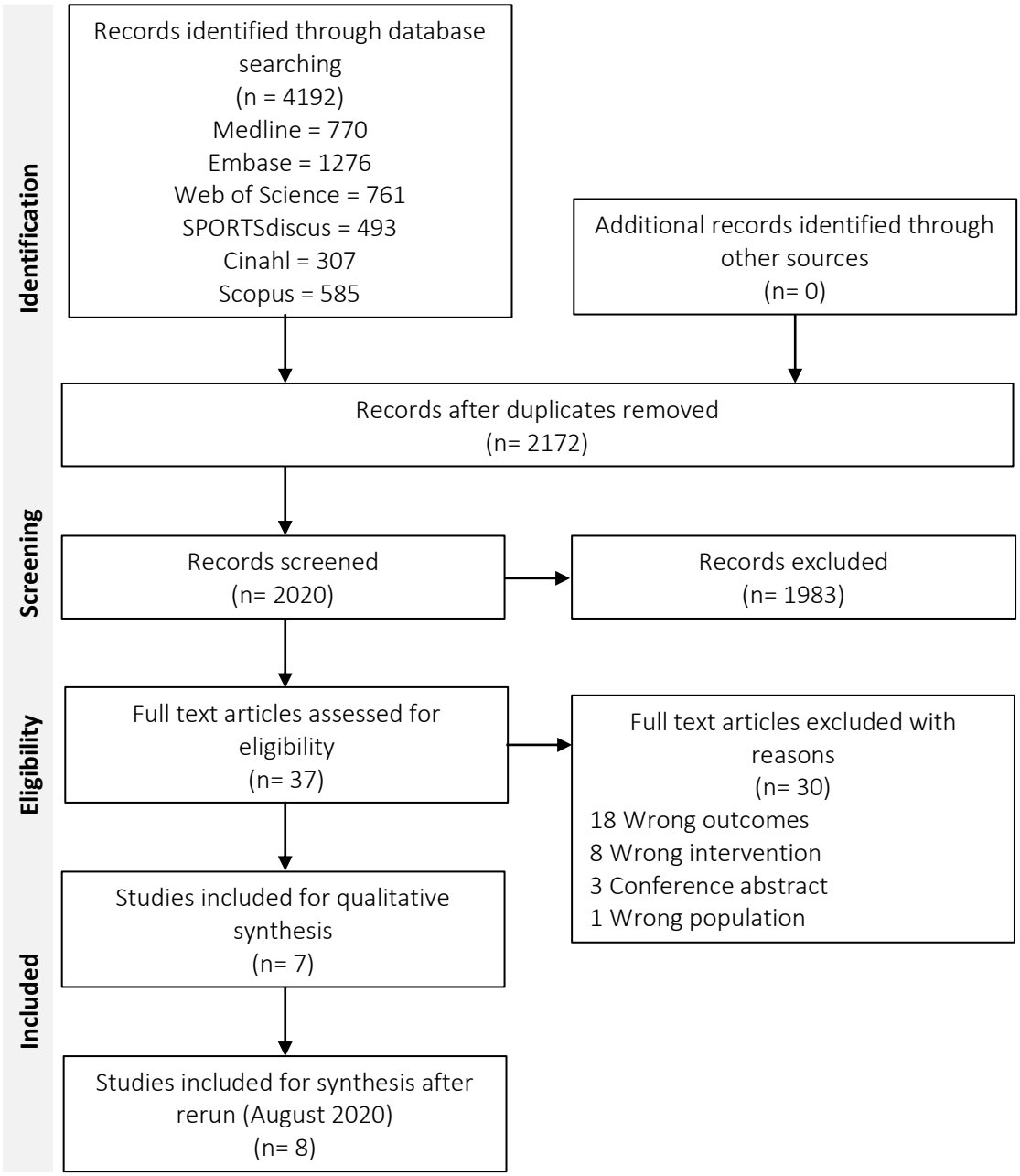
The majority of the reviewed studies also investigated the effect of protein and carbohydrate co-ingestion on post-exercise glycogen resynthesis. Where a maximum of 0.7 g carbohydrate/kg BM/h and a minimum of 0.3 g protein/kg BM/h was provided, rates of muscle glycogen resynthesis are comparable to those observed with isocaloric intake of carbohydrate (74,92). However, where carbohydrate intake is >0.8 g/kg BM/h, protein intake does not further enhance muscle glycogen resynthesis.

Figure 2.1 PRISMA diagram, indicating the systematic review process, and subsequent inclusion and exclusion of respective research papers for A) muscle glycogen resynthesis, B) muscle protein synthesis, C) rehydration, D) immune function and E) gastrointestinal integrity and function.

A



B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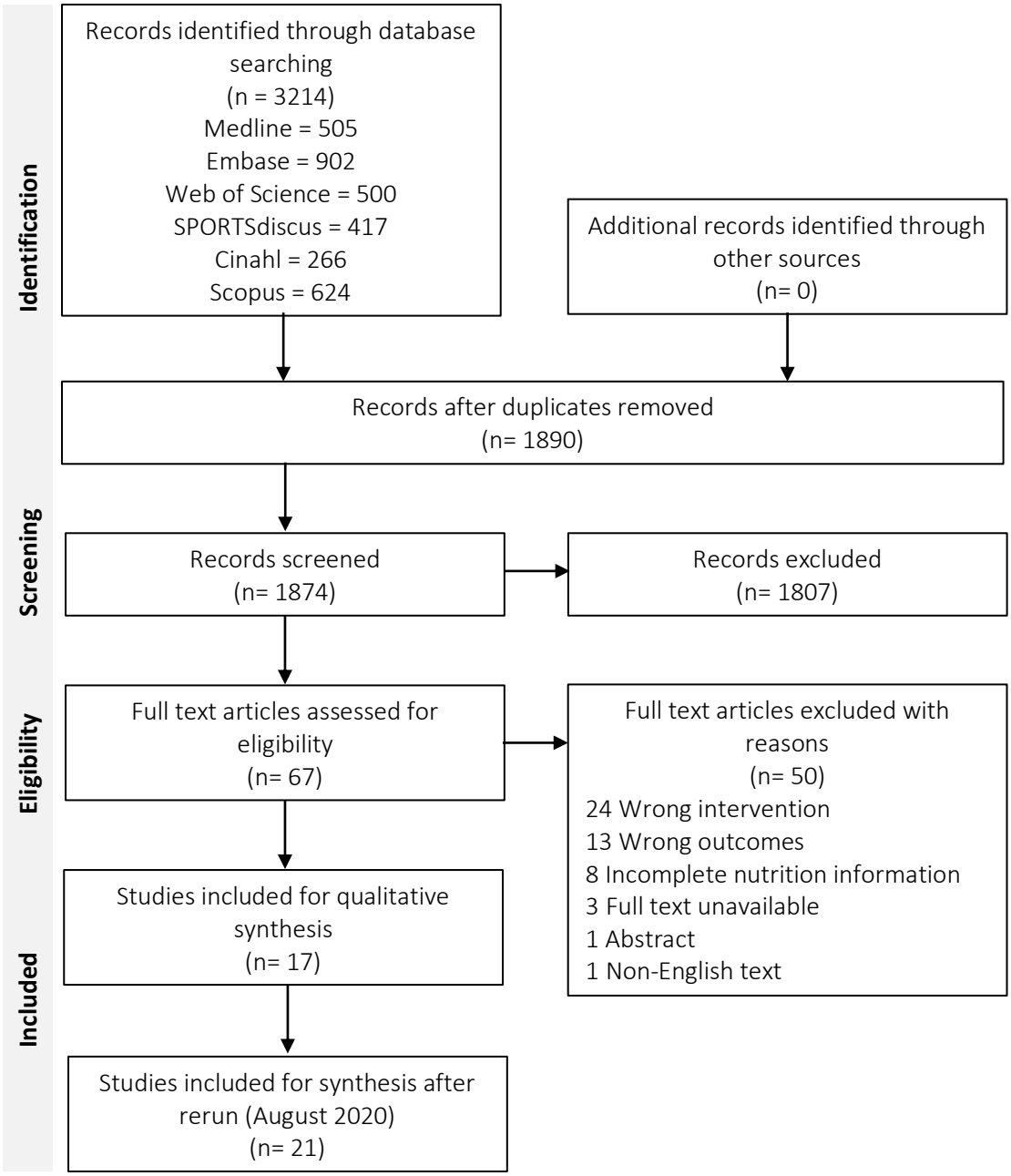
SPORTSdiscus = 493
Cinahl = 307
Scopus = 585
Additional records identified through other sources
(n= 0)
Records after duplicates removed
(n= 2172)
Screening
Records screened
(n= 2020)
Records excluded
(n= 1983)
Eligibility
Full text articles assessed for eligibility
(n= 37)
Full text articles excluded with reasons
(n= 30)
18 Wrong outcomes
8 Wrong intervention
3 Conference abstract
1 Wrong population
Included
Studies included for qualitative synthesis
(n= 7)
Studies included for synthesis after rerun (August 2020)
(n= 8)

C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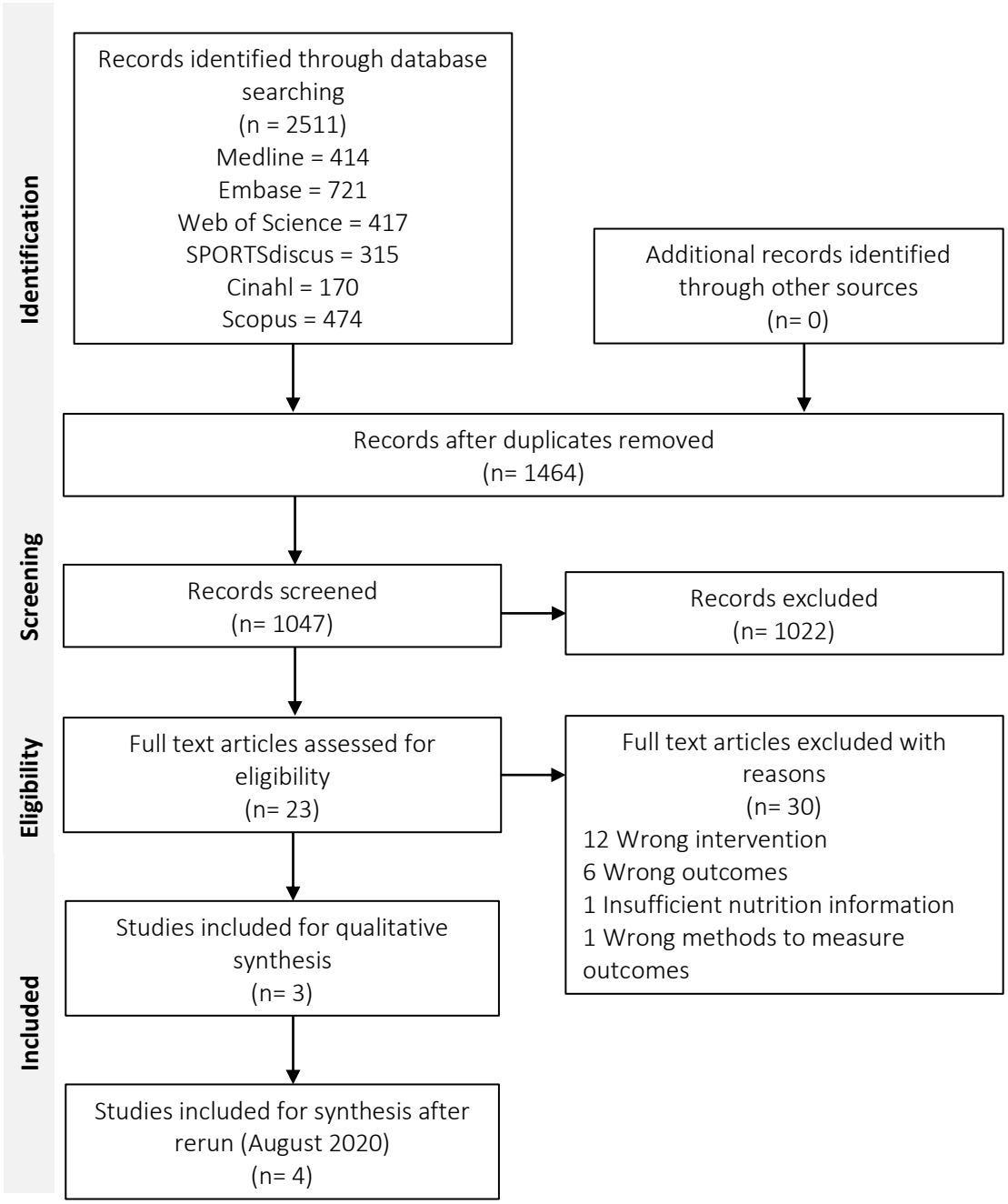
SPORTSdiscus = 417
Cinahl = 266
Scopus = 624
Additional records identified through other sources
(n= 0)
Records after duplicates removed
(n= 1890)
Screening
Records screened
(n= 1874)
Records excluded
(n= 1807)
Eligibility
Full text articles assessed for eligibility
(n= 67)
Full text articles excluded with reasons
(n= 50)
24 Wrong intervention
13 Wrong outcomes
8 Incomplete nutrition information
3 Full text unavailable
1 Abstract
1 Non-English text
Included
Studies included for qualitative synthesis
(n= 17)
Studies included for synthesis after rerun (August 2020)
(n= 21)

D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SPORTSdiscus = 315
Cinahl = 170
Scopus = 474
Additional records identified through other sources
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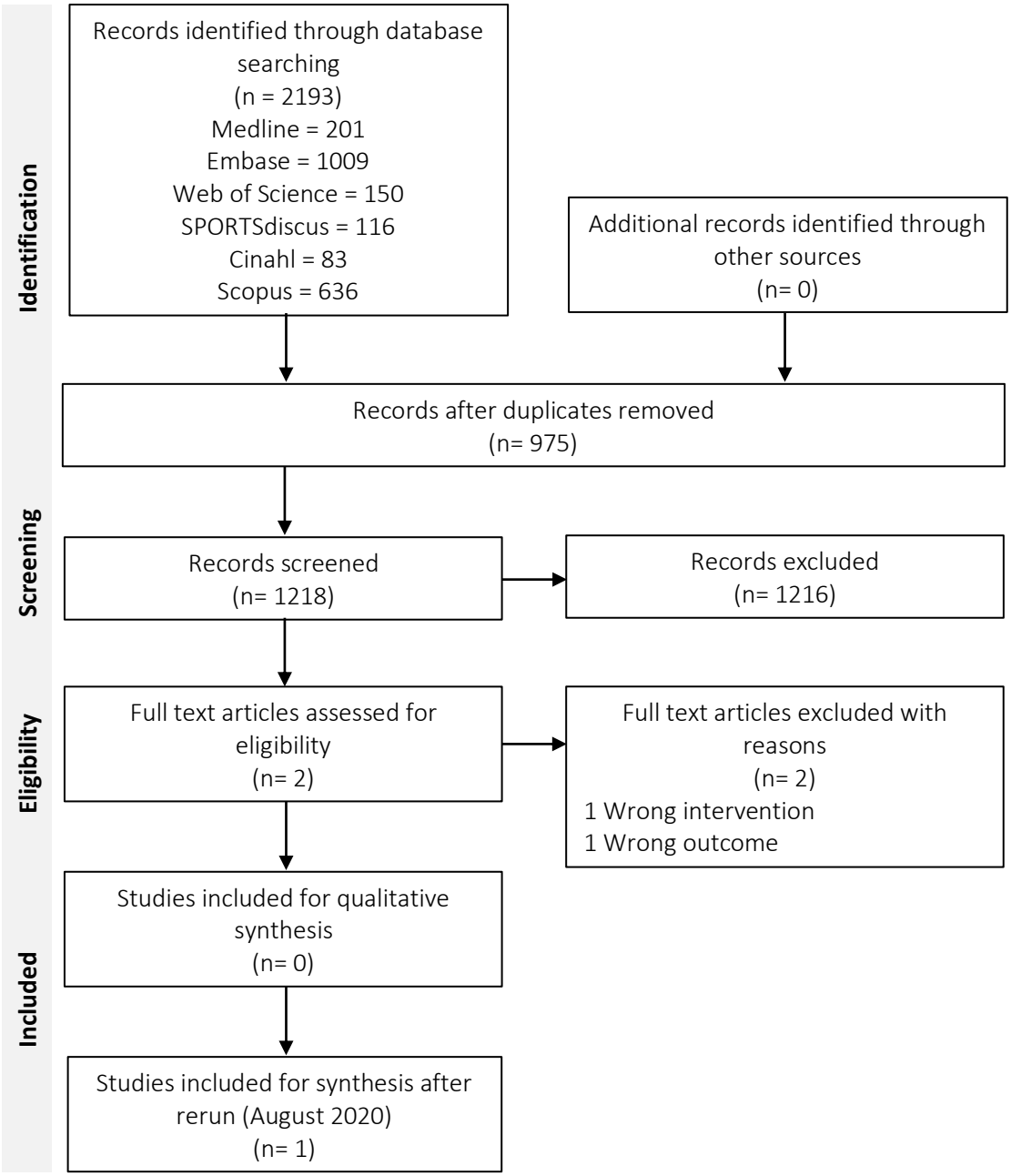
Screening
Records after duplicates removed
(n= 1464)
Records screened
(n= 1047)
Records excluded
(n= 1022)
Eligibility
Full text articles assessed for eligibility
(n= 23)
Full text articles excluded with reasons
(n= 30)
12 Wrong intervention
6 Wrong outcomes
1 Insufficient nutrition information
1 Wrong methods to measure outcomes
Included
Studies included for qualitative synthesis
(n= 3)
Studies included for synthesis after rerun (August 2020)
(n= 4)

E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SPORTSdiscus = 116
Cinahl = 83
Scopus = 636
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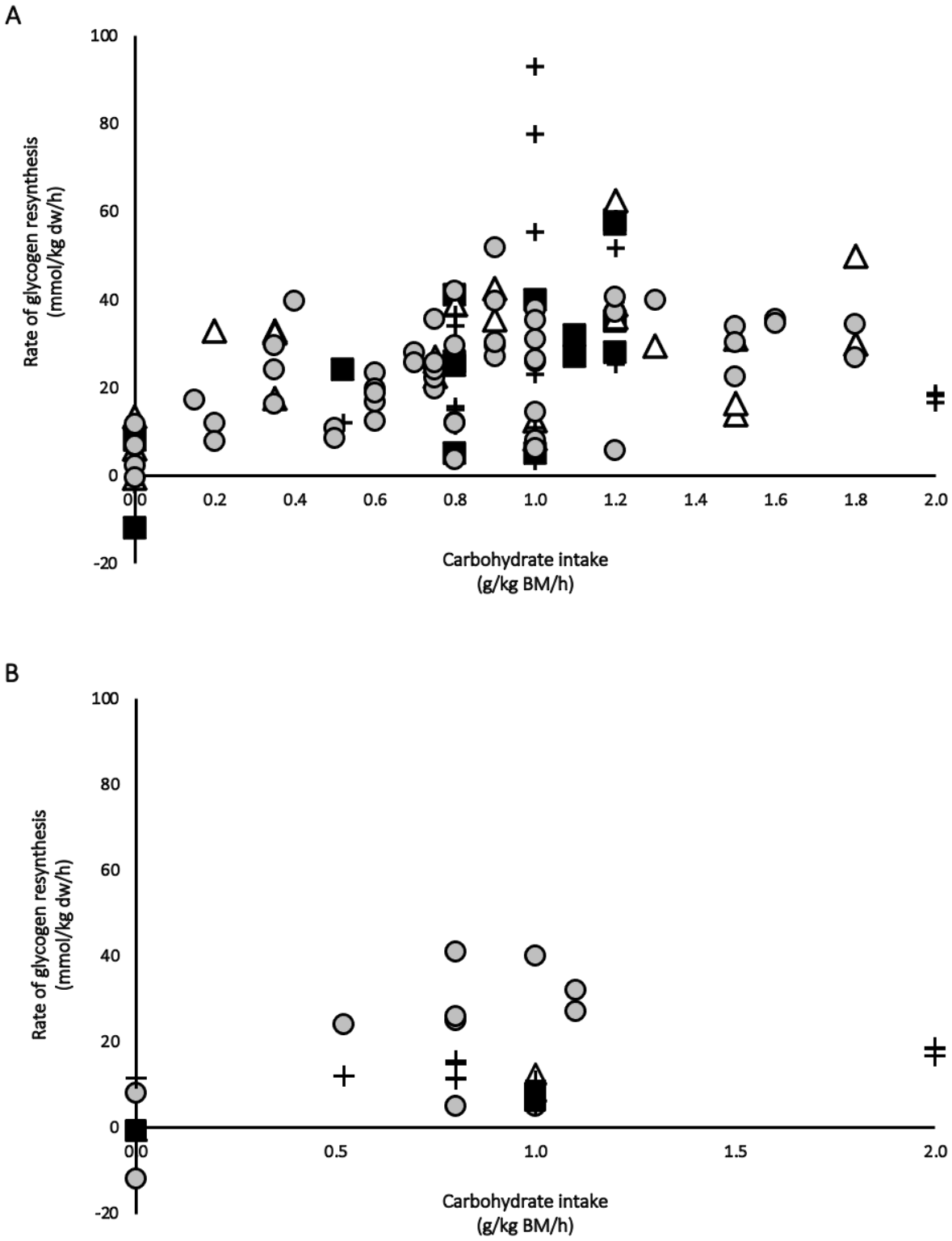
Screening
Records screened
(n= 1218)
Records excluded
(n= 1216)
Eligibility
Full text articles assessed for eligibility
(n= 2)
Full text articles excluded with reasons
(n= 2)
1 Wrong intervention
1 Wrong outcome
Included
Studies included for qualitative synthesis
(n= 0)
Studies included for synthesis after rerun (August 2020)
(n= 1)

Figure 2.2 The rate of muscle glycogen resynthesis (mmol/kg dw/h) compared to A) multiple carbohydrate feedings and B) a single carbohydrate feeding.



Mean rate of resynthesis from studies.0-1 h post-exercise (●), 0-2 h post-exercise (△), 0-3 h post-exercise (+) and 0-4 h post-exercise (■).

Table 2.3A Summary of studies investigating the effect of post exercise nutrition interventions on muscle glycogen resynthesis with multiple feedings throughout acute recovery period.

Reference	Participants	Exercise	Recovery nutrition intervention	Post-exercise muscle glycogen* (mmol/kg dw)	Muscle glycogen rate of resynthesis (mmol/kg dw/h)	Mean blood glucose value between biopsies (mmol/L)	Mean plasma insulin value between biopsies (µIU/ml)
(7)	Trained male athletes (n= 6) VO_{2max} = 64 ml/kg BM/min	70% VO_{2max} to volitional fatigue on treadmill (81-84 min) T_{amb} and RH not reported	1) 0.8 g CHO + 0.4 g PRO/kg BM/h 2) 1.2 g CHO/kg BM/h Consumed every 30 min from 0-4 h post-exercise	1) 99 2) 103	1) 41.9 2) 37.3 (0-4 h)	1) 4.4 2) 5.0 (0-4 h)	1) 26.9 2) 18.3 (0-4 h)
(73)	Recreationally active makes (n= 10) VO_{2max} = 61 ml/kg BM/min	Cycle to exhaustion (mean time= 91 min) T_{amb} and RH not reported	1.2 g CHO/kg BM/h Consumed every h from 0-4 h post-exercise	50	62.5 (0-2 h)	5.0 (0-2 h)	24.9 (0-2 h)
(74)	Trained male athletes (n= 6) VO_{2max} = 61 ml/kg BM/min	90 min running at 70% VO_{2max} T_{amb}= 20.5°C, RH= 55.6%	1) 0.8 g CHO/kg BM/h 2) 0.8 g CHO + 0.3 g PRO/kg BM/h Consumed every 30 min from 0-4 h post-exercise	1) 203 2) 235	1) 12.3 2) 12.0 (0-4 h)	1) 5.7 2) 4.2 (0-4 h)	1) 34.6 2) 30.9 (0-4 h)

(75)	Recreationally active males (n= 27) VO_{2max} not reported	Cycle to exhaustion (78-113 min) T_{amb} and RH not reported	1) 0.2 g glucose/kg BM/h 2) 0.35 g glucose/kg BM/h 3) 0.8 g glucose/kg BM/h 4) 0.35 g sucrose/kg BM/h 5) 0.35 g fructose/kg BM/h Consumed every 2 h from 0-4 h post-exercise	1) 139 2) 65 3) 100 4) 35 5) 100	1) 33.0, 12.0 2) 33.0, 29.5 3) 39.0, 29.5 4) 32.5, 24.0 5) 17.5, 16.3 (0-2, 0-4 h)	1) 5.4, 5.6 2) 6.4, 6.6 3) 6.9, 6.8 4) 4.7, 5.0 5) 4.8, 4.9 (0-2, 0-4 h)	1) 11.1, 14.7 2) 17.7, 21.2 3) 26.3, 37.5 4) 7.0, 10.3 5) 9.6, 9.8 (0-2, 0-4 h)
(76)	Trained male cyclists (n= 7) VO_{2max} = 4.2 L/min	45 min cycling at 70% VO_{2max} evening before trial. 75 min interval cycling 70% - 125% VO_{2max} on day of trial T_{amb} and RH not reported	1) 1.0 g CHO/kg BM/h 2) 0.7 g CHO + 0.2 g PRO/kg BM/h 3) 0.9 g CHO + 0.1 g PRO/kg BM/h Consumed every 30 min from 0-4 h post-exercise	1) 107 2) 118 3) 87	1) 31.0 2) 28.0 3) 29.5 (0-4 h)	1) 5.9 2) 5.6 3) 5.5 (0-4 h)	1) 39.1 2) 35.5 3) 26.0 (0-4 h)
(77)	Trained male cyclists (n= 11) VO_{2max} = 61 ml/kg BM/min	2 h cycling at 70% VO_{2max} T_{amb} and RH not reported	1) 0.6 g CHO/kg BM/h 2) 0.5 g CHO/kg BM/h 3) 0.5 g CHO + 0.1 g PRO/kg BM/h Consumed immediately and 2 h post-exercise	1) 80 2) 80 3) 65	1) 12.5 2) 10.8 3) 8.5 (0-4 h)	1) 6.2 2) 6.1 3) 6.3 (0-4 h)	1) 17.3 2) 15.6 3) 20.2 (0-4 h)

(78)	Recreationally active males (n= 11) VO_{2max} = 4.2 L/min	90 min interval cycling 50-80% W_{max} T_{amb} and RH not reported	1) 1.5 g CHO + 0.2 g PRO/kg BM/h 2) 1.5 g CHO + 0.2 g PRO/kg BM/h Consumed immediately and 2 h post-exercise	1) 245 2) 207	1) 34.0 2) 30.3 (0-4 h)	1) 5.9 2) 5.7 (0-4 h)	1) 29.4 2) 30.7 (0-4 h)
(79)	Trained male cyclists (n= 7) VO_{2max} = 58 ml/kg BM/min	2 h interval cycling 70-120% VO_{2max} T_{amb} and RH not reported	1) 1.2 g CHO/kg BM/h 2) 0.8 g CHO + 0.4 g PRO/kg BM/h Consumed every 30 min from 45-225 min post-exercise	1) 27 2) 36 (C MRS)	1) 5.8 2) 3.8 (0-4 h)	1) 6.2 2) 5.2 (0-4 h)	1) 26.6 2) 60.7 (0-4 h)
(80)	Trained male cyclists (n= 15) VO_{2max} = 62 ml/kg BM/min	Intermittent cycling to exhaustion 50-90% $W_{max,}$ (mean time= 89-93 min) T_{amb} and RH not reported	1) 1.5 g CHO (glucose)/kg BM/h 2) 1.5 g CHO (sucrose)/kg BM/h Consumed every h from 0-5 h post-exercise	1) 87 2) 86 (C MRS)	1) 14.0 2) 16.6 (0-2 h)	1) 7.4 2) 6.9 (0-2 h)	1) 19.9 2) 14.9 (0-2 h)
(81)	Trained male cyclists (n= 12) VO_{2max} = 4.2 L/min	70 min interval cycling 68-88% VO_{2max} $T_{amb=}$ 22°C, RH not reported	1) 1.0 g CHO/kg BM/h consumed immediately post-exercise 2) 1.0 g CHO/kg BM/h consumed 1 h post-exercise	1) 163 2) 140	1) 26.0 2) 14.5 (0-4 h)	1) 5.3 2) 5.6 (0-4 h)	1) 22.0 2) 24.3 (0-4 h)

(82)	Recreationally active males (n= 8) VO_{2max} = 3.9 L/min	120 min interval cycling 62-75% VO_{2max} $T_{amb=}$ 22°C, RH not reported	1) 0.75 g CHO/kg BM/h 2) 1.5 g CHO/kg BM/h 3) 0 g CHO/kg BM/h Consumed immediately and 2 h post-exercise	1) 163 2) 145 3) 165	1) 22.5, 19.8 2) 31.0, 22.5 3) 6.0, 2.3 (0-2, 0-4 h)	1) 5.3, 5.2 2) 5.5, 5.4 3) 3.3, 3.4 (0-2, 0-4 h)	1) 25.9, 36.7 2) 36.8, 55.9 3) 6.7, 6.9 (0-2, 0-4 h)
(83)	Trained male cyclists (n= 8) VO_{2max} = 63 ml/kg BM/min	Intermittent cycling to exhaustion alternating 50-90% W_{max} T_{amb}=22-25°C, RH=50-60%	1) 1.2 g CHO + 0.4 g PRO/kg BM/h 2) 1.2 g CHO/kg BM/h Consumed every 30 min from 0-3 h post-exercise	1) 176 3) 106	1) 35.0, 37.3 2) 35.0, 39.7 (0-1, 0-3 h)	1) 5.3, 5.9 2) 5.8, 5.6 (0-1, 0-3 h)	1) 30.7, 80.6 2) 19.2, 39.3 (0-1, 0-3 h)
(84)	Recreationally active males (n= 9) VO_{2max} = 4.3 L/min	60 min cycling at 60%W_{max} T_{amb}= 32°C or 22 °C RH≤20%	1) 0.9 g CHO/kg BM/h 2) 0.9 g CHO/kg BM/h Consumed immediately and 2 h post-exercise	1) 288 2) 309	1) 42.5, 27.0 2) 35.5, 39.8 (0-2, 0-4 h)	1) 7.4, 7.3 2) 6.7, 6.3 (0-2, 0-4 h)	1) 42.7, 51.3 2) 47.5, 55.6 (0-2, 0-4 h)
(85)	Trained male cyclists/triathletes (n= 7) VO_{2max} = 60 ml/kg BM/min	Interval cycling to exhaustion evening before trial. 70% VO_{2peak} to volitional fatigue (57-60 min) T_{amb}= 21-22°C, RH= 40-50%	1.3 g CHO/kg BM/h Consumed hourly from 0-3 h post-exercise	2) 74	29.5, 40.0 (0-1, 0-4 h)	5.2, 5.1 (0-1, 0-4 h)	17.7, 29.0 (0-1, 0-4 h)

(86)	Trained males (n= 13) VO_{2max} not reported	60 min running + 60 min submaximal cycling + sprints to exhaustion T_{amb} and RH not reported	1) 1.8 g CHO/kg BM/h (high molecular weight) 2) 1.8 g CHO/kg BM/h (low molecular weight) Consumed every 30 min from 0-90 min post-exercise	1) 58 52	1) 50.0, 34.5 2) 26.0, 22.3 (0-2, 0-4 h)	1) 6.9, 6.5 2) 4.9, 5.0 (0-2, 0-4 h)	1) 27.5, 28.4 2) 61.5, 61.5 (0-2, 0-4 h)
(87)	Trained male athletes (n= 8) VO_{2max} = 3.8 L/min	120 min interval cycling at 60-75% VO_{2max} T_{amb} and RH not reported	1) 0.75 g CHO/kg BM/h (solid) 2) 0.75 g CHO/kg BM/h (liquid) Consumed immediately and 2 h post-exercise	1) 124 2) 111	1) 26.0, 22.3 2) 27.0, 24.0 (0-2, 0-4 h)	1) 4.9, 5.0 2) 4.6, 4.9 (0-2, 0-4 h)	1) 61.5, 61.5 2) 47.4, 47.4 (0-2, 0-4 h)
(88)	Trained male cyclists (n= 6) VO_{2max} = 58 ml/kg BM/min	90 min interval cycling at 50-80% VO_{2max} T_{amb} and RH not reported	1) 0.9 g CHO/kg BM/h 2) 0.9 g CHO/kg BM/h Consumed at 15- and 135-min post-exercise	1) 257 2) 227	1) 30.3 2) 51.8 (0-4 h)	1) 5.9 2) 6.1 (0-4 h)	1) 52.0 2) 52.4 (0-4 h)
(89)	Recreationally active males (n= 7) VO_{2max} = 56 ml/kg BM/min	90 min running at 70% VO_{2max} T_{amb}= 22.1°C	1) 0.2 g CHO/kg BM/h 2) 0.6 g CHO/kg BM/h Consumed every 30 min from 0-3 h post-exercise	1) 252 2) 259	1) 7.8 2) 18.8 (0-4 h)	1) 5.7 2) 5.6 (0-4 h)	1) 15.0 2) 27.9 (0-4 h)

(90)	Trained male athletes (n= 8) VO_{2max} not reported	Intermittent cycling to exhaustion alternating 50-90% W_{max} T_{amb}=20°C	1) 0.8 g CHO/kg BM/h 2) 0.8 g CHO + 0.3 g PRO/kg BM/h 3) 0.8 g CHO + 0.3 g PRO/kg BM/h 4) 0.8 g CHO + 0.3 g PRO/kg BM/h Consumed at 15, 60 and 120 min post-exercise	1) 108 2) 81 3) 107 4) 74	1) 29.0 2) 27.3 3) 34.0 4) 36.7 (0-3 h)	1) 6.0 2) 6.1 3) 5.8 4) 6.5 (0- 3 h)	1) 28.7 2) 35.9 3) 47.3 4) 48.1 (0-3 h)
(91)	Trained male athletes (n= 5) VO_{2max} = 61 ml/kg BM/min	Intermittent cycling to exhaustion alternating 50-90% W_{max} T_{amb} and RH not reported	1) 1.2 g CHO + 0.4 g PRO/kg BM/h 2) 1.2 g CHO/kg BM/h 3) Non-caloric placebo Consumed every 15 min from 0-4 h post-exercise	1) 69 2) 90 3) 78	1) 39.0, 37.3 2) 36.0, 40.5 3) 13.5, 11.8 (0-2, 0-4 h)	1) 6.5 2) 7.0 3) 5.0 (0-4 h)	1) 58.5 2) 37.1 3) 3.3 (0-4 h)
(92)	Trained male cyclists (n= 8) VO_{2max} not reported	Intermittent cycling to exhaustion alternating 50-90% W_{max} T_{amb} and RH not reported	1) 0.8 g CHO/kg BM/h 2) 0.8 g CHO + 0.4 g PRO/kg BM/h 3) 1.2 g CHO/kg BM/h Consumed every 30 min from 0-5 h post-exercise	1) 190 2) 174 3) 138	1) 16.6 2) 35.4 3) 44.8 (0-5 h)	1) 5.6 2) 5.3 3) 5.6 (0-5 h)	1) 33.1 2) 59.0 3) 43.2 (0-5 h)

(93)	Trained male athletes (n= 6) VO_{2max} = 67 ml/kg BM/min	Intermittent cycling to exhaustion alternating 50-90% W_{max} T_{amb} and RH not reported	1) 1.2 g CHO/kg BM/h (glucose) 2) 1.2 g CHO/kg BM/h (glucose and fructose) Consumed every 30 min from 0-4 h post-exercise	1) 128 2) 112	1) 28.0, 58.3 2) 35.0, 39.7 (0-1, 0-4 h)	1) 5.9, 5.6 2) 6.3, 5.9 (0-1, 0-4 h)	1) 33.4, 50.7 2) 30.7, 49.5 (0-1, 0-4 h)
(94)	Trained male athletes (n= 8) VO_{2max} = 55 ml/kg BM/min	90 min cycling at 65% VO_{2max} T_{amb} and RH not reported	1) 1.0 g CHO + 0.1g PRO/kg BM/h 2) 1.0 g CHO + 0.1g PRO/kg BM/h Consumed every 15 min from 0-3 h post-exercise	1) 130 1) 228	1) 27.0 2) 35.3 (0-3 h)	1) 5.7 2) 5.6 (0-3 h)	1) 36.5 2) 36.5 (0- 3 h)
(95)	Trained males (n= 16) VO_{2max} = 62 ml/kg BM/min	120 min cycling at 65-75% VO_{2max} T_{amb}= 20°C	1) 0.4 g CHO + 0.1 g PRO/kg BM/h 2) 0.2 g CHO/kg BM/h Consumed immediately and 2 h post-exercise	1) 227 2) 236	1) 39.8 2) 17.3 (0-4 h)	1) 4.8 2) 5.1 (0-4 h)	1) 28.5 2) 14.4 (0-4 h)
(96)	Trained male cyclists (n= 12) VO_{2max} = 67 ml/kg BM/min	120 min cycling at 60-85% VO_{2max} T_{amb}= 22°C	1) 1.0 g CHO + 0.1 g PRO/kg BM/h 2) 1.0 g CHO/kg BM/h Consumed hourly from 0-3 h post-exercise	1) 139 2) 151	1) 35.3 2) 26.5 (0-4 h)	1) 5.6 2) 5.7 (0-4 h)	1) 54.8 2) 49.7 (0-4 h)

(97)	Trained male cyclists (n= 9) VO_{2max} = 67 ml/kg BM/min	120 min interval cycling at 50-80% VO_{2max} T_{amb} and RH not reported	1) 0.75 g CHO/kg BM/h 2) 0.25 g PRO/kg BM/h 3) 0.75 g CHO + 0.25 g PRO/kg BM/h Consumed immediately and 2 h post-exercise	1) 223 2) 289 2) 217	1) 25.8 2) 7.0 3) 35.5 (0-4 h)	1) 5.9 2) 4.2 3) 5.4 (0-4 h)	1) 38.1 2) 5.1 3) 49.7 (0-4 h)
(104)	Trained males (n= 4) VO_{2max} = 58 ml/kg BM/min	45 min cycling at 70% VO_{2max} evening before trial. 90 min interval cycling 70% - 125% VO_{2max} on day of trial T_{amb}= 5°C, 25°C or 35°C	1) 1.0 g CHO/kg BM/h 2) 1.0 g CHO/kg BM/h 3) 1.0 g CHO/kg BM/h	1) 84 2) 93 3) 199	1) 93.0 2) 55.3 3) 77.7	1) 7.9 2) 6.9 3) 6.2	1) 15.3 2) 27.4 3) 12.5
	Trained females (n= 4) VO_{2max} = 46 ml/kg BM/min		4) 1.0 g CHO/kg BM/h 5) 1.0 g CHO/kg BM/h 6) 1.0 g CHO/kg BM/h Consumed immediately and 1 h post-exercise	3) 164 4) 121 5) 228	4) 25.7 5) 23.0 6) 3.3 (0-3 h)	4) 6.4 5) 6.0 6) 5.9 (0-3 h)	4) 12.8 5) 15.9 6) 39.1 (0-3 h)
(105)	Trained male (n= 5) and female (n= 5) cyclists/triathletes VO_{2max} = 53 ml/kg BM/min	100 min interval cycling at 45-90% VO_{2max} T_{amb} and RH not reported	1) 0.7 g CHO/kg BM/h 2) Non-caloric placebo Consumed immediately and 2 h post-exercise	6) Not reported	1) 7.6 2) 7.1 (0-4 h)	1) 4.6 2) 3.8 (0-4 h)	1) 31.1 2) 10.7 (0-4 h)

(106)	Recreationally active males (n= 8) VO_{2max} = 57 ml/kg BM/min and females (n= 8) VO_{2max} = 47 ml/kg BM/min	90 min interval cycling 50-80% W_{max} $T_{amb=}$ 20°C, RH 25-30%	1) 1.6 g CHO + 0.2 g PRO/kg BM 2) 1.6 g CHO + 0.2 g PRO/kg BM Consumed immediately and 2 h post-exercise	1) 213 2) 226	1) 35.5 2) 34.8 (0-4 h)	1) 6.0 2) 5.8 (0-4 h)	1) 13.2 2) 10.6 (0-4 h)
(108)	Trained male (n= 9) and female athletes (n= 8) VO_{2max} = 55 ml/kg BM/min VO_{2max} = 50 ml/kg BM/min	90 min cycling at 65% VO_{2max} T_{amb} and RH not reported	1) 0.7 g CHO + 0.2 g PRO/kg BM/h 2) 1.0 g CHO/kg BM/h 3) Non-caloric placebo Consumed immediately and 1 h post-exercise	1) 142 2) 163 3) 210	1) 25.8 2) 37.8 3) 6.8 (0-4 h)	1) 4.8 2) 5.6 3) 4.2 (0-4 h)	1) 23.2 2) 26.2 3) 6.7 (0-4 h)
(109)	Recreationally active males (n= 7) and females (n= 3) VO_{2max} = 52 ml/kg BM/min (M) VO_{2max} = 46 ml/kg BM/min (F)	120 min cycling at 70% VO_{2max} + 5x1 min sprints 85% (1 min rec at 45%) T_{amb}=19-21°C	1) 0.6 g CHO/kg BM/h 2) 0.6 g CHO + 0.03 g PRO/kg BM/h 3) 0.6 g CHO + 0.06g PRO/kg BM/h Consumed immediately and 2 h post-exercise	1) 119 2) 152 3) 129	1) 23.5 2) 19.8 3) 16.8 (0-4 h)	1) 5.5 2) 5.0 3) 5.0 (0-4 h)	1) 31.8 2) 32.3 3) 45.2 (0-4 h)

M= male, F= female, T_{amb}= ambient temperature, RH= relative humidity, CHO= carbohydrate, PRO= protein, BM= body mass, min= minute(s), h= hour(s), dw= dry weight. C MRS= carbon magnetic resonance spectroscopy

*Muscle glycogen values obtained from muscle biopsy technique unless otherwise stated.

Table 2.3B Summary of studies investigating the effect of post-exercise nutrition interventions on muscle glycogen resynthesis following a single feeding.

Reference	Participants	Exercise	Recovery nutrition intervention	Post-exercise muscle glycogen (mmol/kg dw)	Muscle glycogen rate of resynthesis (mmol/kg dw/h)	Mean blood glucose value between biopsies (mmol/L)	Mean plasma insulin value between biopsies (µIU/ml)
(98)	Healthy males (n= 7) VO_{2max} = 39 ml/kg BM/min	93 min interval cycling at 70% VO_{2max} + sprints) T_{amb} and RH not reported	1) 1.0 g PRO/kg BM/h 2) 0.8 g CHO/kg BM/h 3) 0.8 g CHO + 0.1 g PRO/kg BM/h Consumed 30 min post-exercise	1) 51 2) 74 3) 59	1) 11.7 2) 15.0 3) 11.7 (0-2 h)	1) 4.2 2) 6.0 3) 5.7 (0-2 h)	1) 12.2 2) 46.0 3) 57.5 (0-2 h)
(99)	Healthy males (n= 7) VO_{2max} = 42 ml/kg BM/min	93 min interval cycling at 70% VO_{2max} + sprints) T_{amb} and RH not reported	1) 0.8 g CHO/kg BM/h 2) 0.8 g CHO/kg BM/h 3) 0.5 g CHO/kg BM/h Consumed 30 min post-exercise	1) 51 2) 39 3) 20	1) 15.7 2) 11.3 3) 12.0 (0-2 h)	1) 5.5 2) 5.1 3) 4.9 (0-2 h)	1) 50.5 2) 33.4 3) 21.2 (0-2 h)

(100)	Trained males (n= 10) VO_{2max} = 4.0 L/min	Cycling to exhaustion at 70% VO_{2max} (mean time= 83 min) T_{amb} and RH not reported	1) 1.0 g CHO/kg BM/h (glucose) 2) 1.0 g CHO/kg BM/h (sucrose) 3) Non-caloric placebo Consumed immediately post-exercise	1) 272 2) 250 3) 318	1) 40.0, 12.5, 11.0, 8.0 2) 5.0, 8.5, 5.7, 6.3 3) -12.0, -0.5, -2.0, -0.5 (0-1, 0-2, 0-3, 0-4 h)	1) 5.2 2) 4.7 3) 4.4 (0-1 h)	1) 14.5, 50.7, 44.2 2) 16.0, 36.8, 31.3 3) 8.3, 13.0, 15.6 (0-1, 0-2, 0-3 h)
(101)	Healthy males (n= 8) VO_{2max} = 46 ml/kg BM/min	60 min cycling at 75% VO_{2max} T_{amb} and RH not reported	2.0 g CHO/kg BM/h Consumed immediately post-exercise	180	18.3 (0-3 h)	5.9 (0-3 h)	39.2 (0-3 h)
(107)	Healthy males (n= 8) VO_{2max} = 43 ml/kg BM/min	60 min cycling at 75% VO_{2max} T_{amb} and RH not reported	2.0 g CHO + 0.3 g PRO/kg BM/h Consumed within 10 min post-exercise	1) 185	18.7 (0-3 h)	6.3 (0-3 h)	61.0 (0-1 h)
(102)	Healthy males (n= 12) VO_{2max} = 49 ml/kg BM/min	60 min cycling at 75% VO_{2max} T_{amb} and RH not reported	2.0 g CHO + 0.3 g PRO/kg BM/h Consumed within 10 min post-exercise	119	16.7 (0-3 h)	5.5 (0-3 h)	24.3 (0-1 h)

(103)	Trained male (n= 8) and female (n= 4) cyclists/triathletes VO_{2max} = 61 ml/kg BM/min (M) VO_{2max} = 46 ml/kg BM/min (F)	120 min cycling at 60% VO_{2max} T_{amb} and RH not reported	1) 1.1 g CHO/kg BM/h 2) 1.1 g CHO + 0.3 g PRO/kg BM/h Consumed from 0-20 min post-exercise	2) 238 266	1) 27.0 2) 32.0 (0-1 h)	1) 4.6 2) 4.5 (0-1 h)	1) 18.1 2) 24.3 (0-1 h)

M= male, F= female, T_{amb}= ambient temperature, RH= relative humidity, CHO= carbohydrate, PRO= protein, BM= body mass, min= minute(s), h= hour(s), dw= dry weight. C MRS= carbon magnetic resonance spectroscopy

*Muscle glycogen values obtained from muscle biopsy technique unless otherwise stated.

Muscle Protein Synthesis

A total of 18 trials derived from eight studies were included for review of post-exercise nutrition interventions for muscle protein synthesis (**Figure 2.1B**). Exercise protocols were heterogeneous with regards to modality, intensity and duration (**Table 2.4**). Four studies measured myofibrillar FSR between 2-6 h after endurance exercise (110-113). Provision of 0.2-0.3 g protein/kg BM/h achieved FSR between 0.060 – 0.090%/h. One trial provided 0.55 g protein/kg BM/h, however the FSR induced by this dose (0.103%/h) was 'likely bioequivalent' to 0.2 g protein/kg BM/h (0.092%/h) (113). Three studies measured mitochondrial protein synthesis, two of which did not observe any effect with protein intake (0.3 g/kg BM/h) compared to non-nitrogenous interventions (110,111). The third study did not observe any differences in mitochondrial protein synthesis with isocaloric intake of different quality proteins (112). Two studies observed enhanced mixed muscle FSR (i.e., ranging from 0.065%/h to 0.088%/h) with protein intake (0.4-0.8 g/kg BM/h) compared to non-nitrogenous interventions (i.e., ranging from 0.052%/h to 0.067%/h) (114,115). Wilkinson et al., observed mixed FSR similar to that of non-nitrogenous intervention trials (0.06%/h) with a protein intake equivalent to 0.1 g/kg BM/h (94). Conversely, Lunn et al. observed high mixed FSR (0.080%/h) after consumption of a non-nitrogenous beverage (116).

Six of the reviewed studies analysed the changes in concentration of muscle intracellular signalling proteins including mTOR (n= 5), Akt (n= 3), eukaryotic elongation factor 2 (eEF2) (n=4), eukaryotic initiation factor 4E-binding protein (4E-BPI) (n= 4), ribosomal protein p70 S6 kinase (p70S6K) (n= 4), rp-S6 (n= 3), AMPK (n= 2), and p38-MAPK (n=1) and the expression of mRNA for proteins including muscle ring finger protein 1 (MuRF1) (n= 2), PCG-1α (n= 2) and forkhead box O (FoxO-3A) (n= 1) (110-114, 116). Activation of mTOR and downstream targets (including rpS6 and 4E-BPI) was greater with increasing protein intake (110,111,113), while markers of MPB (i.e., MuRF1, FOXO-3A) were lessened (111,114).

Table 2.4 Summary of studies investigating the effect of post-exercise nutrition interventions on muscle protein synthesis.

Reference	Participants	Exercise	Recovery nutrition intervention	Myofibrillar FSR Value (%/h)	Mitochondrial FSR Value (%/h)	Mixed FSR Value (%/h)
(94)	Trained male cyclists (*n*= 12) VO_{2max}= 60 ml/kg BM/min	100 min intermittent cycling (80-100%W_{max}) T_{amb} and RH not reported	1) 1.2 g CHO + 0.55 g PRO/kg BM/h 2) 1.2 g CHO + 0.2 g PRO/kg BM/h 3) 1.8 g CHO/kg BM/h Consumed every 30 min from 0-90 min post-exercise	1) 0.103 2) 0.092 3) 0.069 (0-4 h)	NR	NR
(110)	Trained male athletes (*n*= 8) VO_{2max}= 55 ml/kg BM/min	90 min cycling at 65% VO_{2max} T_{amb} and RH not reported	1) 1.0 g CHO + 0.1 g PRO (glutamine)/kg BM/h 2) 1.0 g CHO + 0.1 g PRO (glycine & alanine)/kg BM/h Consumed every 15 min from 0-3 h post-exercise	NR	NR	1) 0.063 2) 0.062 (0-3 h)
(111)	Recreationally active males (*n*= 8) VO_{2max}= 52 ml/kg BM/min	60 min cycling at 70% VO_{2max} T_{amb} and RH not reported	1) 1.6 g CHO + 0.8 g PRO/kg BM/h 2) Non-caloric placebo Consumed immediately and 1 h post-exercise	NR	NR	1) 0.065 2) 0.052 (2-6 h)

(112)	Recreationally active males (*n*= 6) VO_{2max}= 4.4 L/min	2 h intermittent cycling (50-80% VO_{2max}) T_{amb} and RH not reported	1) 1.2 g CHO/kg BM/h 2) 1.2 g CHO + 0.4 g PRO/kg BM/h 3) 1.6 g CHO/kg BM/h Consumed every 15 min from 0-3 h post-exercise	NR	NR	1) 0.067 2) 0.088 3) 0.060 (0-4 h)
(113)	Recreationally active males (n= 12 per group) VO_{2max} not reported	4x8 leg press + 4x8 leg extensions at 80% 1RM + 30 min cycling at 60% W_{max} T_{amb} and RH not reported	1) 0.6 g CHO + 0.3 g PRO/kg BM 2) 0.6 g CHO + 0.3 g PRO/kg BM 3) 0.6 g CHO + 0.3 g PRO/kg BM Consumed immediately post-exercise	1) 0.061, 0.052, 0.053 2) 0.055, 0.048, 0.052 3) 0.063, 0.054, 0.056 (0-2 h, 2-6 h, 0-6 h)	1) 0.057, 0.057, 0.058 2) 0.083, 0.060, 0.058 3) 0.083, 0.051, 0.061 (0-2 h, 2-6 h, 0-6 h)	NR
(114)	Trained male cyclists (*n*= 10) VO_{2max}= 67 ml/kg BM/min	80 min steady state cycling (80% W_{max}) T_{amb}= 21°C, RH= 40%	1) 0.7 g CHO/kg BM/h 2) 0.7 g CHO + 0.3 g PRO/kg BM/h Consumed immediately and 30 min post-exercise	1) 0.057 2) 0.087 (0-4 h)	1) 0.086 2) 0.082 (0-4 h)	NR
(115)	Healthy males (*n*= 8) VO_{2max}= 47 ml/kg BM/min	8x5 leg extensions 80% 1RM + 15 min rest + 30 min cycling at 70% VO_{2max} T_{amb} and RH not reported	1) Non-caloric placebo 2) 0.3 g PRO/kg BM/h Consumed immediately post-exercise	1) 0.053 2) 0.073 (1-4 h)	1) 0.070 2) 0.082 (1-4 h)	NR

(116)	Recreationally active males (*n*= 8) VO_{2max}= 53 ml/kg BM/min	45 min running at 65% VO_{2max} T_{amb} and RH not reported	1.0 g CHO/kg BM/h Consumed immediately post-exercise	NR	NR	0.080 (0-3 h)

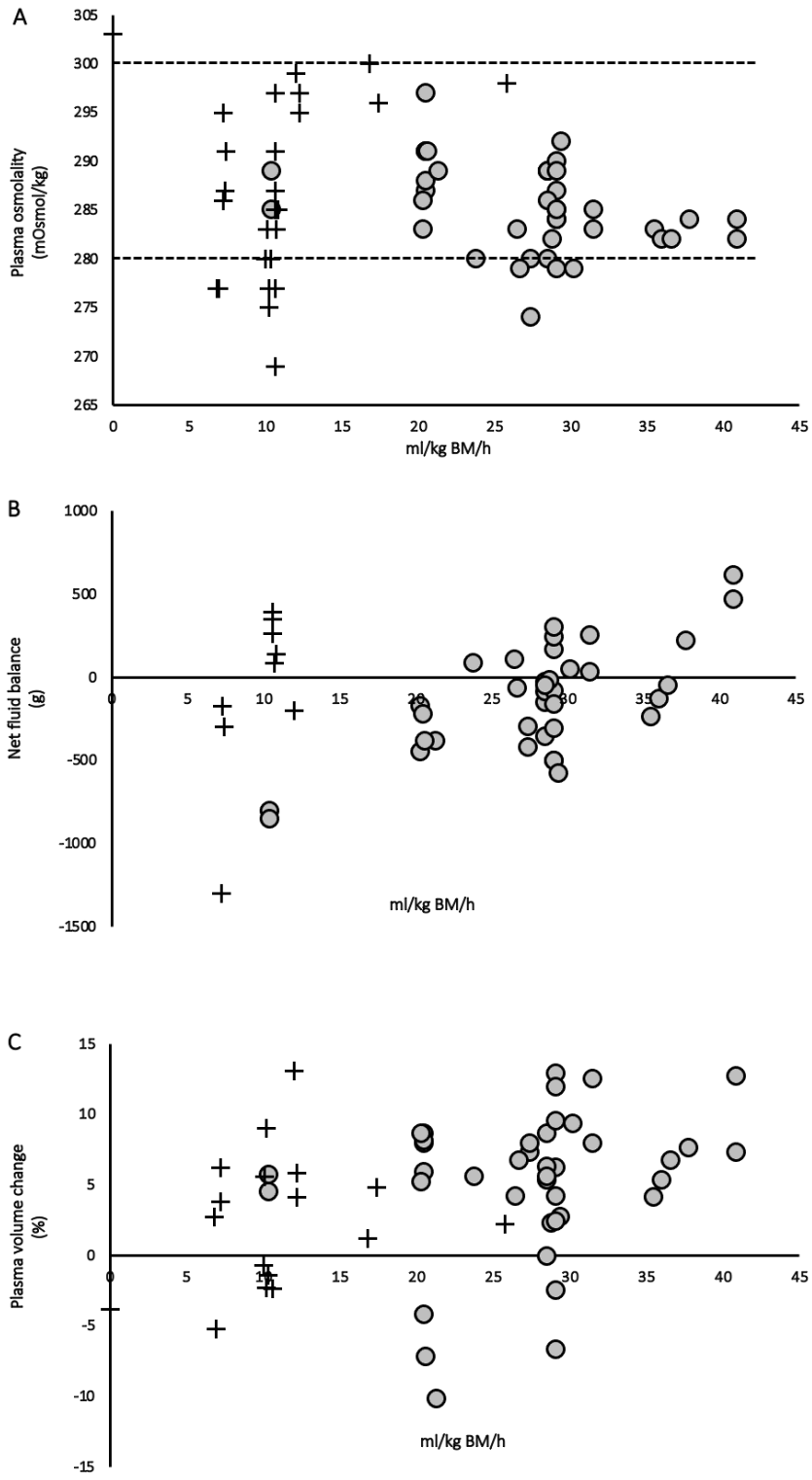
M= male, F= female, RM= repetition maximum, W_{max}= maximum wattage, T_{amb}= ambient temperature, RH= relative humidity, CHO= carbohydrate, PRO= protein, BM= body mass, min= minute(s), h= hour(s), FSR= fractional synthetic rate.

Hydration Status

The search strategy yielded 21 studies for a total of 68 trials that investigated the effect of rehydration inventions on P_{Osmol}, in addition to NFB and (or) P_V change, after prolonged strenuous exercise (**Figure 2.1C)**. The study designs and results are summarised in **Table 2.5**. All of the reviewed studies applied exercise protocols conducted in warm and humid conditions (i.e., 24-35°C ambient temperature and 50-90% relative humidity). BM losses were reported between 2.0% to 3.0%, with the exception of one study that failed to sufficiently reduce fluid by >2% BM loss amongst female participants (i.e., 1.4% BM loss) (117). Fluid prescription was calculated relative to fluid losses to provide 50 (n= 2) (118), 75 (n= 1) (119), 100 (n= 10) (118, 120-122), 120 (n= 1) (119), 130 (n= 3) (123), 150 (n= 42) (8, 117, 118, 124-135), or 200% BM loss (n= 4) (119,135), giving an average of 1982 ± SD 640 ml fluid intake. One trial did not provide any fluid (119), and one study provided fluid *ad libitum* (136). Fifteen studies dispensed fluid over a 1 h period, while the remaining eight studies spread fluid intake over a 3-4 h rehydration period.

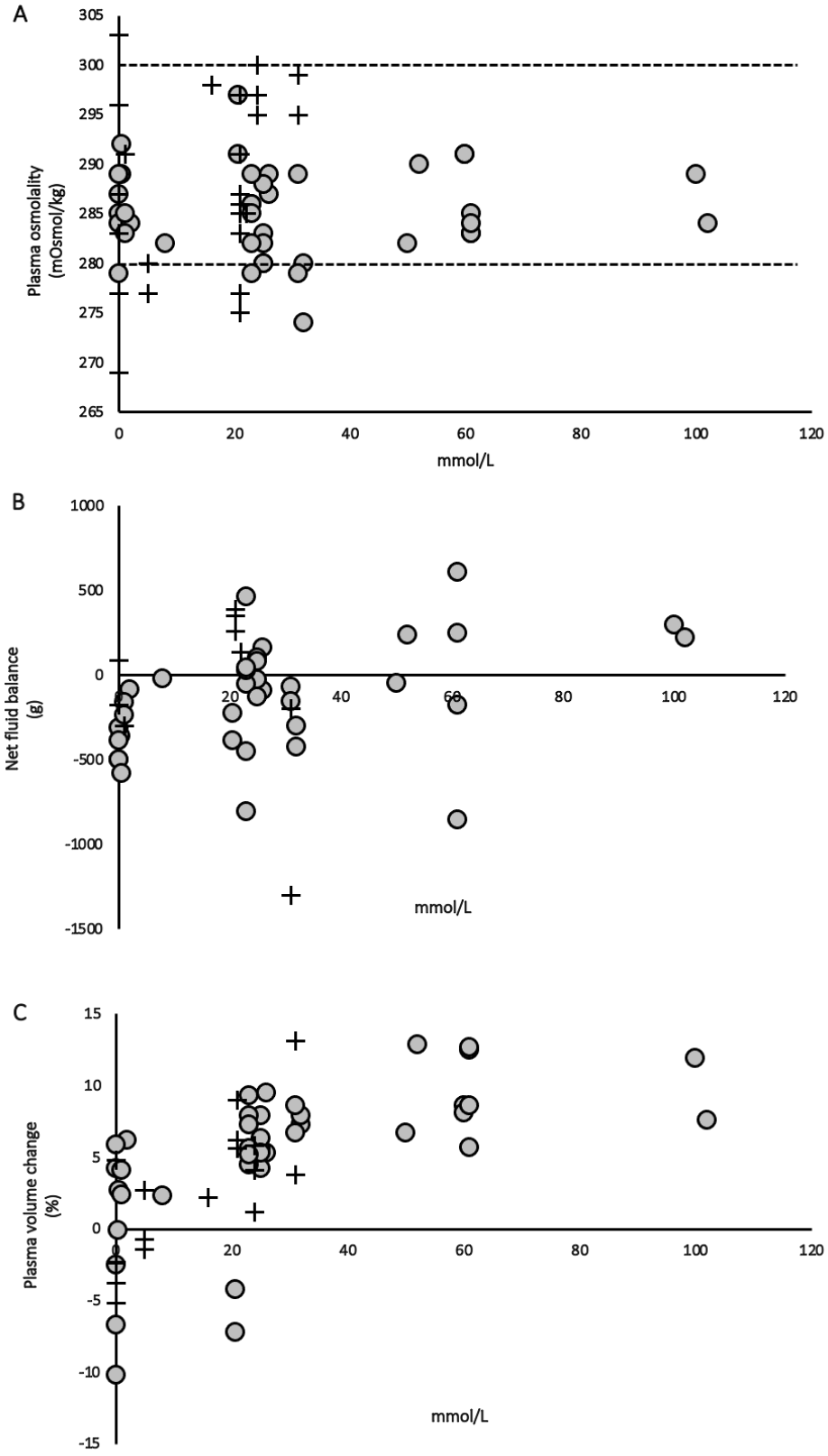
Figure 2.3 compares fluid intake (ml/kg BM/h) to P_{Osmol}, NFB, and P_V change recorded 2 h after the onset of rehydration. When fluid is provided over a 1 h period and BM loss is between 2-3%, a fluid intake between 20-35 ml/kg BM/h appears sufficient to achieve euhydration, with no further benefit with intakes above 35 ml/kg BM/h. When comparable overall volumes (i.e., 100-150% BM loss replacement) of fluid are provided but at a slower rate over 3-4 h (i.e., 7-17 ml/kg BM/h), euhydration is similarly achieved. **Figure 2.4** compares sodium concentration (mmol/L) to P_{Osmol}, NFB, and P_V change recorded 2 h after the onset of rehydration. P_{Osmol} was within range of euhydration 2 h after the onset of rehydration on the vast majority of trials, suggesting that the exercise stress was not sufficient to perturb this marker.

Figure 2.3 Fluid intake (ml/kg BM/h) effect on A) plasma osmolality, B) net fluid balance and C) plasma volume change recorded 2 h after the onset of rehydration.



Hydration provided over 1 h period (●) and 3 h period (✚).
------ indicates reference range for euhydration (137)

Figure 2.4 Sodium concentration of fluid (mmol/L) effect on A) plasma osmolality, B) net fluid balance and C) plasma volume change recorded 2 h after the onset of rehydration.



Hydration provided over 1 h period (●) and 3 h period (+).
------ indicates reference range for euhydration (137)

Table 2.5 Summary of studies investigating the effect of post-exercise nutrition interventions on hydration status.

Reference	Participants	Exercise	Recovery nutrition intervention		Plasma osmolality (mOsmol/kg)	Net fluid balance / Δ BM (g)	Plasma volume change (%)
			Nutrients	Volume (ml/kg BM/h)			
(117)	Recreationally active males (*n*= 9) VO_{2max} = 46 ml/kg BM/min	60 min running at 60% VO_{2max} T_{amb}= 29°C, RH= 71%	1) 0.7 g CHO/kg BM/h, 21 mmol/L sodium 2) Non-caloric beverage 3) 1.0 g CHO/kg BM/h, 5 mmol/L sodium	1) 10.1 2) 10.2 3) 10.0	1) 283 2) 277 3) 280****	NR	1) 5.6 2) -2.3 3) -0.7[b]
	Recreationally active females (*n*= 10) VO_{2max}= 38 ml/kg BM/min		4) 0.5 g CHO/kg BM/h, 21 mmol/L sodium 5) Non-caloric beverage 6) 0.7 g CHO/kg BM/h, 5 mmol/L sodium Consumed every 30 min from 30-180 min post-exercise	4) 7.2 5) 6.9 6) 6.8	4) 286 5) 277 6) 277****	NR	4) 6.2 5) -5.2 6) 2.7[b]
(118)	Healthy males Group L (*n*= 6) VO_{2max}= 59 ml/kg BM/min	Intermittent cycling to 2% BM loss + 10 min immersed in 41°C water prior to exercise T_{amb}=30-32°C, RH=70-90%	1) 0.2 g CHO/kg BM/h, 23 mmol/L sodium 2) 0.3 g CHO/kg BM/h, 23 mmol/L sodium 3) 0.5 g CHO/kg BM/h, 23 mmol/L sodium 4) 0.7 g CHO/kg BM/h, 23 mmol/L sodium	1) 10.4 2) 20.3 3) 31.5 4) 40.9	1) 289 2) 286 3) 285 4) 282	1) -804 2) -451 3) 29 4) 466	1) 4.5[a] 2) 5.2[a] 3) 7.9[a] 4) 7.3[a]

	Group H (*n*= 6) VO_{2max}= 58 ml/kg BM/min		5) 0.2 g CHO/kg BM/h, 61 mmol/L sodium 6) 0.3 g CHO/kg BM/h, 61 mmol/L sodium 7) 0.5 g CHO/kg BM/h, 61 mmol/L sodium 8) 0.7 g CHO/kg BM/h, 61 mmol/L sodium Consumed every 15 min from 30-90 min post-exercise	5) 10.4 6) 20.3 7) 31.5 8) 40.9	5) 285 6) 283 7) 283 8) 284	5) -852 6) -175 7) 250 8) 611	5) 5.7[a] 6) 8.6[a] 7) 12.5[a] 8) 12.7[a]
(119)	Trained cyclists (*n*= 8 males) VO_{2max} not reported	50% W_{max} cycling to 3.0% BM loss (100-120 min) T_{amb}= 28°C, RH= 50-60%	1) 0.9 g CHO/kg BM/h, 31 mmol/L sodium 2) 0.5 g CHO/kg BM/h, 31 mmol/L sodium Consumed 0, 1 and 2h post-exercise	1) 12.0 2) 7.2	1) 299 2) 295	1) -200 2) -1300	1) 13.1[a] 2) 3.8[a]
(120)	Healthy males (*n*= 10) VO_{2max} not reported	Cycling at 50% age predicted max HR to 2% BM loss (mean time= 74 min) in heated (45°C) body suit. T_{amb}= 25.0°C, RH= 50%	1) Non-caloric beverage, neg sodium 2) 0.4 g CHO/kg BM/h, 20.6 mmol/L sodium 3) 1.3 g CHO/kg BM/h, 20.6 mmol/L sodium Consumed over 30 min immediately post-exercise	1) 21.3 2) 20.6 3) 20.5	1) 289 2) 291 3) 297	1) -386 2) -384 3) -222	1) -10.2 2) -7.2 3) -4.2

(121)	Trained endurance male athletes (*n*= 8) VO_{2max}= 58 ml/kg BM/min	Running at 65% VO_{2max} to 2.5% BM loss (time not reported) T_{amb}= 25-26°C, RH= 35-45%	1) Food and fluid withheld 2) 1.3 g CHO/kg BM/h, 16 mmol/L sodium Consumed at 90 and 120 min post-exercise	1) Nil 2) 25.8	1) 303 2) 298***	NR	1) -3.8[b] 2) 2.2[b]
(122)	Recreationally active males (*n*= 9) VO_{2max}= 51 ml/kg BM/min	Intermittent cycling to 2% BM loss (mean time= 51 min) T_{amb}= 31°C, RH= 73%	1) 0.3 g CHO/kg BM/h 2) Non-caloric beverage, 60 mmol/L sodium 3) Non-caloric beverage, 25 mmol/L sodium 4) 0.3 g CHO/kg BM/h, 60 mmol/L sodium Consumed 1 h post-exercise	1) 20.5 2) 20.5 3) 20.5 4) 20.5	1) 287 2) 291 3) 288 4) 291	NR	1) 5.9[a] 2) 8.6[a] 3) 7.9[a] 4) 8.1[a]
(123)	Healthy males (*n*= 6) VO_{2max}= 3.6 L/min	Moderate cycling at 58% VO_{2max} to 1.9% BM loss (mean time= 45 min) T_{amb}= 35.3°C, RH= 59%	1) Non-caloric, 32 mmol/L sodium 2) 0.5 g CHO/kg BM/h, 32 mmol/L sodium 3) 2.6 g CHO/kg BM/h, 31 mmol/L sodium Consumed every 15 min from 30-90 min post-exercise	1) 27.4 2) 27.4 3) 26.7	1) 280 2) 274 3) 279	1) -421 2) -300 3) -67	1) 7.3[a] 2) 7.9[a] 3) 6.7[a]
(124)	Healthy males (*n*= 5) and females (*n*= 3) VO_{2max} not reported	Cycling at 2 W/kg BM until 1.8% BM loss (mean time= 56 min) T_{amb}= 35.0°C, RH= 50%	1) 0.5 g PRO/kg BM/h, neg sodium 2) 0.5 g CHO/kg BM/h, neg sodium Consumed every 15 min from 30-90 min post-exercise	1) 29.1 2) 29.1	1) 285**** 2) 284****	1) -500 2) -500	1) -6.7**** 2) -2.5****

(125)	Healthy males (*n*= 13) and females (*n*= 3) VO_{2max} not reported	Intermittent cycling to 1.7% BM loss (time not reported) T_{amb}= 35°C, RH= 60%	1) 1.7 g CHO/kg BM/h, 31 mmol/L sodium 2) 1.7 g CHO + 0.6 g protein/kg BM/h, 26 mmol/L sodium Consumed every 15 min from 30-90 min post-exercise	1) 28.5 2) 28.5	1) 289 2) 289	1) -154 2) -89	1) 8.6[b] 2) 5.3[b]
(126)	Healthy males (*n*= 7) and females (*n*= 3) VO_{2max} not reported	Intermittent cycling to 1.7% BM loss (mean time= 55 min) T_{amb}= 35°C, RH= 60%	1) Non-caloric beverage, 0.4 mmol/L sodium 2) 0.6 g protein/kg BM/h, 0.5 mmol/L sodium Consumed every 15 min from 30-90 min post-exercise	1) 28.5 2) 29.4	1) 289 2) 292	1) -358 2) -579	1) -0.1[b] 2) 2.7[b]
(127)	Trained males (*n*= 10) VO_{2max}= 60 ml/kg BM/min	60 min running on treadmill at 65% VO_{2max} T_{amb}= 24°C, RH= 60%	1) 0.7 g CHO/kg BM/h, 21 mmol/L sodium 2) 0.3 g CHO/kg BM/h, 21 mmol/L sodium 3) 0.3 g CHO + 0.3 g PRO/kg BM/h, 21 mmol/L sodium 4) 0.5 g CHO + 0.2 g PRO/kg BM/h, 21 mmol/L sodium 5) 0.6 g CHO + 0.2 g PRO/kg BM/h, 21 mmol/L sodium Consumed every 30 min from 0-150 min post-exercise	1) 10.6 2) 10.6 3) 10.6 4) 10.6 5) 10.6	1) 285 2) 277 3) 291 4) 287 5) 297	1) 260 2) 260 3) 350 4) 350 5) 390	NR

(128)	Recreationally active males (*n*= 6) VO_{2max}= 56 ml/kg BM/min	Intermittent cycling to 2.0% BM loss (mean time= 94 min) T_{amb}= 32°C, RH= 54%	1) 0.5 g CHO/kg BM/h, 2 mmol/L sodium 2) 0.5 g CHO/kg BM/h, 26 mmol/L sodium 3) 0.5 g CHO/kg BM/h, 52 mmol/L sodium 4) 0.5 g CHO/kg BM/h, 100 mmol/L sodium Consumed every 10 min from 30-60 min post-exercise	1) 29.1 2) 29.1 3) 29.1 4) 29.1	1) 284** 2) 287** 3) 290** 4) 289**	1) -84* 2) 163* 3) 238* 4) 297*	1) 6.2[a] 2) 9.5[a] 3) 12.9[a] 4) 11.9[a]
(129)	Recreationally active females (*n*= 5) VO_{2max}= 42 ml/kg BM/min	Intermittent cycling to 2% BM loss (mean time= 54 min) + 10 min submersion in 42°C water prior to exercise T_{amb}= 33-34°C, RH= 60%	1) 1.7 g CHO/kg BM/h, 25 mmol/L sodium 2) 1.5 g CHO/kg BM/h, 25 mmol/L sodium 3) 1.8 g CHO/kg BM/h, 25 mmol/L sodium Consumed every 15 min from 30-90 min post-exercise	1) 26.5 2) 23.8 3) 28.5	1) 283 2) 280 3) 280	1) 103 2) 83 3) -28	1) 4.2[a] 2) 5.6[a] 3) 6.3[a]
(130)	Recreationally active males (*n*= 7) VO_{2max} not reported	Cycling to 1.8% BM loss (time= 93-94 min) T_{amb}= 30°C, RH= 58%	1) 0.4 g CHO/kg BM/h, 22 mmol/L sodium 2) Non-caloric beverage Consumed every 30 min from 30-120 min post-exercise	1) 10.8 2) 10.7	1) 285 2) 283	1) 138 2) 85	NR

(131)	Recreationally active males (*n*= 4) and females (*n*= 2) $\dot{V}O_{2max}$= 54 ml/kg BM/min	Intermittent cycling to 2% BM loss (median time= 43 min) T_{amb}= 34°C, RH= 60-70%	1) Non-caloric beverage, 1 mmol/L sodium 2) Non-caloric beverage, 25 mmol/L sodium 3) Non-caloric beverage, 50 mmol/L sodium 4) Non-caloric beverage, 102 mmol/L sodium Consumed from 0-60 min post-exercise	1) 35.5 2) 36.0 3) 36.6 4) 37.8	1) 283 2) 282 3) 282 4) 284	1) -237 2) -130 3) -49 4) 221	1) 4.1[a] 2) 5.3[a] 3) 6.7[a] 4) 7.6[a]
(132)	Recreationally active males (*n*= 4) and females (*n*= 4) $\dot{V}O_{2max}$= 56 ml/kg BM/min	Intermittent cycling to 2% BM loss T_{amb}= 36.0°C, RH= 65%	1) Non-caloric beverage 2) 1.9 g CHO/kg BM/h, 8 mmol/L sodium 3) 1.7 g CHO/kg BM/h, 23 mmol/L sodium 4) Non-caloric beverage, 1 mmol/L sodium Consumed every 15 min from 30-90 min post-exercise	1) 29.1 2) 28.8 3) 28.5 4) 29.1	1) 279 2) 282 3) 286 4) 285	1) -310 2) -18 3) -50 4) -162	1) 4.2[a] 2) 2.3[a] 3) 5.6[a] 4) 2.4[a]
(133)	Recreationally active males (*n*= 7) $\dot{V}O_{2max}$= 4.3 L/min	Intermittent cycling to 1.8% BM loss (mean time= 36.2 min) T_{amb}= 35°C, RH= 60-70%	1.8 g CHO/kg BM/h, 23 mmol/L sodium Consumed every 15 min from 30-90 min post-exercise	30.2	279	46	9.3[a] 5)

(8)	Endurance trained males (*n*= 9) VO_{2max}= 59 ml/kg BM/min	90 min running at 70% VO_{2max} T_{amb}=18-19°C, RH=54-55%	1) 0.8 g CHO/kg BM/h, 24 mmol/L sodium 2) 0.9 g CHO/kg BM/h, 24 mmol/L Consumed every 30 min from 30-180 min post-exercise	1) 12.2 2) 12.2	1) 297 2) 295	NR	1) 4.1 2) 5.8[b]
(134)	Endurance trained males (*n*= 13) VO_{2max}= 65 ml/kg BM/min	60 min running at 70% VO_{2max} T_{amb}= 21°C, RH= 71%	1) 0.7 g CHO/kg BM/h, 21 mmol/L sodium 2) Non-caloric beverage 3) 1.2 g CHO/kg BM/h, 5 mmol/L sodium Consumed every 30 min from 30-180 min post-exercise	1) 10.2 2) 10.6 3) 10.3	1) 275 2) 269 3) 280****	NR	1) 9.0 2) -2.4 3) -1.4[b]
(135)	Endurance trained males (*n*= 9) VO_{2max}= 60 ml/kg BM/min	90 min running at 70% VO_{2max} T_{amb}= 20°C, RH= 55-56%	1) 1.2 g CHO/kg BM/h, 24 mmol/L sodium 2) Non-caloric beverage Consumed every 30 min from 30-180 min post-exercise	1) 16.8 2) 17.4	1) 300 2) 296	NR	1) 1.2 2) 4.8[b]
(136)	Endurance trained female cyclists (*n*= 8) VO_{2max}= 46 ml/kg BM/min	60 min cycling at 60% PPO T_{amb}= 24°C, RH= 66%	1) Non-caloric beverage 2) 0.4 g CHO/kg BM/h, 1 mmol/L Consumed *ad libitum* throughout 4 h recovery period	1) 7.3 2) 7.4	1) 287* 2) 291*	1) -175 2) -300	NR

M= male, F= female, W_{max}= maximum wattage, T_{amb}= ambient temperature, RH= relative humidity, neg= negligible, CHO= carbohydrate, PRO= protein, BM= body mass, min= minute(s), h= hour(s).

[a] change expressed relative to post-exercise plasma volume; [b] change expressed relative to pre-exercise plasma volume; *value taken at t= 60 min (where t= 0 is the first beverage given); **value taken at t= 90 min; ***value taken at t= 160 min; **** value taken at t= 150 min.

Immune Function

The search strategy generated four studies to be included for review **(Figure 2.1D)**. All four reviewed studies required male runners ($\dot{V}O_{2max}$ 59-63 ml/kg/min) to complete 2 h running at 70-75% $\dot{V}O_{2max}$ **(Table 2.6)**. Three studies found that provision of 1.2 g carbohydrate/kg BM in addition to 0.4 g protein/kg BM immediately after prolonged exercise prevented a decrease in bacterially-stimulated neutrophil degranulation and (or) increased saliva lysozyme concentration (64,138,139). However, these nutrition interventions had no impact on circulating stress hormones, leukocyte trafficking, and salivary α-amylase and salivary immunoglobulin A (s-IgA) responses. In addition, Costa et al., 2011 found that provision of carbohydrate alone (1.2 g/kg BM) was sufficient to prevent the depression in bacterially-stimulated neutrophil degranulation during the post-exercise recovery period (25).

Gut Integrity

The search strategy originally did not retrieve any studies that met the selection criteria for post-exercise nutrition interventions and gastrointestinal function and integrity (**Figure 2.1E**). One paper was identified during the rerun, published by the present authors. Costa et al., 2019 demonstrated that following 2 h running exercise at 70% $\dot{V}O_{2max}$ in temperate ambient conditions, there was a 108% increase in intestinal fatty acid binding protein (I-FABP). During recovery, clinically relevant (i.e., breath hydrogen (H_2) >10 ppm) carbohydrate malabsorption occurred 3 h after consumption of a dairy milk beverage containing 1.2g carbohydrate/kg BM and 0.4 g protein/kg BM and isovolumetric plain water (13).

Table 2.6 Summary of studies investigating the effect of post-exercise nutrition interventions on immune function.

Reference	Participants	Exercise	Exercise and nutrition intervention	Cell count at 0 h and 2 h post-exercise and ($x10^9$/L)	Functional changes in immune response
(25)	Trained runners (n= 12 males) VO_{2max}= 63 ml/kg BM/min	2 h running at 75% VO_{2max} T_{amb}= 20°C, RH=59%	1) 1.2g CHO + 0.4g PRO/kg BM/h 2) 1.2 g CHO/kg BM/h 3) Non-caloric placebo Consumed immediately post-exercise	Circulating leukocytes 1) 5.0, 11.3 2) 5.1, 10.8 3) 5.4, 11.5 Circulating lymphocytes 1) 2.8, 1.5 2) 2.8, 1.6 3) 2.8, 1.5	Δ bacterially-stimulated elastase release from pre-exercise to 120 min post-exercise (µg/ml) 1) 7.9 2) 8.1 3) 4.7
(138)	Trained runners (n= 9 males) VO_{2max}= 61 ml/kg BM/min	2 h running at 75% VO_{2max} T_{amb}= 20°C, RH=59%	1) 1.2 g CHO + 0.4 g PRO/kg BM/h 2) 1.2 g CHO + 0.4 g PRO/kg BM/h 3) Non-caloric placebo Consumed immediately post-exercise and 2) consumed 1 h post-exercise	Circulating leukocytes 1) 9.8.1,12.3 2) 9.1, 12.0 3) 9.4, 12.0 Circulating lymphocytes 1) 1.8, 1.3 2) 1.8, 1.3 3) 2.0, 1.3 T-lymphocyte $CD3^+$ 1) 1.6, 1.0 2) 1.4, 1.0 3) 1.6, 0.9	Δ bacterially-stimulated elastase release from pre-exercise to 120 min post-exercise (µg/ml) 1) 3.0 2) 1.8 3) 2.6

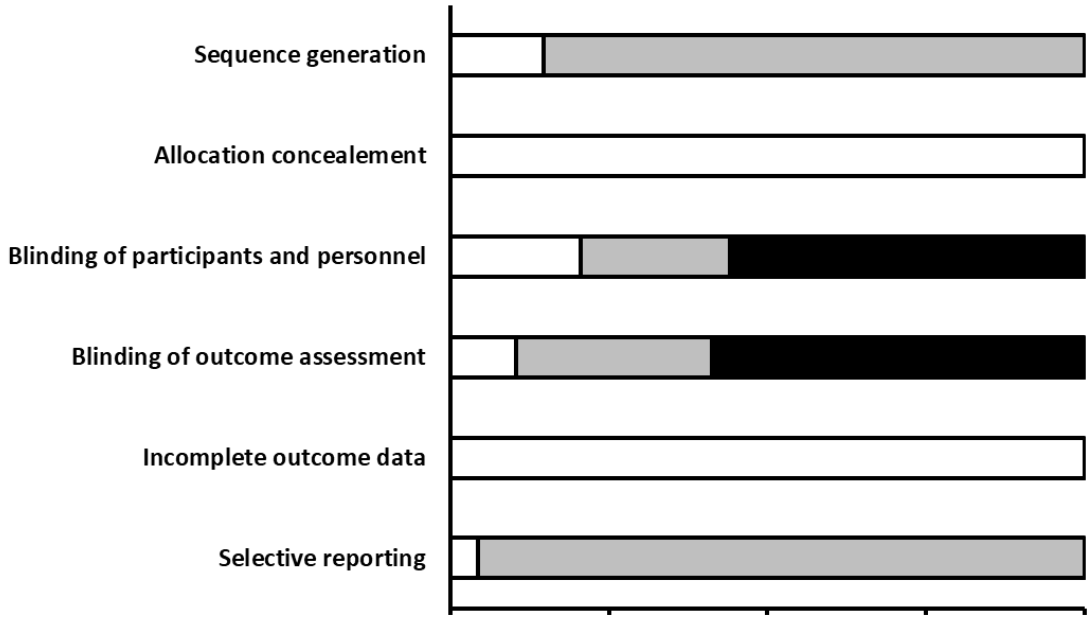
(139)	Trained runners (n= 11 males) VO_{2max}= 62 ml/kg BM/min	2 h running at 75% VO_{2max} T_{amb}= 20°C, RH=59%	1) 1.2 g CHO + 0.4 g PRO 2) Non-caloric placebo Consumed immediately post-exercise	NR	Δ saliva lysozyme concentration from pre-exercise to 120 min post-exercise (µg/ml) 1) 0.5 2) -13.1 Δ saliva α-amylase activity from pre-exercise to 120 min post-exercise (U/ml) 1) 13.1 2) 5.0
(64)	Trained male runners (n= 11) VO_{2max}= 59 ml/kg BM/min	2 h running at 70% VO_{2max} T_{amb}= 25°C, RH=43%	1) 1.2 g CHO + 0.4 g PRO 2) Non-caloric placebo Consumed immediately post-exercise	NR	Δ bacterially-stimulated elastase release from pre-exercise to 120 min post-exercise (%) 1) +27% 2) -38%

M= male, F= female, W_{max}= maximum wattage, T_{amb}= ambient temperature, RH= relative humidity, CHO= carbohydrate, PRO= protein, BM= body mass, min= minute(s), h= hour

Risk of Bias Assessment

Bias was identified across all included studies and is summarised in Figure 2.5. Many studies did not specify blinding or randomisation procedures, therefore risk of performance and detection bias, and order effect (included under 'other bias') has been judged as 'unclear'. In order to improve the strength and rigour of scientific methodology of randomised crossover trials, it is recommended that authors specify the method of randomisation of trial order (e.g. Latin square, computer generated), blinding procedures of participants and personnel (e.g. opaque bottles and flavour-matched beverages for provision of fluids, blinding of sample analysis), and prospective trial registration of aims and methods for transparency in reporting of outcomes.

Figure 2.5 Risk of bias assessment results for low (□), high (■), or unclear (■) risk of bias, in accordance with Cochrane Risk of Bias assessment tool.



Discussion

The aim of the current review was to systematically review the literature investigating the optimal acute recovery nutrition intervention to support i) muscle glycogen resynthesis ii) muscle protein synthesis iii) rehydration iv) immune function and v) gastrointestinal function and integrity after prolonged, strenuous exercise. Results from this review suggest that an intake of 0.8-1.2 g carbohydrate/kg BM/h

and 0.2-0.4 g protein/kg BM/h during the acute recovery period (i.e., within 4 h after strenuous exercise) will maximally stimulate muscle glycogen resynthesis and muscle protein synthesis, and support functional immune responses. Additionally, fluid intake at a rate of 20-35 ml/kg BM/h with 20-40 mmol/L sodium is optimal for rehydration when mild dehydration (e.g. <3% BM loss) is experienced. Some preliminary evidence showed mild gastrointestinal injury and carbohydrate malabsorption during the recovery period of nutritive recovery beverages and (or) pre-exercise meals.

Muscle glycogen resynthesis

Restoration of skeletal muscle glycogen stores is a primary goal of recovery from prolonged, strenuous exercise (140). Consistent with previous reviews and current nutrition guidelines, results from the current review suggest that consumption of 0.8-1.2 g/kg BM/h will achieve maximal muscle glycogen synthesis rates, with intakes greater than 1.2 g/kg BM/h having no additional benefit (6,141). When carbohydrate intake is less than 0.8 g/kg BM/h, provision of protein, including whole food protein (e.g., dairy beverages), protein hydrolysates (i.e., casein or whey) and free amino acids (e.g., glutamine, leucine and (or) isoleucine), to compensate energy intake will elicit similar rates of muscle glycogen synthesis.

Results from the current review demonstrated extreme variation in the degree of depletion of muscle glycogen stores observed within and between trials. The extent of muscle glycogen depletion is a factor of intensity and duration, and muscle glycogen content at the onset of exercise, which in turn is dictated by diet and training status (142,143). On a mixed diet, resting muscle glycogen stores are typically 460-530 mmol/kg dw for endurance-trained athletes, with concentrations as high as 840 mmol/kg dw observed after carbohydrate-loading protocols (144). Without super-compensation of stores, 2 h of endurance-type exercise at 70% $\dot{V}O_{2max}$ has been shown to deplete muscle glycogen (i.e., <300 mmol/kg dw) for both trained and untrained athletes (143). All trials reduced muscle glycogen to $\leq$300 mmol/kg

dw. It is likely that this wide variation can be attributed to heterogeneity of exercise stimuli, fitness level and energy availability (143).

Although not within the scope of this review, it is important to also consider how manipulation of carbohydrate intake may act as a modulator of skeletal muscle training adaptation. Carbohydrate does not contribute to MPS directly; however, carbohydrate intake post-exercise can help reduce MPB (145). This effect may be due to the rise in blood insulin levels elicited by carbohydrate intake, and facilitation of amino acid transport into the muscle cell (38,146). Furthermore, over recent years, there has been a growing body of evidence to support the advantages of training with low carbohydrate availability to increase mitochondrial enzyme activity (i.e., citrate synthase, 3-hydroxyacyl-CoA dehydrogenase, succinate dehydrogenase) and concentration (i.e., cytochrome c oxidase) (20), and improve endurance performance (147,148). This is an emerging area of research, and as such, evidenced-based guidelines have not yet been developed (149).

Muscle protein synthesis

Skeletal muscle is the main site of training adaptations. Unlike other systems for which the aim is to return to homeostasis, it is in the interest of the athlete to increase MPS above resting rates, in order to accumulate new muscle protein towards long-term training adaptations. Results from this review indicate a protein dose of 0.2-0.4 g/kg BM/h will stimulate myofibrillar protein synthesis above resting levels. For a 70 kg athlete, this translates to ~20 g protein, which is consistent with current recommendations (6). Indeed, it has been shown that consumption of 15-25 g of high biological value protein (i.e., egg or whey protein), or ~10 g essential amino acids, in close temporal proximity to RE (e.g., ≤30 min) will maximally stimulate myofibrillar protein synthesis (150-152). Likewise, a recent review of protein requirements for endurance athletes suggests that similar doses of protein to those prescribed after RE (i.e., 20 g) will enhance mixed and myofibrillar protein synthesis following prolonged, strenuous exercise (153).

The training objectives of endurance athletes is to optimise the work capacity, energy efficiency and fatigue resistance of the muscle. Post-exercise protein intakes specific to maximising endurance training adaptations have only recently begun to be investigated, with only three studies retrieved in the current review (110-112). Mitochondrial protein synthesis was not influenced by the quantity or quality of protein provided in these studies. As such, there is currently insufficient evidence to provide quantitative recommendations for protein intake to support mitochondrial biogenesis.

While most of the included studies measured changes in phosphorylation of muscle intracellular signalling proteins and mRNA during the recovery period, it was not possible to synthesise these results. It appears that provision of protein increased activation of the mTOR signalling pathway. This is unsurprising given role of protein as a trigger and substrate for MPS. Fluctuations in the concentration of phosphorylated signalling proteins and mRNA provide an indication of the direction of change of muscle protein synthesis. However, given the multiple, complex signalling pathways involved in protein synthesis, and as an indirect measure, fluctuations in these protein concentrations cannot establish the magnitude of change in MPS elicited by a protein dose (154).

Rehydration

The current review investigated the optimal fluid and sodium intake required to return the body to a euhydrated state, as indicated by P_{Osmol}, in addition to NFB and (or) P_V change. Results from the 21 studies included for review suggest that after mild fluid losses (i.e., 2-3% BM loss), fluid intake equivalent to 20-35 ml/kg BM containing 20-40 mmol/L sodium is sufficient to achieve euhydration within 4 h of exercise.

Current guidelines state that 125-150% of fluid losses should be replaced during the acute exercise recovery period to account for ongoing urine losses (52). In the context of a 70 kg athlete losing 3.0%

BM, this is equal to 2.1 L or 30 ml/kg BM of fluid. However, the current review has only considered mild fluid losses, and therefore these findings are not applicable where greater fluid losses may be experienced. From a practical perspective, *ad libitum* fluid intake during exercise according to thirst (e.g. 200-800 ml) is likely to prevent fluid losses >3% BM (155). Indeed, it has been shown that consuming 3 ml water/kg BM/h when exercising for 2 h at 75% $\dot{V}O_{2max}$ with ambient conditions of 20°C and 59% relative humidity resulted in BM loss of ~2.5% (25,138).

Guidelines related to sodium intake during and after exercise are qualitative only. Sodium losses are more difficult to assess and more variable than fluid losses, and a variety of factors influence sodium needs and losses in response to exercise stress (e.g., environmental conditions, clothing, individual sweat sodium concentration, habitual diet) (156,157). Official statements advise that athletes experiencing large fluid losses should consume sodium-containing snacks and fluids during the recovery period, to aid intracellular fluid retention, drive thirst and minimise risk of hyponatremia (52). However, caution is warranted, as there is minimal scientific evidence derived from controlled laboratory settings (155). In conjunction with sodium intake, these guidelines recommend fluids are consumed 'at a modest rate' to avoid excessive diuresis (6).

Immune function

Results from the current review suggest that 1.2 g carbohydrate/kg BM/h with or without 0.4 g protein/kg BM/h will prevent a decrease in bacterially-stimulated neutrophil degranulation and increase salivary lysosome concentration. Authors proposed that the concomitant increase in plasma insulin following consumption of carbohydrate and (or) protein prevented the decrease in bacterially-stimulated neutrophil degranulation, however this mechanism has not been established (158,159). While the clinical significance of a decreased in bacterially-stimulated neutrophil degranulation has not been established, sub-optimal neutrophil function may be indicative of increased susceptibility to illness and infection in the post-exercise period. The mechanism by which carbohydrate-protein feeding

post-exercise increases salivary lysozymes has not been established either, however may be related to delayed gastric emptying and rehydration leading to a concentrating effect of the saliva (139). Provision of carbohydrate and (or) protein did not appear to impact on other immune indices such as cytokine responses, salivary immunoglobulin A (sIgA) and salivary α-amylase.

There are currently no nutritional guidelines specific to supporting immunocompetency in the post-exercise period. The results from the current review are aligned with the current protein and carbohydrate recommendations for muscle protein synthesis and glycogen resynthesis. Therefore, consumption of 0.4 g protein/kg BM and 1.2 g carbohydrate/kg BM immediately post-exercise can provide benefits across all three aspects of exercise recovery, for those participating in immunodepressive exercise.

Gastrointestinal integrity and function

The search strategy only yielded one study that has investigated the effects of gastrointestinal damage on the absorption and digestion of nutrients after prolonged, strenuous exercise. As previously discussed, damage caused by intense prolonged exercise can impair digestion and absorption of nutrients, while nutrition provided during exercise can also attenuate by maintaining patency of the gastrointestinal tract. Future research is needed to determine the degree to which post-exercise nutrition tolerance and assimilation is impacted by impaired gastrointestinal function and integrity.

Limitations

The authors acknowledge certain limitations to the applicability of the presented results. Firstly, most included studies required participants to perform the exercise protocol in the fasted state. This is not representative of common training and competition practices, as athletes will often consume a meal or snack in the hours preceding exercise. Secondly, 92% of participants in the current review were male, with no female participants included in the studies investigating muscle protein synthesis and immune function. Given the known body composition and metabolic differences between males and females,

these results might not reflect optimal recovery nutrition for female athletes. Nutrition interventions described in this review are presented as g/kg BM/h or ml/kg BM/h, and therefore do not take into consideration the quality or type of nutrients, nor the specific timing of intake or time permitted for recovery. Finally, the exercise-induced perturbations (i.e., body water and substrate losses, muscle damage, exercise-induced immunodepression) relative to the recovery nutrition consumed, have not been considered in the current review.

Conclusion

Results from this review suggest that an intake of 0.8-1.2 g carbohydrate/kg BM/h and 0.3 g protein/kg BM/h, in addition to 20-35 ml sodium-containing fluid/kg BM/h during the acute recovery period (i.e., within 4 h after strenuous exercise) will maximally stimulate muscle glycogen resynthesis and muscle protein synthesis, promote rehydration and support functional immune responses. Gastrointestinal damage and subsequent nutrient malabsorption may occur following prolonged strenuous exercise.

Part II

Systematic literature review: The effect of dairy milk on markers of recovery optimisation in response to endurance exercise.

Abstract

The food and fluid provided in the acute post-exercise period plays an essential role in endurance exercise recovery and adaptation. The current systematic literature review aimed to identify and synthesize research that investigated the effect of dairy milk beverages in comparison to alternative post-exercise beverages on markers of '*exercise recovery optimisation*' (i.e., muscle glycogen resynthesis, muscle protein synthesis, and rehydration), which may influence subsequent endurance exercise performance. Seventeen papers met the inclusion criteria. Quality assessment was undertaken using the Cochrane Collaboration's tool for assessing risk of bias. Intervention beverages included fresh dairy milk (n= 3), chocolate flavoured dairy milk (n= 6), dairy milk-based sports beverages (n= 4), or a combination of the aforementioned beverages (n= 4). Results indicate dairy milk enhanced muscle protein synthesis (i.e., mixed fractional synthetic rate: 0.11%/h dairy milk *vs* 0.08%/h control), and elicited similar rates of muscle glycogen resynthesis (5.9 mmol/kg ww/h) compared to a carbohydrate replacement beverage (7.6 mmol/kg ww/h). Seven studies investigated the effect of dairy milk beverages on hydration status, three of which found no differences in restoring net fluid balance after consumption of a dairy milk or dairy milk-based beverage compared to a carbohydrate-electrolyte beverage and (or) water, when consumed *ad libitum.* The remaining four studies observed a greater net fluid balance after consumption of a dairy milk or dairy milk-based beverage compared to an isovolumetric dose of a carbohydrate-electrolyte beverage and (or) water. To date, no study has investigated the effect of dairy milk consumption after endurance exercise on markers of immune competency or gastrointestinal status. Five studies observed enhanced exercise time-trial or time-to-exhaustion performance (7.4% to 52.4%) with a dairy milk beverage compared to an isocaloric beverage, while another study found no differences. It is concluded that dairy milk may provide either comparable or superior recovery nutrition qualities with regards to muscle protein synthesis, glycogen replenishment, rehydration, and subsequent endurance exercise performance, when compared to non-nutritive beverages, carbohydrate replacement beverages, and (or) carbohydrate-electrolyte beverages.

Introduction

It is well established that the food and fluid consumed in the acute period after endurance exercise plays an essential role in metabolic and physiological recovery, and biological adaptation processes to the respective exertional stress. Replacement of carbohydrate due to muscle glycogen depletion (e.g., oxidative phosphorylation of endogenous carbohydrate) and water due to exercise-associated body water losses (e.g., sweat, respiration, and obligatory urine losses), in the initial hours following exertional stress is required to prevent decrements in performance during subsequent training or competitive events (6,51,160). Moreover, promotion of adaptive responses to endurance training, including accretion of mitochondrial and myofibrillar muscle proteins can be modulated through post-exercise nutritional intake, most appreciably due to the quantity and quality of protein intake (153,161). There has been a considerable amount of research investigating the effects of nutritional strategies on recovery from prolonged endurance exercise, which has led to the development of nutrition guidelines and recommendations for the optimal quantity and (or) quality of carbohydrate, protein, and water to optimally replenish muscle glycogen stores, maximally stimulate net positive muscle protein synthesis, and restore hydration status, respectively (6,141). However, the majority of exercise recovery nutrition research has neglected immunocompetency in their recovery assessment, and also neglected the integrity and function of the gastrointestinal tract, with feeding tolerance and GIS being measured occasionally in an inconsistent manner and (or) briefly mentioned (162). Considering immunocompetency and a competent gastrointestinal tract are essential pre-requisites for optimal nutrient intake and exercise recovery (11,13,163,164), the current research may have underestimated the potential for further muscle glycogen replenishment, muscle protein synthesis, and rehydration, leading to suboptimal exercise recovery nutrition guidelines and recommendations.

There is increasing evidence to suggest that dairy milk, in particular chocolate flavoured dairy milk (CM), has the potential to provide a '*gold standard*' recovery formulation, since it has similar nutritional properties to current exercise recovery nutrition guidelines and recommendations (165-167). Partly

skimmed (2% fat) CM contains carbohydrate and protein (casein and whey) in an approximate ratio of 3:1. Post-exercise consumption of carbohydrate and protein in this ratio (i.e., 0.8-1.2 g/kg and 0.3-0.4 g/kg, respectively) has been reported to enhance muscle protein synthesis, and have comparable effects on muscle glycogen resynthesis, and subsequent endurance exercise performance, compared to a beverage containing an isocaloric dose of carbohydrate (95,115,168). Moreover, the natural mineral content of CM (i.e., sodium and potassium) is comparable to the concentration found in carbohydrate-electrolyte beverages (CEB) and may aid water retention in plasma and (or) intracellular compartments (20,132). Considering this nutritional profile, the effectiveness of dairy milk as an exercise recovery beverage has been reviewed extensively using narrative methodologies, with a primary focus on anabolic processes (e.g. muscle glycogen and protein synthesis) and performance (165-169). These reviews highlight the potential for dairy milk beverages to support recovery processes in both endurance and resistance exercise. A recent literature review examined the effectiveness of dairy milk recovery beverages on hydration status and subjective rating of thirst (172). While there is some evidence to suggest that milk may be a more efficient hydration beverage than water or carbohydrate-electrolyte beverage (132), the search strategy implemented by this review only yielded four studies, and as a result, the authors concluded there was insufficient evidence to differentiate the effectiveness of dairy milk from other recovery beverages for rehydration. To date, only one systematic literature review (SLR) has examining the effects of CM on subsequent exercise performance, in adjunct with associated physiological strain and (or) recovery markers (170). The meta-analysis within found that consuming CM after a preload exercise bout increases time to exhaustion (TTE) in a subsequent performance test, when compared to a placebo. However, this SLR did not evaluate the effects of dairy milk recovery beverages on muscle glycogen resynthesis, rehydration, immune function, or gastrointestinal status.

In summary, the existing literature lacks a complete and systematic review of the effects of dairy milk and (or) dairy milk-based beverages on '*exercise recovery optimisation*' (i.e., to maximise desired and

minimise detrimental outcomes within the complex and interrelated physiological and metabolic homeostatic systems) that may be targeted by nutrition interventions. Therefore, the aim of the current systematic literature review was to systematically identify and synthesize research that investigated the effect of dairy milk in comparison to alternative post-exercise beverages on markers of '*exercise recovery optimisation*' (i.e., muscle glycogen resynthesis, muscle protein synthesis, hydration status, immunocompetency, and gastrointestinal status) and subsequent endurance exercise.

Methods

This review was conducted in accordance with the PRISMA Statement (71). The review protocol was registered (http://www.crd.york.ac.uk/PROSPERO) with PROSPERO, registration number CRD42017083594.

Search Strategy

A three-step search strategy was developed with the assistance of an academic librarian. This search of published English-language studies was implemented across six electronic databases from inception until June 2019: Ovid MEDLINE, EMBASE, Cinahl, SportsDISCUS, Web of Science, and Scopus. The reference lists of all identified studies were searched to identify any additional studies for inclusion. The keywords applied in the literature search are shown in Table 2.7.

Table 2.7 Search strategy for the systematic literature review on the effect of dairy milk in comparison to alternative post-exercise beverages on aspects of endurance exercise recovery.

Field One- Intervention and Comparison		Field Two (combine with OR)- Population
Keyword: Milk MeSH headings: Beverages, Milk	AND	Keywords: exercise* OR recover* OR postexercise OR post exercis* OR sport* OR athl* MeSH headings: Athletes, Exercise, Physical Exertion, Physical Activity, Exercise, Sports

* Used to retrieve unlimited suffix variations.

Eligibility Criteria

In order to obtain the level of methodological detail required, only laboratory controlled studies and field studies were considered for review. The PICOS design format was used to determine whether studies were eligible for inclusion **(Table 2.8)**. Elite athletes and habitually trained adolescents or adults, free of known diseases, were the focus of this review. Interventions considered for inclusion were those that required participants to undergo prolonged exercise of endurance, interval or mixed-model (e.g., circuit, crossfit, and (or) cardiovascular-resistance exercise) nature, followed by the provision of a quantified volume of a dairy milk or dairy milk-based beverage, *vs* a comparator, within 6 h post-exercise. Excluded interventions included resistance only exercise, extended training programs, pharmacological interventions, chronic interventions of dietary supplementation or dietary manipulation interventions, or interventions that offered pre- or mid-exercise supplementation. Studies with incomplete information regarding the dose, timing, and nutritional composition of the post-exercise recovery beverage provided were also excluded.

Study Selection

The database search was imported into Endnote and duplicates were manually removed. The searches were then imported into an online software program (Covidence) for management of studies and synthesis of evidence. Two reviewers (IR and VC) worked independently and in duplicate to assess papers against the PICOS. A third reviewer (RC) resolved conflicts where these arose. Full texts were also assessed against the PICOS statement by reviewers (IR and VC) independently and in duplicate, with consensus reached by a third reviewer (RC).

Table 2.8 PICOS statement outlining inclusion and exclusion criteria.

	Inclusion	Exclusion
Population	Habitually trained and elite level active individuals (including masters, adolescents (13+ years), and adults).	Infants and children, sedentary individuals (i.e. no adherence to exercise or structured physical activity programs), diagnosed disease state, and (or) untrained participants.
Intervention	Acute (immediately to 4 h) post-exercise (prolonged or interval based endurance, and mixed model (i.e., circuit, crossfit and (or) CV-resistance programs)) dairy milk or dairy milk-based supplement consumption.	Pre- or mid-exercise nutrition intervention only, chronic supplementation or dietary patterns, pharmaceutical interventions, short duration high intensity (e.g., maximal effort), resistance exercise, alcoholic intervention beverages, or incomplete information regarding dose, timing, and nutritional composition of intervention.
Comparator	Placebo, alternative sports recovery beverage, and (or) no nutrition intervention.	Failure to meet comparator inclusion criteria.
Outcome	Myofibrillar, mitochondrial or mixed protein fractional synthetic rate, muscle intracellular signalling proteins concentration (e.g., mTOR, rps60), rate of muscle glycogen resynthesis, hydration status (e.g., bioelectrical impedance analysis, plasma osmolality (P_{Osmol}), plasma volume change (ΔP_V), and (or) net fluid balance (NFB)), gastrointestinal status (e.g., integrity, function, systemic, and (or) symptoms), immune function (e.g., circulating stress hormones, leukocyte trafficking, leukocyte function, and (or) salivary immune defences), endurance exercise performance (i.e., time trial (TT) and TTE), appetite, and (or) energy intake.	Failure to meet outcome inclusion criteria.
Study design	Laboratory controlled trials and field studies.	All other study designs.

Data Extraction and Synthesis

Data were independently extracted in duplicate (IR and VC) from each included study into a standardised table developed for reporting this review. Extracted data related to study characteristics, exercise stress, nutrition intervention, primary and secondary outcomes. Results were analysed descriptively due to the heterogeneity of study designs and reported outcomes; and therefore, extracted data were ineligible for further analysis (i.e., meta-analysis).

Risk of Bias Assessment

Risk of bias assessment was performed using the Cochrane 'risk of bias' assessment tool (72). Two reviewers independently (IR and SG) and in duplicate conducted the assessments by referring to the criteria for judging risk of bias in the assessment tool.

Results

Search results

The search strategy yielded 1,940 non-duplicate studies, including an additional study retrieved through screening of reference lists. Upon title and abstract screening, 1899 were excluded. The full texts of 41 studies were reviewed. Reasons for exclusion following full text screening include ineligible intervention design (e.g., resistance exercise intervention (n= 5), extended training program (n= 4), and non-milk intervention (n= 3)), abstract text or thesis only (n= 10), ineligible method for measuring outcomes (i.e., whole body protein turnover estimated through nitrogen excretion) (n= 1) or ineligible outcomes (i.e., blood glucose, insulin, cortisol and IL-6 concentration only) (n= 1). A total of 17 randomised crossover trials were included for review (Figure 2.6).

Effect of dairy milk on muscle glycogen resynthesis and muscle protein synthesis

Two studies assessed the effect of dairy milk exercise recovery beverages on muscle protein synthesis and glycogen resynthesis. Lunn et al. demonstrated that consumption of CM (0.2 g protein (PRO)/kg BM/h) after 45 min treadmill running at 65% $\dot{V}O_{2max}$ stimulated mixed muscle protein synthesis greater than an energy-matched non-nitrogenous CEB (0.11%/h CM *vs* 0.08%/h CEB; $P<0.05$) (116). In this study, the CM and CEB provided 0.8 g carbohydrate/kg BM/h and 1.0 g CHO/kg BM/h, respectively. There was no significant difference in the rate of muscle glycogen resynthesis between trials. It was also found that CM containing 0.6 g CHO/kg BM/h + 0.2 g PRO/kg BM/h and carbohydrate replacement beverage (CRB) containing 0.8 g CHO/kg BM/h elicited similar rates of muscle glycogen resynthesis (105). Phosphorylation of the signalling proteins mTOR, eIF4E-BP1 and rpS6 was increased during the

dairy milk trial, which is indicative of up-regulation of the mTOR muscle protein synthetic pathway (105,116).

Four studies investigated the effects of dairy milk and (or) dairy milk-based beverages on blood glucose response, compared to a carbohydrate replacement beverage, soymilk, and (or) a non-caloric placebo. The blood glucose response during the recovery period for each study was variable, with no consistent pattern in terms of beverage type, energy or carbohydrate content (Table 2.9).

Effect of dairy milk on hydration status

The search strategy retrieved seven studies that examined the effects of dairy milk or milk-based sports beverage (MBSB) on hydration status. Of these, three studies investigated the effects of *ad libitum* intake of a MBSB (174,175), or a low sugar (LS-MILK) and a high protein (HP-MILK) dairy milk beverage (136), compared to water and (or) CEB. Two studies found intake of the MBSB was significantly less than water and (or) CEB. Another study found no significant differences in the volume of exercise recovery beverages consumed; however, significantly less water was consumed during both dairy milk trials, due to the higher energy density of the milk beverages ($P \leq 0.022$) (136). Moreover, it was found that total water intake during the exercise recovery period (i.e., *ab libitum*) was significantly less during the dairy milk trials compared to CEB and water (136,175).

Despite differences in total fluid intake, none of the aforementioned studies observed differences in NFB at any time point throughout the recovery time period measured. Offsets in NFB were likely due to corresponding increases in urine output with greater water intake, although these differences were not always significant (175). McCartney et al., however, observed a significantly higher P_{Osmol} 4 h after rehydration with the dairy milk trials (HP-MILK and LS-MILK > water, and HP-MILK > CEB), possibly associated with the nutrient density and bioavailability of dairy milk (136) (Table 2.9). Interestingly, a

higher percentage of fluid retention with LS-MILK compared with water was also observed, but there were no significant differences when corrected to total volume of fluid retained.

Four studies provided participants with isovolumetric doses of exercise recovery beverages during each trial (130,132,133,176). These studies observed a significantly greater positive NFB after consumption of dairy milk or MBSB, compared to water or CEB. With the exception of Watson et al., the percentage of fluid retained was also significantly greater during the milk trials (133). Similar to the observations by McCartney et al., two additional studies found that P_{Osmol} was significantly higher 2 h after the onset of rehydration during the dairy milk trials compared to CEB and (or) water (130,133).

Effect of dairy milk on immune function

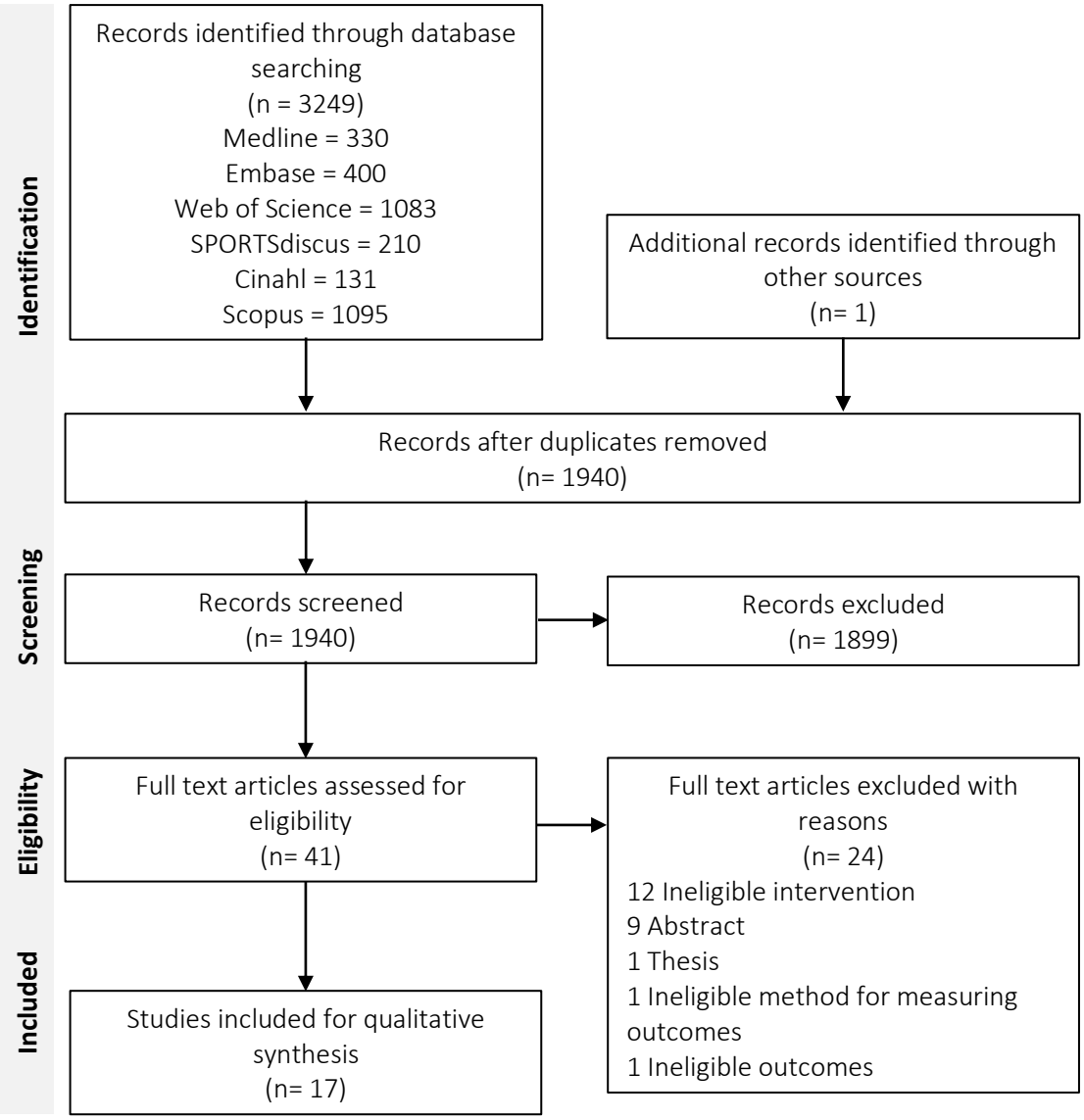
The search strategy did not yield any studies investigating the effect of post-exercise consumption of dairy milk on immune indices, such as total and differential leukocyte trafficking, immune cell function (i.e., *in vitro* or *in vivo*), and (or) inflammatory cytokine responses.

Effect of dairy milk on gastrointestinal status and intake tolerance

None of the reviewed studies observed any differences between trials in gastrointestinal discomfort *per se*. To date, no study has comprehensively assessed gastrointestinal discomfort and (or) GIS during the exercise recovery period in response to recovery beverage consumption using a validated and reliability verified GIS assessment tool (162).

Nine studies reported on appetite and thirst throughout the exercise recovery period, five of which observed significantly lower levels of hunger and (or) increased levels of fullness and bloating after consumption of dairy milk compared to other trials (130,132,133,176,177), with another study reporting a trend towards significance (178). Three studies found no differences in hunger between trials, two of which provided *ad libitum* food to participants during the exercise recovery period (174,175,179).

Figure 2.6 PRISMA diagram, indicating the systematic review process, and subsequent inclusion and exclusion of respective papers.



Three studies investigated the effects of dairy milk or dairy milk-based exercise recovery beverages on *ad libitum* energy intake. All studies found energy intake from food during the exercise recovery period was significantly less during the dairy milk and (or) MBSB trial when compared with water (175,177), CRB (179), and CEB trials (175). However, only two studies observed significantly lower overall energy intake during the recovery time period compared to the water (175), and CRB trials (179).

Effect of dairy milk on endurance exercise performance

Nine studies investigated the effect of dairy milk and (or) MBSB on subsequent endurance exercise performance or capacity, which included a 20 km and 40 km cycling ergometer time trial (TT) (105,180), or TTE at a given power output or speed (116, 178, 181-184), respectively. The majority of these studies conducted the performance test at the end of the 2-4 h recovery period, whereas two studies conducted the test the following day (182,183). Where water or a non-caloric placebo was provided as a comparator (n= 4), participants demonstrated greater endurance exercise performance (i.e., increased TTE or reduced TT) after consuming dairy milk as an exercise recovery beverage (8.6% to 93.3% greater performance) (105,180,182,184). Six studies provided an isocaloric CRB or CEB as a comparator (105, 116, 178, 182-184), five of which observed greater performance during the dairy milk trials (7.4% to 52.4% greater performance) (105,116,178,181,184). Moreover, three studies compared the effects of dairy milk beverages to a lower-energy CEB. One study observed a greater TTE compared with the CEB (178), the remaining two studies found no significant differences (133,181).

Risk of bias assessment

Many studies did not specify blinding or randomisation procedures, therefore risk of performance and detection bias with or without order effect respectively, has been judged as *'unclear'* (Figure 2.7)

Table 2.9 Systematic literature review study inclusion data extraction and qualitative synthesis: the effect of dairy milk in comparison to alternative post-exercise beverages on aspects of endurance exercise recovery.

Reference	Participants	Exercise stress	Recovery beverages	Intake timing	Dairy *vs* Comparator
(116)	n= 8 habitually active males. $\dot{V}O_{2max}$= 53 mL/kg BM/min.	45 min running at 65% $\dot{V}O_{2peak}$, 4 h recovery period, TTE at 100% $\dot{V}O_{2peak}$. T_{amb} and RH not reported.	CM *vs* CRB	Immediately post-exercise.	↑ FSR (CM 0.11%/h, CRB 0.08%/h), ↑ TTE (CM 250 s, CRB 203 s), ^{NS} Muscle glycogen resynthesis (CM 6.4 g/100g, CRB 5.4 g/100g), ↑ Phosphorylation of eIF4E-BP1.
(105)	n= 10 (5 males, 5 females) trained cyclists/ triathletes. $\dot{V}O_{2max}$= 53 mL/kg BM/min.	90 min cycling at 70% $\dot{V}O_{2max}$ + 10 min interval at 45-90% $\dot{V}O_{2max}$, 3 h recovery period, TT 40 km. T_{amb} and RH not reported.	CM *vs* CRB *vs* W	0 h and 2 h post-exercise.	↑ Muscle glycogen resynthesis (CM 7.6 mmol/kgww/h, CRB 5.9 mmol/kgww/h, W 1.8 mmol/kgww/h), ↓ Blood glucose and plasma insulin (CRB > CM and W), ↑ Phosphorylation of mTOR and rpS6 (CM > CRB and W), ↓ TT (CM 79 min, CRB 86 min, W 87 min), ^{NS} RPE during TT, ↑ HR during TT (CM > CRB and W).
(174)	n= 8 recreationally active males. $\dot{V}O_{2max}$ not reported.	Aerobic run (1) or cycle (2) until 1.7% BM loss (duration and intensity not specified). T_{amb}= 23.0°C, RH= 57%.	MBSB *vs* CEB	*Ad libitum* throughout 2 h recovery period.	↓ Fluid intake (MBSB1 1.4 L, MBSB2 1.4 L, CEB1 2.2 L, CEB2 2.6 L), ^{NS} NFB at all time points, ↓ Total urine output (MBSB1 0.2 L, MBSB2 0.2 L, CEB1 1.1 L, CEB2 1.5 L), ↓ Perceived pleasantness and ↑thirst.
(175)	n= 10 trained male cyclists. $\dot{V}O_{2max}$= 53 mL/kg BM/min	Cycling at 65% PPO until 1.8% BM loss. T_{amb}= 23.0°C, RH= 70%.	MBSB *vs* CEB *vs* W *vs* W2	*Ad libitum* throughout 4 h recovery period.	↓ Fluid intake (MBSB 1.8 L, CEB 1.1 L, W 2.7 L, W2 2.1 L) from beverage and overall (MBSB 1.9 L, CEB 2.8 L, W 2.3 L, W2 2.2 L), ^{NS} NFB, P_{Osmol}, urine output and fluid retention, ↑ Overall energy intake (MBSB 10.6 MJ, CEB 10.2 MJ, W 7.8 MJ, W2 7.6 MJ), ↑ Thirst (MBSB > W) and bloating (MBSB > W).

(176)	n= 15 recreationally active males. $\dot{V}O_{2max}$ not reported.	Cycling 70-80% age predicted HR to 1.8% BM loss. T_{amb}= 22.0°C, RH= 60-70%.	MILK *vs* MBSB *vs* CEB *vs* SM	1 h period commencing 30 min post-exercise.	↑ NFB (MILK -0.9 kg, SM -0.9 kg, MBSB -0.5 kg, CEB -1.2 kg), ↓ Total urine output (MBSB 0.7 L, MILK 1.3 L, SM 1.1 L, CEB 1.8 L), ↑ Fluid retention (MBSB 65.1%, MILK 40.0%, SM 46.9%, CEB 16.6%), ^{NS} Δ P_V, ↑ Fullness (MBSB > MILK, SM and CEB), ↓ Blood glucose 4 h post-exercise (SM > MBSB, MILK and CEB).
(130)	n= 7 males. $\dot{V}O_{2max}$ not reported	Continuous cycling to 1.8% BM loss. T_{amb}= 30°C, RH= 58%.	MILK *vs* CEB *vs* W	3 h period commencing immediately post-exercise.	↑ NFB (MILK 0.1 L, CEB -0.4 L, W -0.6 L), ↑ Fluid retention (MILK 71%, CEB 52%, W 47%), ↑ P_{Osmol} 2 h after rehydration (MILK 288 mOsmol/kg, CEB 287 mOsmol/kg, W 286 mOsmol/kg), ↓ Hunger (MILK < CEB and W), ^{NS} GIS.
(132)	n=11 participants (5 males, 6 females). $\dot{V}O_{2max}$ not reported.	Intermittent cycling at 2 W/kgBM to 1.7% BM loss. T_{amb}= 35°C, RH= 57%.	MILK *vs* MILK+Na *vs* CEB *vs* W	1 h period commencing 20 min post-exercise.	↑ NFB (MILK 0.0 L, MILK+Na 0.6 L, W -0.6 L, CEB -0.6 L), ↓ Total urine output (MILK 0.6 L, MILK+Na 0.5 L, W 1.2 L, CEB 1.2 L), ↑ Fluid retention after 4h (MILK 69%, MILK+Na 72%, W 36%, CEB 38%), ↓ Hunger (MILK, MILK+Na and CEB < W).
(133)	n= 7 males. $\dot{V}O_{2max}$= 4.3 L/min.	Intermittent cycling to 1.8% BM loss, 3 h recovery period, TTE at 61% $\dot{V}O_{2peak}$. T_{amb}= 35°C, RH= 60-70%.	CM *vs* CEB	1 h period commencing 20 min post-exercise.	^{NS} NFB and Δ P_V, ↑ P_{Osmol} 0-3 h post-exercise (CM 282 mOsmol/kg, CEB 280 mOsmol/kg), ↑ Fluid retention (CM 77%, CEB 62%), ^{NS} TTE, ^{NS} RPE during TTE. ↑ Blood glucose at 0 h, 2 h and 3 h post-exercise,

(136)	n= 8 females. $\dot{V}O_{2max}$= 46 mL/kg BM/min.	Preheat + continuous cycling at 60% PPO until 1.8% BM loss. T_{amb}= 24.2°C, RH= 66%.	LS-MILK *vs* HP-MILK *vs* CEB *vs* W	*Ad libitum* throughout 4 h recovery period.	[NS] Fluid intake and NFB, ↓Water intake (HP-MILK 1.6 L, LS-MILK 1.5 L, W 1.8 L, CEB 1.8 L), ↑ P_{Osmol} 4 h after rehydration (HP-MILK 299 mOsmol/kg, LS-MILK 295 mOsmol/kg, W 288 mOsmol/kg, CEB, 288 mOsmol/kg), ↑ Fluid retention (HP-MILK 83.7%, LS-MILK 84.1%, W 70.2%, CEB 74.2%), ↓ Urine output (HP-MILK 0.2 L, LS-MILK 0.2 L, W 0.6 L, CEB 0.5 L) [NS] Total volume of fluid retained, [NS] Total energy intake, ↑ Thirst (HP-MILK and LS-MILK > W and CEB), ↓ Hunger (HP-MILK and LS-MILK > W and CEB).
(181)	n= 9 males. $\dot{V}O_{2max}$ = 65 mL/kg BM/min.	Interval cycling to exhaustion (50-90% P_{max}), 4 h recovery period, TTE at 70% $\dot{V}O_{2max}$. T_{amb} and RH not reported.	CM *vs* CRB *vs* CEB	0 h and 2 h post-exercise.	↑ TTE (CM 40 min, CEB, 41 min, CRB 27 min), [NS] GIS and total body water, [NS] RPE during TTE.
(183)	n= 10 regional-level cyclists and triathletes $\dot{V}O_{2max}$= 55 mL/kg BM/min.	Interval cycling for 48 min (60% $\dot{V}O_{2max}$ to 'all-out' sprint), 15-18 h recovery period, TTE at 85% $\dot{V}O_{2max}$. T_{amb} and RH not reported.	CM *vs* CRB	0 h and 2 h post-exercise.	[NS] TTE and muscle soreness. ↓ Δ CK pre- to post-exercise (CM 27.9 U/L, CRB 211.9 U/L).
(178)	n= 9 trained male cyclists, $\dot{V}O_{2max}$= 4.3 L/min.	Interval cycling to exhaustion (50-90% P_{max}), 4 h recovery period, TTE at 70% P_{max}. T_{amb} and RH not reported.	CM *vs* CRB *vs* CEB	0 h and 2 h post-exercise.	↑ TTE (CM 32 min, CEB 23 min, CRB 21 min), [NS] GIS. [NS] RPE during TTE.

(180)	n= 8 male cyclists. $\dot{V}O_{2max}$= 61 mL/kg BM/min.	Interval cycling to exhaustion (50-90% P_{max}), 4 h recovery period, TT 20 km. T_{amb} and RH not reported	CM *vs* MILK *vs* SM *vs* HEMP *vs* P	2 h period immediately post-exercise.	↓ 20 km TT (CM 35 min, MILK 35 min, SM 35 min, HEMP 35 min, P 38 min).
(179)	n= 9 females. $\dot{V}O_{2max}$= 45.7 mL/kg BM/min.	30 min cycling at 65% $\dot{V}O_{2max}$. T_{amb} and RH not reported.	CM *vs* CRB	1 h commencing immediately post-exercise.	↓ Absolute (CM 2.4 MJ, CRB 3.2 MJ) and relative energy intake (CM 1.5 MJ, CRB 2.3 MJ), ^{NS} Hunger and fullness ratings.
(182)	n= 10 male climbers. $\dot{V}O_{2max}$ not reported	Exhaustive 'tredwall' climb to volitional exhaustion, 24 h recovery period, Repeated exhaustive 'tredwall' climb to volitional exhaustion. T_{amb} and RH not reported.	MBSB *vs* W	0 h and 2 h post-exercise.	↑ TTE (MBSB 18 min, W 14 min) and distance climbed (MBSB 218 metres, W 170 metres).
(184)	n= 10 males. $\dot{V}O_{2max}$= 44 mL/kg BM/min	Interval cycling to exhaustion (50-90% P_{max}), 2 h recovery period, TTE at 70% $\dot{V}O_{2max}$. T_{amb}= 25°C, RH= 56%	MILK *vs* CEB *vs* W	0 min, 30 min, and 60 min post-exercise.	↑ TTE (MILK 70 min, CEB 52 min, W 36 min). ↓ RPE during TTE.

(177)	n= 13 females. $\dot{V}O_{2max}$= 44 mL/kg BM/min	60 min cycling at 65% $\dot{V}O_{2max}$. T_{amb} and RH not reported.	MBSB *vs* CRB *vs* W	Immediately post-exercise.	[NS] Overall energy intake ↓ Time-averaged rating of hunger (MBSB 63/100, CRB 72/100, W 83/100), ↑ Time-averaged rating of fullness (MBSB 42/100, CRB 27/100, W 14/100), ↓ Time averaged-blood glucose (MBSB 3.4 mmol/L, CRB 4.8 mmol/L, W 4.0 mmol/L), ↑ Insulin (MBSB > W), ↑ Glucagon (MBSB > CRB), ↑ GLP-1_{7-36} (MBSB > W).

↑= significance increase dairy milk *vs* comparator, ↓= significant decrease dairy milk *vs* comparator, and [NS]= no significant difference between dairy milk *vs* comparator.

CEB= carbohydrate-electrolyte sports drink, CM= chocolate milk, CRB= carbohydrate replacement beverage, HEMP= hemp seed chocolate milk, HP-MILK= high-protein milk, LS-MILK= low-sugar milk, MBSB= Milk-based sports beverage, MILK= bovine milk, MILK+Na= bovine milk + sodium, P= non-caloric placebo, SM= soy milk, W= water, W2= second water trial.

BM= body mass, CCO= counterbalanced cross-over, CK= creatine kinase, FSR= fractional synthetic rate, GIS= gastrointestinal symptoms, HR=heart rate, NFB= net fluid balance, P_{max}= maximal power output, P_{Osmol}= plasma osmolality, PPO= peak power output, RCT= randomised cross-over trial, RH= relative humidity, RPE= Rate of perceived exertion, T_{amb}= ambient temperature, TBW= total body water, TT= time trial, TTE= time to exhaustion, $\dot{V}O_{2max}$ = maximal oxygen uptake, and ww= wet weight.

Figure 2.7 Risk of bias for the systematic literature review on the effect of dairy milk in comparison to alternative post-exercise beverages on aspects of endurance exercise recovery, using the Cochrane Collaboration 'risk of bias'.

	Sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting
Lunn et al. 2012	?	⊕	⊖	?	⊕	?
Ferguson-Stegall et al. 2011	?	⊕	⊖	?	⊕	?
Baguley et al. 2016	?	⊕	⊖	?	⊕	?
Campagnolo et al. 2017	⊕	⊕	⊖	?	⊕	?
Desbrow et al. 2014	⊕	⊕	⊖	?	⊕	?
Seery & Jakeman, 2016	?	⊕	⊖	?	⊕	?
Shirreffs, Watson & Maughan, 2007	⊕	⊕	⊖	?	⊕	?
Watson et al. 2008	?	⊕	⊖	?	⊕	?
Karp et al. 2006	?	⊕	⊖	⊕	⊕	?
Pritchett et al. 2009	?	⊕	⊖	?	⊕	?
Thomas, Morris & Stevenson, 2009	?	⊕	⊖	?	⊕	?
Upshaw et al. 2016	?	⊕	⊖	?	⊕	?
Rumbold et al. 2015	?	⊕	⊖	?	⊕	?
Potter & Fuller, 2015	?	⊕	⊖	?	⊕	?
McCartney et al. 2019	⊕	⊕	⊖	?	⊕	?
Sudsa-ard et al. 2014	?	⊕	⊖	?	⊕	?
Brown et al. 2016	⊕	⊕	⊖	?	⊕	?

⊕ Low risk; ⊖ high risk; ? unclear risk

Discussion

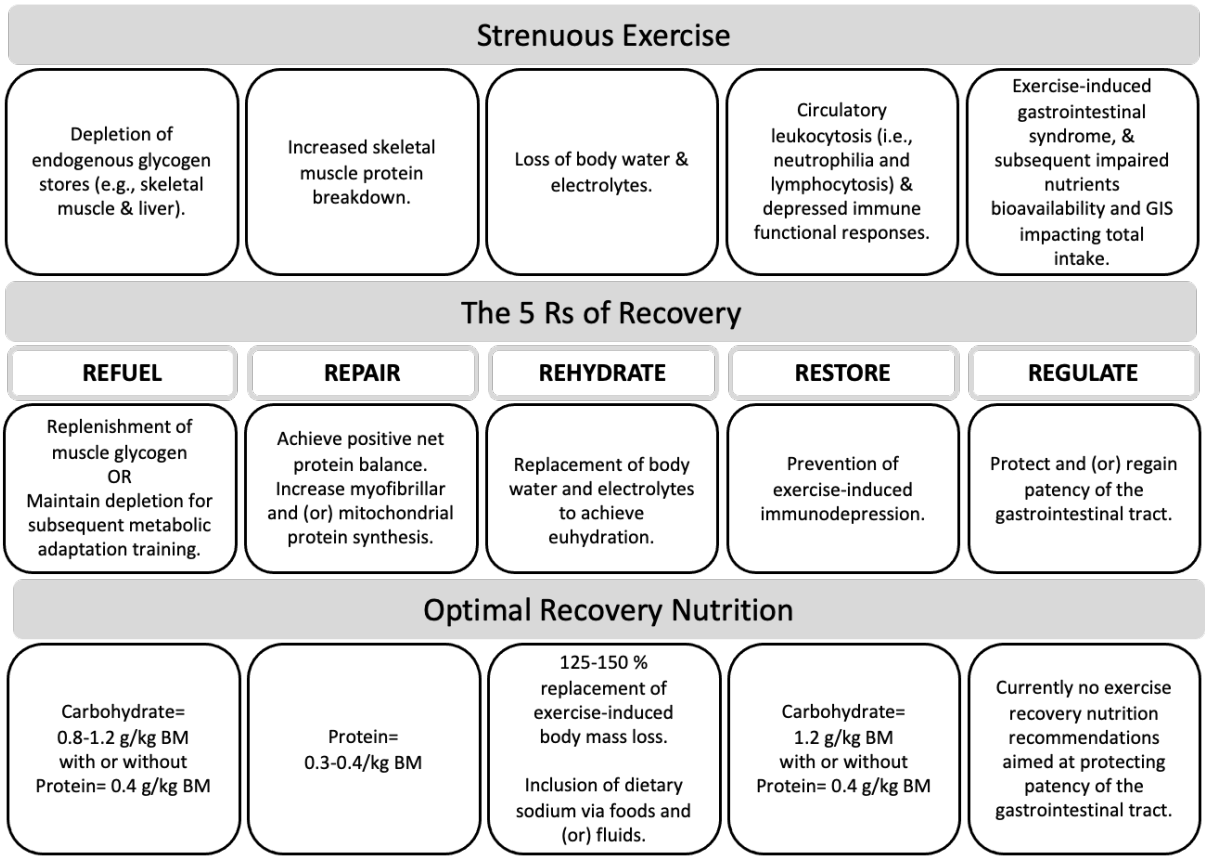
The aim of the current SLR was to systematically identify and synthesize research that investigated the effect of dairy milk in comparison to alternative post-exercise beverages on markers of '*exercise recovery optimisation*', including muscle protein synthesis, muscle glycogen resynthesis, hydration status, immune competency, gastrointestinal status, and endurance exercise performance. Results from the current SLR found that dairy milk enhanced muscle protein synthesis (muscle protein synthesis (i.e., mixed fractional synthetic rate) and elicited similar rates of muscle glycogen resynthesis compared to an isocaloric non-nitrogenous carbohydrate replacement beverage. Moreover, a dairy milk or dairy milk-based beverage resulted in similar restoration of NFB compared to a CEB and water, when consumed *ad libitum,* and a more positive NFB when consumed in equal volumes. Five studies observed enhanced endurance performance with a dairy milk beverage compared to an isocaloric beverage, while two studies found no differences. To date, no study has investigated dairy milk consumption after endurance exercise on markers of immune competency, or gastrointestinal status. Based on the current SLR findings and additional research investigating the effect of carbohydrate, protein, and water provisions in the time period after endurance exercise, recommended nutritional intake for '*exercise recovery optimisation*' is depicted in Figure 2.8, which is in accordance with the nutritional composition of CM reported in the inclusion studies.

Muscle Protein Synthesis

Skeletal muscle is a highly dynamic tissue and is the main site of training adaptations. NPB is the difference between muscle protein synthesis and muscle protein breakdown. A positive NPB is required for muscle accretion and training adaptations, with both exercise stimulus and nutritional intake acting as a modulator of this balance (39,185,186). The current SLR found that consuming dairy milk after endurance type exercise will enhance mixed muscle protein synthesis (i.e., myofibrillar and mitochondrial protein synthesis) compared to a non-nitrogenous carbohydrate beverage. These findings are not surprising given the role of dietary protein as a mechanistic stimulator and nitrogenous substrate for muscle protein synthesis (37,146,187). The observed anabolic effect in the reviewed

studies is likely due to the high essential amino acid (EAA) content of dairy milk, in particular leucine (188). For example, increasing the concentration of plasma EAAs has been shown to stimulate muscle protein synthesis through the mTOR pathway, independent of exercise (189). Both of the reviewed studies examined changes in the concentration of phosphorylated intramuscular signalling proteins along the mTOR signalling pathway, and observed increases in phosphorylation of translational signalling proteins after consumption of a dairy milk recovery beverage, including eIF4E-BP1, mTOR, and rp-S6 (105,116). Such findings suggest that the protein quantity and quality of the dairy milk consumed after endurance exercise is a potent stimulator for muscle protein synthesis.

Figure 2.8 Evidence based rationale and justification for the nutritional composition aimed at 'exercise recovery optimisation'.



BM: body mass, GIS: gastrointestinal symptoms.

While these findings provide evidence of an overall increase in muscle protein synthesis, the specific protein fractions (i.e., myofibrillar or mitochondrial proteins) in response to consumption of a dairy milk

beverage after endurance exercise remains unclear. Adaptations to endurance exercise are characterised by an increase in the density of mitochondrial proteins, thereby increasing the oxidative capacity and metabolic fatigue resistance of the muscle. Endurance exercise has been shown to acutely stimulate mitochondrial protein synthesis to a greater degree than myofibrillar protein synthesis (48). Results from this review suggest that the protein content of a dairy milk recovery beverage (0.2 g/kg BM/h) will stimulate overall muscle protein synthesis to a greater degree than a non-nitrogenous recovery beverage, however further research is required to elucidate the effect of a dairy milk beverage after endurance exercise on mitochondrial protein synthesis specifically.

Muscle Glycogen Resynthesis

Replenishment of muscle glycogen stores has been established as a major determinant of endurance exercise performance during subsequent performance testing (27,190). Therefore, failure to adequately replenish muscle glycogen stores is a major limiting factor for achieving optimal endurance performance, particularly when athletes must undertake multiple endurance training sessions or competitions with limited time for recovery (6). It is recommended that high rates of carbohydrates (i.e., 1.0 g/kg BM/h) are consumed during the acute recovery time period in order to maximise muscle glycogen replenishment (160). Since the carbohydrate content of the dairy milk beverages provided in the reviewed studies did not meet these recommendations (0.6-0.8 g/kg BM/h), it appears that the protein content of the dairy milk beverages aided muscle glycogen resynthesis, as both studies observed comparable rates of resynthesis to isocaloric beverages with higher carbohydrate contents. Previous research, highlighted within several reviews, have established that when carbohydrate intake is less than 0.8 g/kg BM/h, provision of protein within the carbohydrate containing beverage, to compensate energy intake, will elicit similar rates of muscle glycogen resynthesis (74,92,140). This is likely due to the heightened insulin response after consumption of combined carbohydrate and protein, which stimulates glucose uptake at the skeletal muscle plasma membrane (i.e., GLUT-4 transporter) and glycogen synthase activity within the intracellular cytoplasm (97,191). Therefore, results from this

review suggest that co-ingestion of protein (e.g., 0.4 g/kg BM) with carbohydrate (e.g., ≥0.8 g/kg BM) provided by a dairy milk exercise recovery beverage will result in comparable rates of muscle glycogen resynthesis to an isocaloric CEB (97,192,193).

Hydration Status

It appears that consumption of dairy milk or milk-based recovery beverage will result in comparable restoration of NFB to CEB and water when consumed *ad libitum*, and a more positive NFB when consumed in equal volumes. Previous research has demonstrated that fluid retention is enhanced with increasing sodium concentrations (118). For trials in which hydration markers were measured after providing isovolumetric doses of each recovery beverage, the sodium content ranged from 18-59 mmol/L and 12-23 mmol/L for the dairy milk beverages and CEB, respectively. It is likely that the higher sodium and (or) the sucrose derived from added sugar in CM and MBSB (values not specified), may have contributed towards the superior restoration of fluid balance observed in the dairy milk trials (132,176).

Additionally, the higher energy density of milk or milk-based beverages is likely to delay gastric emptying and intestinal transit, and consequently slow the delivery of water into circulation (194). A slower rate of circulatory water delivery avoids acute and transient episodes of hypervolaemia, and subsequently attenuates the sudden drop in plasma osmolality, which leads to increased circulatory water clearance by the kidney through enhanced urine production and output. Three inclusion studies observed such responses, whereby P_{Osmol} was significantly lower during the water and (or) CEB trials than the milk trials, within 1 h of rehydration (130,133,136). Additionally, increased urine output 1-2 h after rehydration was observed during the water and CEB trials, compared with dairy milk beverage trials (130,133).

Evidently, body water-electrolyte balance following strenuous exercise and fluid consumption behaviours are complex and dynamic in nature, and cannot be accurately measured with a single method. It has been suggested that *'gold-standard'* for assessing hydration status at discrete time points in close temporal proximity under controlled laboratory conditions is a combination of TBW and P_{Osmol} (58). Use of additional methods including Δ P_V, Δ BM, urine measures of hydration, and thirst perception may help to provide a more comprehensive evaluation of body water dynamics during the acute recovery period (58,195). Results from this current SLR suggest that provision of an isovolumetric dose of a dairy milk beverage after strenuous exercise will result in a more positive NFB, and a higher P_{Osmol} when compared to water and (or) a CEB. However, the effect of beverage differences on TBW was not investigated. Further research investigating the effects of an exercise recovery beverages on hydration status should utilise multiple methods including P_{Osmol} and TBW, at a minimum. Considering the technical and cost constrains of using an isotope tracer (e.g., deuterium oxide) with mass spectrometry techniques to determine TBW, the application of multi-frequency bioelectrical impedance analysis for TBW provides a practical alternative, bearing in mind the limitations of using such a technique (196,197). However, it is imperative that such devices are validated against an isotope tracer technique, prior to application, thus ensuring accuracy and meaningful outcome interpretations.

Immune Function

A single bout of prolonged and intense exercise may result in an acute immune disturbance, characterised by acute circulatory leukocytosis (e.g., neutrophilia and lymphocytosis followed by lymphopenia), reduced immune cell function (e.g., reduced bacterially stimulated neutrophil degranulation *in vitro*, reduced mitogen or antigen-induced lymphocyte proliferation *in vitro*, and reduced antigen-induced delayed hypersensitivity challenge *in vivo*), perturbed oral-respiratory mucosal immunity, increased inflammatory cytokine responses (25,60,64,138,139,198). While the clinical implications of such a response are inconclusive, it has been theorised that perturbed immune responses following a bout(s) of endurance exercise, or during periods of intensified training, may

decrease an athlete's resistance to the presentation of opportunistic invasion by pathogenic microorganisms, subsequently increasing risk for illness and (or) infection (12). Therefore, targeted nutrition strategies to optimise recovery of immune function after and (or) between bouts of strenuous endurance exercise would appear to be a logical and favourable intervention. At present, there are no nutritional guidelines or recommendations that specifically focus on feeding post-exercise in order to support immune function. However, the consumption of an exercise recovery supplementation beverage containing 1.2 g/kg BM of carbohydrate with or without 0.4 g/kg BM of protein immediately following 2 h running at 75% $\dot{V}O_{2max}$ has been shown to support post-exercise immune functional responses, as evidenced by a more favourable bacterially-stimulated neutrophil degranulation response and salivary antimicrobial status, compared with either a water control or delayed feeding (i.e., 1 h post-exercise) (25,138,139). Considering dairy milk provides similar total carbohydrate and protein nutritional profiles per respective portion, it is plausible that dairy milk consumption immediately after strenuous endurance exercise may result in favourable immune outcomes, however this warrants substantiation (199).

Gastrointestinal Status and Intake Tolerance

The term *'exercise-induced gastrointestinal syndrome'* has recently been used to describe the perturbations to gastrointestinal epithelial integrity, gastrointestinal functional responses, local epithelial and systemic immune interactions that have been reported to present both clinical and performance implications (11,17,64,163). The primary causal mechanisms of EIGS include altered circulatory- and neuroendocrine-gastrointestinal physiological processes in response to exertional stress, which results in secondary outcomes. These secondary outcomes may include, but not limited to: 1) decreased gastrointestinal motility, digestion, and absorption; 2) increased intestinal epithelial cell injury, increased intestinal epithelial tight-junction injury and (or) dysregulation, and subsequent hyperpermeability; and 3) increased local and systemic inflammatory responses. These outcomes have associations with extrinsic (e.g., exercise intensity, duration, modality, and heat exposure) and intrinsic

(e.g., feeding tolerance, predisposition to gastrointestinal disease/disorder, and the gastrointestinal microbiota) exacerbation factors, and clearly linked to GIS that appears to be specific to the primary causal factors and secondary outcomes (11,64). Such exercise-associated perturbations may have implications for post-exercise nutrition availability and feeding behaviours. Indeed, a growing number of recent studies have provided evidence of impaired absorption of carbohydrate and protein following exertional stress (i.e., both endurance and resistance exercise), likely due to exercise-associated enterocyte injury and (or) sympathetic drive suppressing submucosa plexus and brush border nutrient absorption influencing activities (13,68,70,164,200). Carbohydrate malabsorption of a pre-exercise carbohydrate-rich (i.e., 120 g) meal has been observed in response to 2 h running at 70% $\dot{V}O_{2max}$ in temperate ambient conditions with fluid restriction to induced 3.1% BM loss dehydration (breath H_2 peak: 12 ppm) and with euhydration (breath H_2 peak: 6 ppm) (13). Meanwhile, 68% of an endurance athlete participant cohort demonstrated carbohydrate malabsorption of clinical significance (≥10 ppm breath H_2) in response to 90 g/h consumption of a 2:1 glucose-fructose 10% *wv* during 2 h running at 60% $\dot{V}O_{2max}$, followed by a 1 h distance test, in temperate ambient conditions. These outcomes are not surprising considering reduced active and passive carbohydrate absorption has been reported in response to running at 70% $\dot{V}O_{2max}$ compared to rest (68). Moreover, a reduction in post-exercise protein absorption, as measured by *in vivo* combination of 20 g L-[$^{1\text{-}13}$C] phenylalanine labelled protein ingestion with continuous intravenous L-[ring-$^{2}H_5$] phenylalanine infusion, was observed after a single bout of resistance-type exercise (70).

To date, no study has investigated the effect of exercise-associated gastrointestinal injury and functional impairment on dairy milk nutrient bioavailability in the post-exercise recovery period. However, given our current understanding of EIGS, it is possible that ingestion of dairy milk after strenuous endurance exercise may result in malabsorption of both carbohydrate and protein (201). This may have implications for the recovery of perturbed physiological systems, from a nutrient bioavailability perspective; and may also induce GIS, further burdening feeding behaviours and nutrient

provisions. For example, due to the relatively high energy density and lactose (i.e., a high fermentable carbohydrate) content of dairy milk, there is anecdotal belief that athletes may experience some abdominal discomfort. However, despite some studies reporting higher levels of bloating and (or) fullness with dairy milk consumption in the recovery period, this review did not find any consistent evidence of significant differences in abdominal discomfort when compared to alternative beverages.

Finally, there is some evidence to suggest that consumption of dairy milk as a recovery beverage will reduce energy intake of a subsequent meal. It is, however, unclear if overall energy intake (i.e., total energy intake from the beverage and the meal) is offset. Further research in the area is required, as a reduction in overall energy intake following consumption of a dairy milk exercise recovery beverage might be a useful strategy for athletes who are aiming to create an energy deficit. Conversely, this strategy may be harmful to athletes struggling to achieve adequate energy intake.

Endurance Exercise Performance

Four studies included in this review demonstrated that consumption of dairy milk as a recovery beverage resulted in an improvement in a subsequent protocol of exercise capacity or performance when compared to non-caloric alternatives (105,180,182,184). This is not surprising given the importance of carbohydrate availability for prolonged endurance exercise performance or intermittent high-intensity exercise (51,141,169,178) . However, five studies also observed such improvement when dairy milk was compared to energy-matched alternatives (105,116,178,181,184). The carbohydrate content of the dairy milk beverages in these trials were less than or equal to that of the comparator beverages. It can be theorised that the improved endurance exercise performance observed in these trials is attributed to the potential of dairy milk to stimulate MPS and aid rehydration, in addition to enhancing glycogen replenishment. Thus, dairy milk may provide a thorough nutritional base for '*exercise recovery optimisation*', compared to the overall recovery outcomes when only one nutrient

and (or) one recovery variable is targeted (e.g., carbohydrate or protein or water provisions on muscle glycogen resynthesis *vs* muscle protein synthesis *vs* rehydration *vs* gastrointestinal and immune status).

A final consideration around recovery and its link to longer term performance benefits involves the emerging evidence that withholding carbohydrate after a high quality training session might prolong the duration of the post-exercise period of increased transcription of adaptive proteins associated with endogenous fat energy substrate oxidation (202). Undertaking endurance exercise with low endogenous glycogen stores appears to enhance activation of muscle intracellular signalling proteins (e.g., p38-MAPK, AMPK, and PGC-1α) that modulate the expression of enzymes and other proteins that effect mitochondrial biogenesis, fuel utilisation (e.g., increased fat oxidation at the respective exercise workload), and (or) aerobic economy and efficiency (202,203). In addition, since the restoration of glycogen is inversely correlated with the increase in cellular signalling, the deliberate avoidance of carbohydrate in the acute recovery period may extend the period of enhanced adaptation (6,203). It is, however, important to consider the frequency and placement of these dietary strategies. For example, low carbohydrate in the recovery period is not ideal when high intensity training sessions (e.g., HITT) or competition are consecutively planned, but may be more suited to promote adaptation to a high quality session when the subsequent workout involves moderate intensity exercise. As such, alteration of dairy milk to manipulate the carbohydrate content (e.g., addition of carbohydrate when the promotion of glycogen synthesis is desired, and membrane permeability manufacturer processing techniques inducing alteration in dairy milk lactose content for 'recover low' sessions) may add to the versatility of dairy milk as a recovery beverage.

Research limitations, future implications, and translational application

The authors acknowledge certain limitations to the translational applicability of the presented SLR findings. The potential language bias introduced through limiting the search strategy to English language papers only is acknowledged. The overall risk of bias is moderate. Most studies failed to

identify randomisation (n= 12) and blinding procedures (n= 16), and all failed to prospectively register trials. Moreover, due to the nature of the studies testing beverages with different flavours and textures, blinding of participants was not possible. Most studies included in the summary required participants to perform the exercise protocol in the fasted state. This is not representative of common training and competition practices, as athletes will often consume a meal or snack in the hours preceding exercise. Additionally, the majority of studies were conducted amongst male athletes. Given the well-established differences in body composition, metabolism, and physiological responses (i.e., hydration, gastrointestinal, and immune status in response to exertional stress) due to biological sex, these results might not reflect ideal recovery nutrition for female athletes (11,15,197,204,205). The intervention beverages have been categorised based on the general type of beverage (i.e., chocolate milk, milk-based sports beverage, etc), and therefore have not considered the specific nutritional profiles of each beverage. Finally, due to the heterogeneity of the exercise protocols, nutritional composition of beverages and reported outcomes, a meta-analysis of the reviewed studies was not possible.

Overall, the major limitation of all included studies, from the perspective of translational applicability, was the lack of universal or unifying markers of recovery with a transfer to a performance outcome. All studies focused on one or two key recovery components, with or without a subsequent endurance exercise capacity or performance test. Such experimental procedures are imperative for targeted assessment of a singular recovery cluster (i.e., markers for muscle protein synthesis, muscle glycogen resynthesis, or rehydration) with identification of outcomes and mechanisms of action advancing scientific knowledge. Nevertheless, a stronger investigation of the transfer to performance benefits is warranted to avoid misguided, misinterpreted, and (or) inappropriate applications of the research findings.

Future applied research in relation to post-exercise nutrition should employ a comprehensive assessment of recovery markers (Figure 2.8), ensuring experimental designs are able to capture

accurate interactions between the nutritional intervention/s and '*exercise recovery optimisation*' outcomes. In particular, given the failure of previous research to take into account the magnitude of intestinal injury and impairment of nutrient transport associated with sympathetic drive early in the post-exercise period (11,201), it is likely that the potential for recovery may have been underestimated. Conversely, if the magnitude of EIGS is attenuated through pre- and (or) during-exercise nutritional or physiological manipulative strategies, then it is plausible that the nutrient bioavailability of the exercise recovery nutritional strategy will be enhanced (11,15,64,69,163).

Conclusion

The nutritional properties of dairy milk beverages, particularly those with higher carbohydrate and sodium contents, closely align with the current nutrition guidelines for recovery from endurance exercise. Although several quality issues were identified across the included library, the current SLR provides evidence to suggest that dairy milk may provide equivalent or superior recovery of muscle protein synthesis, glycogen replenishment, and rehydration when compared to carbohydrate and carbohydrate-electrolyte alternatives, which appear to have performance benefits (e.g., dairy milk > non-caloric and caloric comparators). However, to date, there has been no research investigating the effects of dairy milk on immune and gastrointestinal status following strenuous endurance exercise.

Chapter Three

Assessing overall exercise recovery processes using carbohydrate and carbohydrate-protein containing recovery beverages.

Abstract

We compared the effect of two different, but commonly consumed, beverages on integrative markers of exercise recovery following a 2h high intensity interval exercise (i.e., running 70-80% $\dot{V}O_{2max}$ intervals and interspersed with plyometric jumps). Participants (n= 11 males, n= 6 females) consumed a chocolate flavoured dairy milk beverage (CM: 1.2 g carbohydrate/kg BM and 0.4 g protein/kg BM) or a carbohydrate-electrolyte beverage (CEB: isovolumetric with 0.76 g carbohydrate/kg BM) after exercise, in a randomised-crossover design. The recovery beverages were provided in three equal boluses over a 30min period commencing 1h post-exercise. Muscle biopsies were performed at 0h and 2h in recovery. Venous blood samples, nude BM and total body water were collected before and at 0h, 2h, and 4h recovery. Gastrointestinal symptoms and breath hydrogen (H_2) were collected before exercise and every 30min during recovery. The following morning, participants returned for performance assessment. In recovery, breath H_2 reached clinical relevance of >10 ppm following consumption of both beverages, in adjunct with high incidence of gastrointestinal symptoms (70%), but modest severity. Blood glucose response was greater on CEB *vs* CM (P< 0.01). Insulin response was greater on CM compared with CEB (P< 0.01). *E.coli* lipopolysaccharide stimulated neutrophil function reduced on both beverages (49%). p-GSK-3β/total-GSK-3β was greater on CM compared with CEB (P= 0.037); however neither beverage achieved net muscle glycogen re-storage. Phosphorylation of mTOR was greater on CM than CEB (P< 0.001). Fluid retention was lower (P= 0.038) on CEB (74.3%) compared with CM (82.1%). Physiological and performance outcomes on the following day did not differ between trials. The present findings expand on recovery nutrition strategies to target functionality and patency of the gastrointestinal tract as a prerequisite to assimilation of recovery nutrition, as well as restoration of immunocompetency. The carbohydrate and protein composition of CM beverage supported greater hydration and expression of cell signalling proteins regulating translation initiation, suggesting greater

overall acute recovery optimisation, however this did not translate to performance benefits the following day.

Introduction

The manipulation of recovery nutrition to promote physiological restoration, adaptation to training and, potentially, performance benefits is an established practice amongst athletes and a key theme in sports nutrition research. Over the past decade, studies have meticulously investigated the optimal nutritional approach for isolated goals such as the replacement of energy substrate, restoration of body water losses, and repair of damaged tissues (e.g., skeletal muscle), leading to the development of generalised recommendations for each element of exercise recovery (6). Such guidelines target individual aspects of exercise recovery, offering prescriptive values for the intakes of carbohydrate, protein, and water to support muscle and liver glycogen replenishment, skeletal muscle protein synthesis, and rehydration, respectively. To date, however, recovery guidelines have failed to consider the impact of exercise-induced gastrointestinal syndrome (EIGS) on regulation of nutrient availability via gastrointestinal integrity and functional responses and (or) the restoration of immunocompetency in response to immunodepressive exercise (11,18,64). These neglected aspects appear to be associated with nutrient circulatory and cellular bioavailability which subsequently determines the overall recovery nutrition outcomes (206).

It is well established that prolonged strenuous exercise reduces muscle glycogen and body water content, and induces skeletal muscle damage. For example, steady state treadmill running for 90 min at 70% $\dot{V}O_{2max}$ in 20-22°C ambient temperature (T_{amb}) and 54-56% relative humidity (RH) has been reported to deplete muscle glycogen content below 250 mmol/kg dw, and induce BM loss >2.5% indicative of mild dehydration (7,8). Muscle damage, as indicated by biochemical (i.e., increased creatine kinase (CK) or myoglobin) and functional (i.e., reduced isometric maximal voluntary contraction and (or) increased muscle soreness) markers is induced by eccentric plyometric contractions (i.e., 5 x 20 drop jumps) and prolonged intermittent running (i.e., 90 min Loughborough Intermittent Shuttle Test) (9,10). From an immune perspective, prolonged strenuous exercise is also known to induce a systemic leukocytosis, and depress several immune cell functional responses (i.e.,

reduced bacterially stimulated neutrophil degranulation *in vitro*, reduced mitogen or antigen-induced lymphocyte proliferation *in vitro*, and reduced antigen-induced delayed hypersensitivity challenge *in vivo*), the extent of which appears to be proportional to exercise intensity and duration (12). Impaired immunocompetency, especially neutrophil functional responses, may result in suboptimal clearance of damaged cell debris required for muscle glycogen resynthesis (160,207), tissue repair and remodelling (16), and clearance of environmental and (or) luminal-derived pathogenic agents (11,13-15). Indeed, bacteria and bacterial endotoxins may enter systemic circulation by physical breaks and (or) hyperpermeability in the gastrointestinal epithelium as a result of EIGS, leading to a pronounced systemic inflammatory response and (or) GIS (14,69,208,209). The recovery of these exercise-induced physiological disturbances, returning to baseline levels or assisting with adaptations to the exercise stress, is highly dependent on nutrient bioavailable during passive rest. Moreover, recovery outcomes are ultimately regulated by food and fluid choice and ingestion (i.e., nutrient density and water volume), and gastrointestinal functional responses to the intake load.

Circulatory nutrient availability is a key feature in immunocompetence during and after prolonged strenuous exercise. For example, frequent carbohydrate ingestion (i.e., 45 g/h) during exercise has shown favourable effects by attenuating perturbations to bacterial endotoxin and systemic inflammatory profiles (69).While, carbohydrate (i.e., 1.2 g/kg BM), with or without protein (i.e., 0.4 g/kg BM) provisions immediately post-exercise has also shown favourable effects in preventing post-exercise reduction in neutrophil functional, albeit *in vitro* (25,64,138), but not other immune functional responses and (or) status concentration changes (25,64). Ultimately these immune restoration outcomes are dependent on the regulation of the gastrointestinal tract in allowing circulatory nutrient availability. As such, EIGS can impair gastrointestinal functional capacity leading to reduced post-exercise nutrient bioavailability (11,64,68,70). Failure to consider the influence of EIGS on nutrient intake, bioavailability and cellular assimilation may have led to suboptimal estimates for acute recovery nutrition guidelines and recommendations targeting glycogen replenishment and tissue repair.

Considering the integrative and inter-dependant nature of exercise recovery processes (i.e., gastrointestinal patency, immune restoration, rehydration, muscle glycogen and protein resynthesis), in assessing the impact of food and (or) fluid provisions after exercise on overall recovery, it is imperative to apply an exercise stress model known to perturb the physiological status of these recovery categories. As such, it appears exercise duration up to 2 h, of intermittent high intensity nature, and inclusion of plyometric jumps meets this criteria (10,14,25,64,138,206,210).

Contemporary sports nutrition recognises the need to personalise and periodise the nutrition support for training and competition including recovery nutrition, whether it refers to acute (e.g., 4-8 h) or longer-term (e.g., following day or >24 h) recovery processes. The concept of 'exercise recovery optimisation' provides an additional contribution to the sophistication of our knowledge and practice by integrating recovery strategies that maximise desired outcomes while minimising those that cause detrimental outcomes within the complex and interrelated recovery responses to exercise (206). It is now well established and generally accepted that the foods and fluids provided immediately after exercise cessation play an important role in subsequent physical performance outcomes when repetitive bouts have a shorter time separation (e.g., several hours), but are less impacting on performance outcomes over longer-periods (e.g., next day and onwards) when habitual dietary intake may support the overall recovery processes (51,206,211). Firstly, it is still unknown how exercise-associated gastrointestinal and immune perturbations impacts recovery nutrition processes and subsequent performance (i.e., the following day), using adequate dietary control experimental models. Secondly, from a practical perspective, daily repetitive exercise bouts of sufficient exertion (e.g., duration and intensity) to induce physiological disturbance that warrants recovery attention is normally associated to training loads, whilst competition is habitually on consecutive days. As such, merging relevant nutritional provisions, recovery process, and timescale seems the most logical translational aspect of professional practice.

Considering the importance of carbohydrate, protein and water provisions, and nutrient bioavailability in supporting exercise recovery processes, flavoured dairy milk beverages and carbohydrate electrolyte beverages are the most commonly consumed beverages amongst athletic populations in the post-exercise recovery period, despite their isovolumetric energy and nutritional composition differences (20,206). These nutritionally diverse, but intake tolerable beverages, provide an ideal nutritional comparison to assess nutrient availability and overall exercise recovery variables (e.g., carbohydrate-protein *vs* carbohydrate). With this in mind, the current study aimed to investigate the impact of a carbohydrate- and protein-containing flavoured dairy beverage and a non-nitrogenous carbohydrate electrolyte beverage on overall and integrative markers of acute recovery following an exercise stress known to perturb many aspects of physiological and metabolic homeostasis. The influence of physiological and nutritive factors on assimilation and bioavailability of recovery nutrition was assessed using global markers of recovery optimisation including EIGS, immune function response previously confirmed to depress after exercise and response to recovery nutrition (e.g., *in vitro* bacterially-stimulated neutrophil elastase release), muscle glycogen resynthesis, protein synthesis, rehydration, and performance outcomes. It was hypothesised that exercise-induced gastrointestinal damage would result in clinically relevant carbohydrate malabsorption and GIS to a greater extent following consumption of the dairy milk beverage. It was also hypothesised that the overall greater nutrient content of the dairy (carbohydrate-protein containing) beverage would result in a greater muscle glycogen resynthesis, expression of cell signalling proteins regulating translation initiation, fluid retention and prevention of declined neutrophil function, than carbohydrate alone, and result in greater exercise performance the following day.

Methods

Participants

Seventeen (n= 11 males, n= 6 females) recreationally trained endurance athletes (mean (SD): age 33.7 (9.3) years, nude BM 69.1 (10.1) kg, height 173.9 (8.1) cm, % body fat 17.8 (6.6) %, $\dot{V}O_{2max}$ 55.8 (6.9) ml/kg BM/min, weekly training volume 413 (201) min, and modality: endurance running, endurance

cycling, ultra-endurance running, HIIT) volunteered to participate in the study and completed the experimental procedures. volunteered to participate in the study. All participants gave written informed consent. The study protocol received approval from the local ethics committee (Monash University Human Research Ethics Committee: 12799) and conformed with the Helsinki Declaration for Human Research Ethics. The trial was prospectively registered with ANZCTR (reference number 375090). All participants confirmed being free from illness, disease (including gastrointestinal infections, dietary intolerances, diseases and (or) disorders) and injury. Individuals were excluded if they confirmed having consumed potential dietary modifiers of gastrointestinal integrity, were adhering to gastrointestinal-focused dietary regimes within the previous three months, or consumed non-steroidal anti-inflammatory medications, antibiotic and (or) stool altering medications within one month before the experimental protocol. An additional 4 participants were recruited but were unable to complete both trials, due to illness/injury (n= 1 male, n= 1 female) or unexpected unavailability (n= 1 male, n= 1 female).

Preliminary measures

One to three weeks prior to the first experimental trial, baseline measurements for height (stadiometer, Holtain Limited, Crosswell, Crymych, United Kingdom), BM (Seca 515 MBCA, Seca Group, Hamburg, Germany), body composition (Seca 515 MBCA, Seca Group, Hamburg, Germany) and $\dot{V}O_{2max}$ (Vmax Encore Metabolic Cart, Carefusion, San Diego, California, US) were recorded. $\dot{V}O_{2max}$ was estimated by means of a continuous incremental exercise test to volitional exhaustion on a motorised treadmill (Forma Run 500, Technogym, Seattle, Washington, US), as previously reported (138). Criteria for attaining $\dot{V}O_{2max}$ included the participant reaching volitional exhaustion (i.e., rating of perceived exertion (RPE) of 19-20 Borg scale), a heart rate (HR) within 10 beats/min of HR_{max}, with observation of $\dot{V}O_2$ plateau is increasing exercise intensity and (or) inclusion of RER (≥1.10). To determine running speeds for the exercise trials, the speed at approximately 50 (7.3 (1.0) km/h), 55-60 (8.7 (1.3) km/h),

70-75 (10.8 (1.4) km/h) and 80-85 (12.7 (1.8) km/h) % $\dot{V}O_{2max}$ and 1% gradient was determined and verified from the $\dot{V}O_2$-work rate relationship.

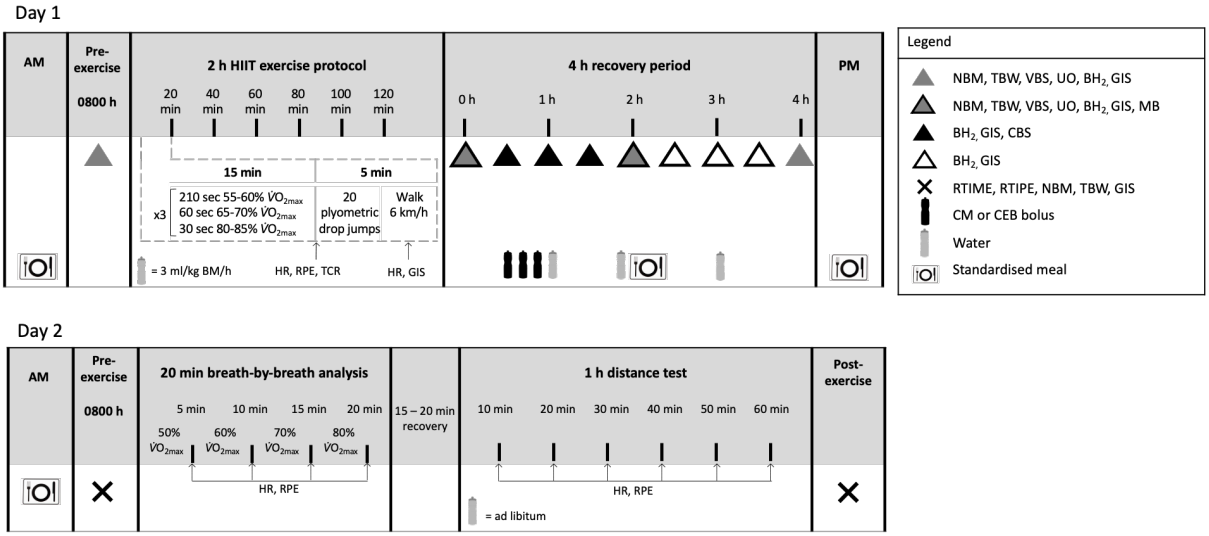
Experimental protocol

To control dietary intake, all foods and fluids were provided throughout the experimental trials, and participants were required to consume a standardised low fermentable carbohydrate (FODMAP) diet during the 24 h before, and throughout the experimental trials. Meals were designed in accordance with current nutrition guidelines for endurance athletes, and calculated to provide <2 g FODMAP per meal using a FODMAP specific database (Monash University, FoodWorks Professional 7, Xyris, Brisbane, Australia) (6,212). Compliance was assessed using a food and fluid diary (overall mean (SD): energy 10.1 (3.0) MJ/day, protein 98 (30) g/day, fat 57 (36) g/day, carbohydrate 353 (87) g/day, fibre 44 (11) g/day, and water 2333 (1358) ml/day). Participants were asked to avoid alcohol and strenuous exercise during the 48 hours before each experimental trial, and to refrain from consuming caffeinated beverages during the 24 h before each experimental trial. In a randomised order (computer generated randomisation), generated by an independent third-party researcher, participants completed two experimental trials separated with at least a 5 days washout period, to accommodate the participants' availability. Trials for female athletes were scheduled during the follicular phase of their menstrual cycle (n= 5) or when taking the active medication of the oral contraceptive pill (n= 1). Resting estrogen levels (DKO003/RUO; DiaMetra, Italy) were measured for verification, were within normal reference range, and did not differ between trials (11.6 (6.0) pg/ml; P= 0.593). Participants reported to the laboratory at 0800h after consuming the standardised mixed carbohydrate breakfast (energy 2.9 (0.8) MJ, protein 28 (9) g, fat 19 (5) g, carbohydrate 99 (28) g, fibre 12 (5) g, and water 363 (264) ml). Before commencing the exercise protocol, participants were asked to void. Pre-exercise nude BM and TBW (Seca 515 MBCA, Seca Group, Hamburg, Germany) were recorded. Participants inserted a thermocouple 12 cm beyond the external anal sphincter to record pre-exercise rectal temperature (T_{re}) (Precision Temperature 4600 Thermometer, Alpha Technics, California, USA). Participants provided a breath sample into a 250 ml

breath collection bag (Wagner Analysen Technick, Bremen, Germany), and completed an exercise-specific mVAS GIS assessment tool (162). Blood was collected by venepuncture from an antecubital vein into three separate vacutainers (6 ml 1.5 IU/ml lithium heparin, 4 ml 1.6 mg/ml K_3EDTA, and 5 ml SST; BD, Oxford, UK). The exercise protocol consisted of a 2 h (initiated at 0900h) HIIT session in T_{amb} 23.4 (1.1) °C and 44 (6) % RH, as described in **Figure 3.1**. The protocol involved 3 rounds of running for 3.5 min at 55-60% $\dot{V}O_{2max}$, 1 min running at 65-70% $\dot{V}O_{2max}$ and 30 sec running at 75-80% $\dot{V}O_{2max}$, followed by 20 plyometric drop jumps of alternating legs. Participants then returned to the treadmill to walk until the 20 min cycle was completed. This was repeated 6 times. The protocol was designed to provide sufficient exercise stress to perturb key markers of physiological and metabolic homeostasis (e.g., muscle glycogen, muscle protein, and hydration), including immune and gastrointestinal status, as previously reported (13,14,64,206). During exercise, participants were provided with water equivalent to 3 ml/kg BM/h (25,138). Heart rate (HR) (Polar Electro, Kempele, Finland), rating of perceived exertion (RPE) (213), and thermal comfort rating (TCR) (214) were measured at the 15 min mark of each 20 min cycle, as previously described (14). Recovered HR and GIS were measured during the final 30 s of the 20 min cycle. Immediately post-exercise, nude BM and T_{re} were recorded. The recovery period commenced 30 min after the end of the exercise protocol to prepare for muscle biopsy sampling. Participants rested in a supine position in a sterile phlebotomy room for venous blood sampling followed by the first muscle biopsy thereafter. Muscle biopsy samples were taken 0 h and 2 h into the recovery period. TBW was measured immediately after muscle biopsy sampling. Blood samples, nude BM and TBW were collected again at 2 h and 4 h of recovery. Breath samples were collected and GIS recorded every 30 min throughout the recovery period. Total urine output was collected throughout the total recovery period. Weight of urine output was recorded at 2 h and 4 h of recovery. After sampling at 2 h post-exercise, participants received a standardised recovery meal (energy 2.8 (0.7) MJ, protein 31 (8) g, fat 4 (2) g, carbohydrate 137 (32) g, fibre 9 (2) g, and water 415 (103) ml), and were instructed to consume as much as tolerable, and the total weight of the meal consumed was recorded. In addition, participants consumed a standardised evening meal after leaving the laboratory (energy

3.1 (1.4) MJ, protein 32 (14) g, fat 18 (17) g, carbohydrate 102 (50) g, fibre 19 (6) g, and water 757 (52) ml).

Figure 3.1 Schematic illustration of experimental design.



NBM: nude body mass, TBW: total body water, VBS: venous blood sampling, UO: urine output, BH_2: breath hydrogen, GIS: gastrointestinal symptoms, HR: heart rate, TCR: thermal comfort rating, RPE: rating of perceived exertion, MB: muscle biopsy, CBS: capillary blood sampling, RTIME: readiness to invest mental effort, RTIPE: readiness to invest physical effort, RER: respiratory exchange ratio, CM: chocolate flavoured dairy milk, CEB: carbohydrate-electrolyte beverage, BM: body mass.

The following morning, participants returned to the laboratory (0800h) to assess psychophysiological parameters and exercise performance. Due to unforeseen circumstances unrelated to the study intervention, 3 participants did not return for the second day of testing on one or both of their trials. Therefore, data for 14 participants (n= 9 males, n= 5 females) was included for analysis. A standardised mixed carbohydrate breakfast (energy 2.9 (1.0) MJ, protein 29 (12) g, fat 18 (6) g, carbohydrate 97 (37) g, fibre 11 (5) g, and water 414 (235) ml) was consumed at 0700h. Nude BM, TBW and GIS were recorded on arrival and again after the performance test. Before and after the performance test, participants completed measures of readiness to invest mental and physical effort, rated from 0-10, with higher ratings indicating greater readiness to invest effort (215). Participants performed a 20 min running exercise bout to measure oxygen uptake and oxidation rates at four submaximal exercise intensities (50%, 60%, 70%, and 80% $\dot{V}O_{2max}$) for 5 min each. Thereafter, in accordance with the cohort population (recreationally trained endurance athletes and varied endurance modalities) they were

asked to complete a 1 h performance test in thermoneutral conditions (T_{amb} 23.0 (1.3) °C and 46 (9) % RH). Participants were instructed to run the maximal distance they were capable of running in 1 h, with the incline set at 1%, as previously reported (163). Total distance, HR, RPE, and water intake (provided ad libitum) were recorded every 10 min.

Muscle biopsy procedure

Nine participants consented to providing muscle samples for both trials (n= 8 males, n= 1 female). Muscle biopsies were performed using a modified 5mm Bergstrom biopsy needle. Samples were obtained from the vastus lateralis of the ipsilateral leg for the first trial, and contralateral leg for the second. The skin of the lateral aspect of the mid-thigh was washed well (10% Povidone- Iodine solution) then 2-3 ml of local anaesthetic (lidocaine 1%) was infiltrated subcutaneously over vastus lateralis to anaesthetise the skin and superficial fascia. After the anaesthetic had taken effect, two 5 mm stab incisions ~15 mm apart were made through skin and fascia, with one incision made for each muscle biopsy sample. Samples were then extracted, immediately submerged in liquid nitrogen and stored at -80°C prior to further analysis.

Recovery beverages

In a randomised, repeated measures design, participants were provided with: 1) chocolate flavoured dairy milk recovery beverage (CM) on one occasion, and a 2) orange flavoured carbohydrate-electrolyte beverage (CEB) on another occasion. The beverages were prepared by a third-party researcher, and served in opaque bottles, at ~7°C beverage temperature (216), in 3 equal boluses every 10 min, beginning 60 min after cessation of exercise. It was not possible to match the recovery beverage to taste and texture (e.g., dairy milk versus water-based solutions); however, participants were blinded to all aspects of the recovery beverage until ingestion, whereby distinct flavour and texture was apparent. The volume of the beverage was calculated to provide 1.2 g/kg BM of carbohydrate and 0.4 g/kg BM of protein on CM (energy 2449 (358) kJ, protein 28 (4) g, fat 11 (2) g, and carbohydrate 83 (12) g), and was in accordance with previous recovery research targeting muscle glycogen resynthesis, muscle

protein resynthesis, and exercise-induced immunodepression prevention and (or) restoration (206). The CEB was matched for volume on the alternate trial (energy 898 (131) kJ, protein 0 (0) g, fat 0 (0) g, and carbohydrate 52 (8) g) and provided 0.76 g/kg BM of carbohydrate and 0.0 g/kg BM of protein. Additional water calculated to provide a total fluid intake of 35 ml/kg BM was provided at equal volume at hourly intervals. Participants were instructed to drink as much as tolerable. Total fluid intake was recorded hourly. The percentage of ingested fluid retained was calculated from the difference between ingested fluid and urine output, as a fraction of total fluid intake (128).

Sample analysis

Blood glucose concentration, haemoglobin, total and differential leukocyte counts, which included neutrophils, lymphocytes and monocytes, were determined by HemoCue system (Glucose 201+, Hb201, and WBC DIFF, HemoCue AB, Ängelholm, Sweden) in duplicate from heparin whole blood samples. Coefficient of variation (CV) for blood glucose concentration, hemogobin, and leukocyte counts were 5.1%, 1.6% and 13.6%, respectively. Hematocrit was determined by capillary method in triplicate from heparin whole blood samples and using a microhaematocrit reader (CV: 0.7%) (ThermoFisher Scientific). Hemoglobin and hematocrit values were used to estimate changes in P_V relative to baseline, and used to correct plasma variables (217). To determine the blood glucose response to the recovery beverage, immediately before and every 30 min thereafter for 2 h, blood glucose concentration was measured in duplicate using a handheld system from capillary blood samples (CV: 4.1%) (Accu-Chek Proforma, Roche Diagnostics, Indianapolis, Indiana, USA). To determine *in vitro* bacterially-stimulated elastase release, 1 ml of whole blood was pipetted into a microcentrifuge tube containing 50 µg of 1 mg/ml bacterial stimulant (lipopolysaccharide from *E.coli*, Sigma, Poole, UK) within 5 min of collection and gently vortex-mixed. Samples were incubated in a water bath (Labline, Thermo Fisher Scientific Australia, Scoresby, Victoria, Australia) at 37°C for 1 h, and further mixed by gentle inversion at 30 min. Bacterially challenged samples were then centrifuged at 4000 rpm (1500 g) for 10 min, and supernatant was aspirated into 1.5 ml micro-storage tubes and stored at -80°C for further

analysis. The remaining whole blood in the heparin and K_3EDTA vacutainers were centrifuged at 4000 rpm (1500 g) for 10 min within 15 min of sample collection. The whole blood collected in the SST serum tube was allowed to clot for 1 h in ~4°C prior to centrifuging at 4000 rpm (1500 g) for 10 mins. 2 x 50 µl of heparin plasma was used to determine P_{Osmol}, in duplicate (CV: 0.7%), by freezepoint osmometry (Osmomat 030, Gonotec, Berlin, Germany). The remaining heparin and K_3EDTA plasma, and SST serum was aspirated into the appropriate 1.5 ml micro-storage tubes and frozen at -80°C until analysis. Circulating concentrations of insulin (DKO076; DiaMetra, Italy), cortisol (DKO001; DiaMetra, Italy), aldosterone (Demeditec Diagnostics GmbH, Kiel, Germany), PMN elastase (BMS269; Affymetrix EBioscience, Vienna, Austria), intestinal fatty acid-binding protein (I-FABP) (HK406; Hycult Biotech, Uden, The Netherlands), sCD14 (HK320; Hycult Biotech), and lipopolysaccharide binding protein (LBP) (HK315; Hycult Biotech) were determined by ELISA. Additionally, systemic cytokine profile (including plasma interleukin (IL)-1β, tumour necrosis factor (TNF)-α, IL-6, IL-8, IL-10, and IL-1 receptor antagonist (ra) concentrations) (HCYTMAG-60K, EMD Millipore, Darmstadt, Germany) were determined by multiplex system. All variables were analysed as per manufacturer's instructions on the same day, with standards and controls on each plate, and each participant assayed on the same plate. The CVs for ELISAs were ≤6.1% and for cytokine profile multiplex was 16.0%. Breath samples (20 ml) were analysed in duplicate (CV: 2.1%) for hydrogen (H_2) content using a gas-sensitive analyser (Breathtracker Digital Microlyzer, Quintron, Milwaukee, Wisconsin, US). Plasma sodium, potassium and calcium concentrations were determined using ion selective electrodes (Cobas c analyser, Roche Diagnostics, Risch-Rotkreuz, Switzerland) and analysed by local pathology services (Cabrini Pathology, Malvern, Victoria, Australia).

Western blot analysis

Approximately 30 mg of skeletal muscle was solubilized in radioimmunoprecipitation (RIPA) buffer (Millipore, Bayswater, Victoria, Australia) with 1 µl/ml protease inhibitor cocktail (Sigma-Aldrich, Castle Hill, New South Wales, Australia) and 10 µl/ml Halt Phosphatase Inhibitor Single-Use Cocktail (Thermo

Scientific, Australia, North Ryde, New South Wales, Australia). The concentration of protein per sample was determined by the bicinchoninic acid assay (BCA Protein Assay Kit#23225, Thermo Scientific). 20 µg of skeletal muscle protein lysate was loaded onto into either Bio-Rad precast Criterion TGX Stain-Free 4-12% gels (Bio-Rad, Gladesville, New South Wales, Australia). SDS-PAGE was conducted following manufacturer's instructions. Protein was then transferred to PVDF membranes and blocked for 1 h in 5% bovine serum albumin (BSA) solution in Tris-buffered saline-Tween, (pH 7.6, 20 mmol/L Tris and 150 mmol/L NaCl, 0.1% Tween) (TBST) at room temperature. Membranes were then incubated in primary antibodies diluted in 5% BSA/TBST overnight at 4°C. Following washing in TBST, membranes were incubated for 1 h with fluorescent secondary antibodies (mTOR^{Ser2448}, Akt^{Ser473}, rpS6$^{Ser235/236}$, and phospho-glycogen synthase kinase 3β (GSK-3β)^{Ser9}) (anti-Rabbit IgG (H+L) DylightTM 800 Conjugate;Anti-mouse IgG (H+L) DylightTM 680 Conjugate) (Cell Signalling Technologies®, Danvers, Massachusetts, USA) diluted 1:10,000 in TBST. Following 2 further washes in TBST and 1 wash in phosphate buffered saline (PBS) membranes were scanned using the LiCOR® Odyssey CLx® Imaging System (Millennium Science, Mulgrave, Victoria, Australia). All targets were normalized to total protein using either the Bio-Rad stain-free system.

Muscle glycogen analysis

One fraction of muscle sample (approx. 20-25 mg ww) was freeze-dried, after which collagen, blood and other non-muscle material were removed from the muscle fibres. Samples were then pulverized and powdered. Samples were extracted with 0.5 M perchloric acid ($HClO_4$) containing 1 nmol EDTA and neutralised using 2.2 M $KHCO_3$. Adenosine triphosphate, phosphocreatine, and creatine was determined from the supernatant by enzymatic spectrophotometric assays (218,219). Muscle glycogen content was determined from 2 aliquots of freeze-dried muscle (2-3 mg), as previously reported (218).

Statistical analysis

Confirmation of adequate statistical power was determined a priori from the applied statistical test, mean, standard deviation, and effect size on markers of 1) gastrointestinal integrity (i.e., plasma I-

FABP), function (i.e., breath hydrogen), and GIS (11,13,14,210); 2) circulating leukocyte, endotoxin and cytokine profiles (13,25,64,138); 3) total body water, plasma osmolality, plasma volume change (13,64); 4) rate of skeletal muscle glycogen resynthesis (7,74); 5) phosphorylation of intramuscular signalling proteins (111,220); and 6) performance (221,222). Using a standard alpha (0.05) and beta value (0.80), the current participant sample size, within a repeated measures cross-over design, is estimated to provide adequate statistical power (power* 0.80-0.99) for detecting significant between- (trial) and within- (time) group differences (G*Power 3.1, Kiel, Germany). Data in the text and tables are presented as mean (SD) for descriptive method, and mean and 95% confidence interval (CI) for primary variable, as indicated. For clarity, data in figures are presented as mean and standard error of the mean (SEM), and (or) mean and individual responses, as indicated. Systemic inflammatory cytokine responses are presented as raw values and systemic inflammatory response profile (SIR-profile), as previously reported (208). Only participants with full data sets within each specific variable were used in the data analysis. There were no outliers for female participant data points for any of the primary and secondary outcome measures. All data were checked for normal distribution (i.e., Shapiro-Wilks test of normality) prior to main within- and between-group comparative analysis. Variables with singular data points were examined using paired sample t-tests, or non-parametric Wilcoxon signed-rank test, when appropriate. Variables with multiple data points were examined using a two-way repeated-measures ANOVA. Assumptions of homogeneity and sphericity were checked, and when appropriate adjustments to the degrees of freedom were made using the Greenhouse-Geisser correction method. Main effects were analysed by Tukey's post hoc HSD. Statistics were analysed using SPSS statistical software (V.26.0, IBM SPSS Statistics, IBM Corp., Armonk, NY, USA) with significance accepted at $P \leq 0.05$. Additionally, Hedge's g measurement of effect size for GIS and feeding tolerance severity between CM and CEB was determined as >0.50 and >0.80 for medium and large effects, respectively.

Results

Exertional strain

During exercise, a main effect of time (MEOTime) was observed for peak (overall mean and 95% CI: 157 (155 to 159) bpm; P< 0.001) and recovered HR (119 (117 to 121) bpm; P< 0.001), RPE (13 (13 to 13); P< 0.001), and TCR (9 (8 to 9); P= 0.005); whereby HR, RPE, and TCR increased as exercise progressed on CM and CEB, with no differences between trials observed, T_{re} increased pre- (36.8 (36.6 to 37.0) °C) to post-exercise (37.9 (37.7 to 38.1) °C) on CM and CEB (P< 0.001), with no difference between trials observed. A trial*time interaction was observed for plasma cortisol concentration (P= 0.012) (**Table 3.1**), whereby Δ pre- to post-exercise plasma cortisol concentrations was greater on CEB (274 (109 to 439) nmol/L) compared with CM (53 (-92 to 197) nmol/L). However, no trial differences were observed during the recovery period. Exercise-induced BM loss (1.9 (1.7 to 2.2) %), Δ PV (-359 3.1 (-5.0 to -1.2) %), pre- and post-exercise P_{Osmol} and TBW (including extracellular and intracellular water) did not differ between CM and CEB (**Table 3.1**).

Gastrointestinal integrity, function and symptoms

A MEOTrial (P= 0.020) and MEOTime (P= 0.003) were observed for plasma I-FABP concentration, whereby levels were generally higher pre- and post-exercise on CM, and increased in response to the exercise stress on both trials; however magnitude of response was not significantly different between trial (**Table 3.1**). There was a MEOTime for breath H_2 (P< 0.001), a peak of clinical significance (CM: 17 (11 to 24) ppm *vs* CEB: 18 (11 to 25) ppm) occurring following consumption of both recovery beverages (**Figure 3.1**). No significant main effects or interaction were observed for plasma sCD14 or LBP concentrations (**Table 3.1**). There were no significant differences in the incidence or severity of GIS during exercise and recovery period between (**Table 3.2**).

Table 3.1 Change in hydration and biomarkers in response to 2 h HIIT exercise (between 60% and 80% $\dot{V}O_{2max}$) in temperate ambient conditions and after consumption of chocolate dairy milk (CM) and the carbohydrate-electrolyte (CEB) recovery beverages.

	CM				CEB			
	Pre-exercise	Post-exercise	2 h post-exercise	4 h post-exercise	Pre-exercise	Post-exercise	2 h post-exercise	4 h post-exercise
Total body water (%)	59.8 (57.6 to 62.0)	60.2 (58.0 to 62.3)	59.3†† (57.2 to 61.4)	59.2†† (57.1 to 61.3)	59.5 (57.5 to 61.6)	60.4 (58.1 to 62.7)	58.9†† (56.8 to 61.1)	59.2†† (57.1 to 61.2)
(L)	40.6 (37.8 to 43.4)	40.2 (37.3 to 43.2)	40.2§§ (37.4 to 43.1)	40.6 (37.8 to 43.4)	40.4 (37.7 to 43.1)	40.2 (37.5 to 43.0)	39.8§§ (37.0 to 42.6)	40.3 (37.5 to 43.0)
Extracellular water (%)	24.5 (23.8 to 25.2)	24.4 (23.7 to 25.1)	24.1§§†† (23.4 to 24.8)	24.1§§†† (23.4 to 24.8)	24.4 (23.7 to 25.1)	24.3 (23.6 to 25.0)	23.9§§†† (23.3 to 24.6)	24.0§§†† (23.4 to 24.7)
(L)	16.8 (15.8 to 17.9)	16.4§§ (15.4 to 17.5	16.4§§ (15.4 to 17.5)	16.6§† (15.6 to 17.7)	16.6 (15.6 to 17.6)	16.2§§ (15.3 to 17.2)	16.2§§ (15.3 to 17.2)	16.4§† (15.4 to 17.4)
P_{Osmol} (mOsmol/kg)	292 (288 to 296)	296 (291 to 301)	293 (290 to 296)	292† (289 to 296)	290 (287 to 293)	293 (289 to 296)	289 (285 to 292)	288† (284 to 291)
Δ P_V (%)	----	-4.8§§ (-7.6 to -1.9)	-1.1†† (-4.0 to 1.8)	-0.1†† (-2.3 to 2.2)	----	-2.3§§ (-3.7 to 0.9)	+3.7†† (1.5 to 6.0)	+3.6†† (0.2 to 7.0)
Cortisol (nmol/L)	349 (305 to 594)	461 (348 to 575)	296*$^{\#\#}$ (216 to 377)	220**$^{\#\#}$ (133 to 307)	449 (304 to 593)	716**^{aa} (544 to 888)	340$^{\#\#}$ (235 to 446)	236**$^{\#\#}$ (148 to 325)
I-FABP‡ (pg/ml)	616 (435 to 796)	1244§§ (862 to 1626)	----	----	494 (363 to 625)	848§§ (647 to 1049)	----	----
sCD14 (µg/ml)	2.21 (1.9 to 2.52)	2.05 (1.72 to 2.39)	----	----	2.42 (2.28 to 2.56)	2.53 (2.38 to 2.67)	----	----

LBP (µg/ml)	11.5 (7.9 to 15.2)	11.6 (8.5 to 14.8)	----	----	12.0 (0.7 to 15.3)	13.2 (9.1 to 17.3)	----	----
IL-1β (pg/ml)	3.8 (1.3 to 6.3)	4.0 (1.7 to 6.3)	4.0 (1.7 to 6.4)	4.7 (2.0 to 7.5)	2.8 (1.2 to 4.4)	2.8 (1.7 to 4.0)	2.6 (1.1 to 4.1)	3.1 (1.2 to 4.9)
TNF-α (pg/ml)	14.9 (10.5 to 19.3)	14.4 (10.3 to 18.5)	14.3 (9.8 to 18.9)	17.3 (10.5 to 24.0)	11.4 (8.8 to 14.1)	13.9 (10.2 to 17.6)	12.9 (9.7 to 16.1)	14.0 (8.5 to 19.5)
IL-6 (pg/ml)	37.3 (< 1.0[c] to 75.7)	40.1 (< 1.0[c] to 79.7)	41.3 (< 1.0[c] to 83.0)	34.8 (< 1.0[c] to 69.1)	35.7 (< 1.0[c] to 72.5)	33.9 (< 1.0[c] to 69.1)	34.8 (< 1.0[c] to 70.9)	33.4 (< 1.0[c] to 68.4)
IL-8 (pg/ml)	19.1 (3.4 to 34.8)	20.1 (4.2 to 35.9)	19.4 (3.2 to 35.7)	18.2 (4.5 to 31.9)	16.2 (1.5 to 30.9)	17.7 (4.2 to 31.2)	16.9 (2.2 to 31.6)	16.5 (2.0 to 31.0)
IL-10 (pg/ml)	25.5 (13.2 to 37.7)	34.2[§] (20.8 to 47.6)	23.0[†] (13.1 to 32.8)	24.1[†] (12.7 to 35.4)	18.2 (10.8 to 25.7)	34.7[§] (22.1 to 47.2)	17.3[†] (11.5 to 23.1)	14.8[†] (9.2 to 20.4)
IL-1rα (pg/ml)	37.0 (21.2 to 52.8)	45.7 (27.6 to 63.7)	52.9[§] (33.6 to 72.2)	44.2 (30.9 to 57.5)	37.7 (20.6 to 54.9)	39.8 (26.7 to 52.9)	44.4[§] (29.0 to 59.8)	44.0 (29.6 to 58.4)
Aldosterone (nmol/L)	96 (67 to 125)	292[§§] (213 to 371)	100[††] (79 to 121)	85[††] (61 to 110)	130 (92 to 168)	462[§§] (292 to 633)	132[††] (84 to 180)	111[††] (63 to 158)
Serum sodium (mmol/L)	140 (139 to 141)	134 (130 to 138)	138[†] (134 to 142)	139[††] (136 to 143)	139 (137 to 141)	135 (133 to 138)	145[†] (141 to 148)	143[††] (139 to 148)
Serum potassium (mmol/L)	4.2 (3.6 to 4.8)	4.2 (3.7 to 4.8)	4.1 (3.5 to 4.7)	4.3 (3.7 to 4.9)	4.6 (4.4 to 4.9)	4.3 (4.1 to 4.6)	4.6 (4.3 to 5.0)	4.4 (4.1 to 4.7)
Serum calcium (mmol/L)	2.34 (2.31 to 2.37)	2.17[§§] (2.10 to 2.23)	2.31[††] (2.24 to 2.39)	2.32[††] (2.26 to 2.37)	2.32 (2.29 to 2.35)	2.21[§§] (2.17 to 2.25)	2.38[††] (2.32 to 2.43)	2.38[††] (2.29 to 2.46)

Mean (95% CI) (n= 17); MEOTime †† P< 0.01 and † P< 0.05 *vs* post-exercise, MEOTime §§ P< 0.01 and § P< 0.05 *vs* pre-exercise, ** P< 0.01 and * P< 0.05 *vs* pre-exercise, [##] P< 0.01 and [#] P< 0.05 *vs* post-exercise, [aa] P< 0.01 and [a] P< 0.05 *vs* CM, [c] under detectable lowest standard.

Table 3.2 Incidence of gastrointestinal symptoms and severity of gut discomfort, total, upper-, and lower-gastrointestinal symptoms in response to 2 h HIIT exercise (between 60% and 80% $\dot{V}O_{2max}$) in temperate ambient conditions and after consumption of chocolate dairy milk (CM) and the carbohydrate-electrolyte (CEB) recovery beverages.

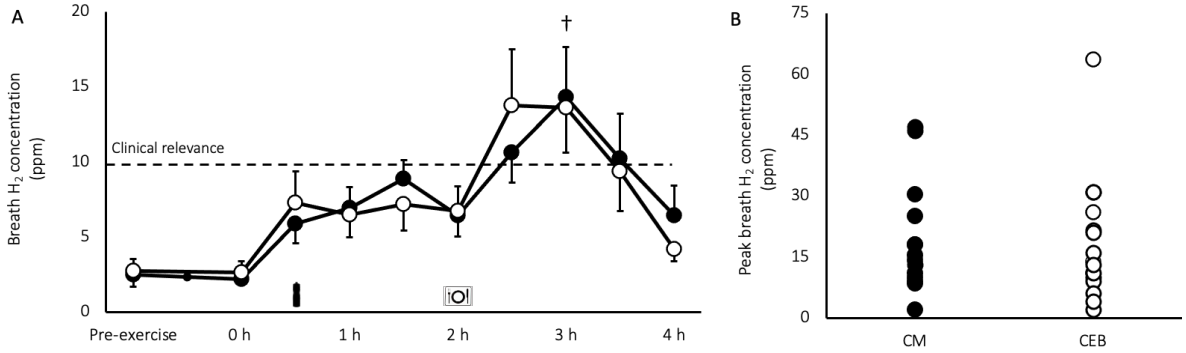
	CM					CEB				
	Exercise		Recovery			Exercise		Recovery		
	Incidence % (severe)	Severity	Incidence % (severe)	Severity Acute (1-2 h)	Severity Total (1-4 h)	Incidence % (severe)	Severity	Incidence % (severe)	Severity Acute 1-2 h	Severity Total (1-4 h)
Gut discomfort	NA	5 (0-13)	NA	5 (0-24)	14 (0-51)	NA	5 (0-18)	NA	4 (0-22)	11 (1-50)
Total GIS[a]	59 (0)	6 (4-18)	76 (47)	6 (3-24)	15 (3-51)	71 (12)	7 (2-31)	64 (35)	4 (1-22)	12 (1-50)
Upper GIS[b]	47 (0)	3 (3-10)	41 (29)	4 (10-24)	11 (2-51)	35 (0)	2 (1-11)	52 (24)	3 (1-22)	8 (2-50)
Belching	35 (0)	3 (3-10)	18 (0)	0 (1-1)	0 (1-2)	29 (0)	2 (1-11)	18 (0)	0 ----	1 (2-4)
Heartburn	6 (0)	0 (3-3)	6 (0)	0 ----	0 (4-4)	0 (0)	0 ----	0 (0)	0 ----	0 ----
Bloating	12 (0)	0 (2-4)	35 (29)	4 (10-24)	10 (2-51)	12 (0)	0 (1-3)	41 (24)	3 (1-22)	7 (3-50)
Stomach pain	0 (0)	0 ----	0 (0)	0 ----	0 ----	0 (0)	0 ----	0 (0)	0 ----	0 ----
Urge to regurgitate	6 (0)	0 (1-1)	0 (0)	0 ----	0 ----	0 (0)	0 ----	0 (0)	0 ----	0 ----

Regurgitation	0 (0)	0 ----	0 (0)	0 ----	0 ----	0 (0)	0 ----	0 (0)	0 ----	0 ----
Lower GIS[b]	24 (0)	1 (1-6)	42 (18)	1 (3-8)	3 (3-20)	18 (0)	1 (7-13)	35 (12)	1 (5-10)	3 (1-25)
Flatulence	12 (0)	0 (1-3)	12 (0)	0 ----	0 (3-5)	18 (0)	1 (7-13)	24 (0)	0 ----	1 (1-7)
Lower bloating	6 (0)	0 (2-2)	18 (0)	0 ----	1 (2-11)	6 (0)	0 ----	6 (6)	0 ----	0 (8-8)
Urge to defecate	12 (0)	0 (1-3)	24 (0)	0 ----	2 (2-12)	0 (0)	0 ----	12 (6)	1 (5-10)	1 (5-10)
Intestinal pain	0 (0)	0 ----	0 (0)	0 ----	0 ----	0 (0)	0 ----	0 (0)	0 ----	0 ----
Abnormal defecation[c]	0 (0)	0 ----	6 (6)	0 (6-6)	0 ----	0 (0)	0 ----	6 (6)	0 ----	0 (10-10)
Others										
Nausea	12 (0)	1 (1-11)	6 (0)	0 (5-5)	0 (5-5)	24 (6)	1 (1-12)	12 (0)	1 (4-7)	1 (4-7)
Dizziness	29 (0)	2 (2-12)	24 (0)	1 (1-7)	1 (1-7)	29 (6)	1 (1-11)	18 (0)	0 (1-3)	0 (1-3)
Stitch[d]	6 (0)	0 (1-1)	0 (0)	0 ----	0 ----	6 (0)	0 (4-4)	0 (0)	0 ----	0 ----
Feeding tolerance										

Appetite	NA	13 (0-39)	NA	21 (3-35)	26 (5-43)	NA	14 (0-38)	NA	25 (8-43)	32[a] (9-58)
Thirst	NA	23 (0-44)	NA	16 (4-26)	21 (6-37)	NA	23 (0-41)	NA	19 (8-37)	26 (14-51)

Values are presented as means and range of participant reporting GIS incidence (*n* = 17). GIS incidence during exercise and recovery are presented as percentage of total participants reporting GIS ≥1 on the mVAS. GIS severity during exercise and recovery are presented as mean summative accumulation of mVAS rating scale of measured time periods and individual range of participant reporting GIS incidence (162,212). Summative accumulation of upper-, lower-, and other gastrointestinal symptoms,[a] summative accumulation of upper- or lower- gastrointestinal symptoms,[b] abnormal defecation including loose watery stools, diarrhoea and blood in stools,[c] and acute transient abdominal pain.[d] NA: not applicable. Wilcoxon signed-rank tests showed no differences between CM and CEB for GIS; however [a] $P < 0.05$ *vs* CM for appetite. Hedge's *g* measurement of effect size for GIS and feeding tolerance severity between CM and CEB was determined as >0.50 and >0.80 for medium and large effects, respectively; however, no medium or large effects size value were detected between CM and CEB.

Figure 3.2 Breath hydrogen response (A) and individual peak breath hydrogen (B) after 2 h HIIT exercise in temperate ambient conditions and consumption of a chocolate dairy milk (CM: ●) or the carbohydrate-electrolyte (CEB: ○) recovery beverage. Mean ± SEM (n= 17).

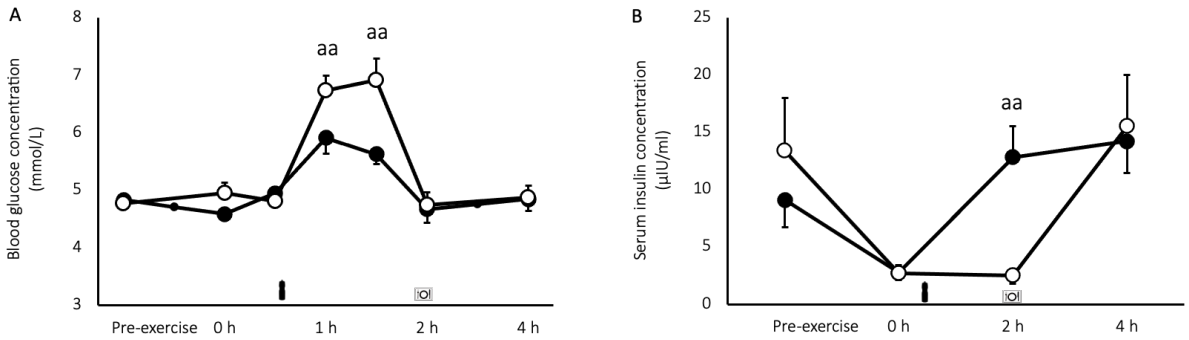


MEOTime † P< 0.05 *vs* post-exercise.

Glucose availability and insulin response

A trial*time interaction was observed for blood glucose (P= 0.001; **Figure 3.3A**) and serum insulin responses (P= 0.007; **Figure 3.3B**) imposed by feeding in the recovery period. Peak blood glucose concentration (P= 0.001) and area under the curve (P= 0.001) were greater during the 2 h after consumption of CEB (7.5 (6.9 to 8.0) mmol/L and 700 (665 to 735) mmol/L/2 h, respectively) compared with CM (6.3 (5.8 to 6.7) mmol/L and 634 (601 to 667) mmol/L/2 h, respectively). Insulin levels were significantly greater at 2 h recovery following consumption of CM compared with CEB (P< 0.01).

Figure 3.3 Blood glucose (A) and serum insulin (B) concentrations after 2 h HIIT exercise protocol in temperate ambient conditions and consumption of a chocolate dairy milk (CM: ●) or the carbohydrate-electrolyte (CEB: ○) recovery beverage.



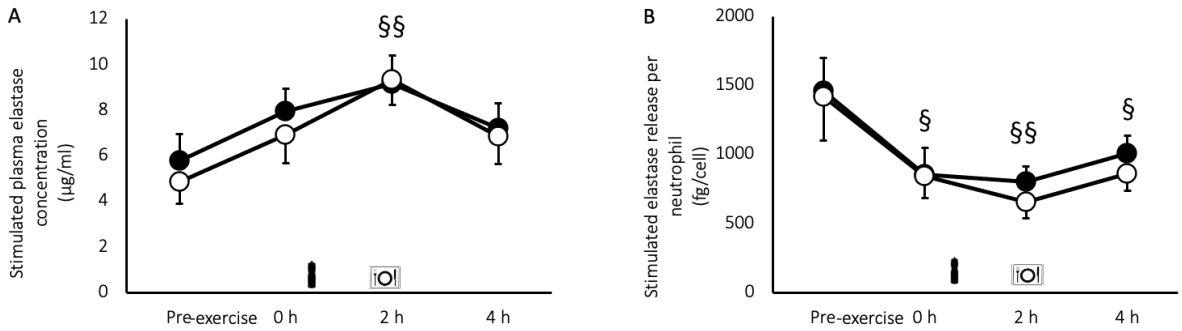
Mean ± SEM (*n*= 17): MEOTime †† P< 0.01 *vs* post-exercise, [aa] P< 0.01 *vs* CM.

Immune responses

An exercise-induced leukocytosis (10.5 (9.6 to 11.4) x10^9/L; P< 0.001), neutrophilia (7.4 (6.5 to 8.2) x10^9/L; P< 0.001), lymphocytosis (2.9 (2.5 to 3.4) x10^9/L; P< 0.001), monocytosis (0.6 (0.6 to 0.7) x10^9/L;

P< 0.001), and increased neutrophil:lymphocyte ratio (3.3 (2.9 to 3.8); P< 0.001) were observed in the recovery period on both trials. A MEOTime was observed for total bacterially-stimulated plasma elastase concentration (P= 0.003; **Figure 3.4**A), and bacterially-stimulated elastase release per neutrophil (P= 0.015 **Figure 3.4**B). Whereby, bacterially-stimulated total elastase release increased (71%) during recovery, peaking at 2 h post-exercise in both trials; while relative per neutrophil elastase release values decreased (49%), toughing at 2 h post-exercise in both trials. No main effects or interaction were observed for unstimulated plasma elastase concentration (146 (124 to 170) ng/ml). A MEOTime was observed for plasma IL-10 (P= 0.006) and IL-1ra (P= 0.016) concentrations; whereby the exercise protocol induced compensatory anti-inflammatory responses in recovery. No main effects or interaction were observed for plasma IL-1β, TNF-α, IL-6, and IL-8 concentrations. No difference in exercise-induced SIR-profile (CM: 45 (26 to 64) arb.unit and CEB: 40 (22 to 58) arb.unit) and recovery beverage post-prandial SIR-profile (CM: 11 (-9 to 30) arb.unit and CEB: -1 (-21 to 18) arb.unit) were observed.

Figure 3.4 Total (A) and per cell (B) bacterially-stimulated neutrophil elastase release after 2 h HIIT exercise in temperate ambient conditions and consumption of a chocolate dairy milk (CM: ●) or the carbohydrate-electrolyte (CEB: ○) recovery beverage.



Mean ± SEM (n= 14): MEOTime §§ P< 0.01 and § P< 0.05 *vs* pre-exercise.

Muscle glycogen

Post-exercise muscle glycogen content was 262 (230 to 294) mmol/kg dw (**Figure 3.5**A). The early rate of muscle glycogen formation did not differ between trials (-30.3 (-39.4 to -21.2) mmol/kg dw/h); however muscle glycogen concentrations were higher on CM (P< 0.001). No main effects or interaction were observed for the ratio of phosphorylated GSK-3β to total GSK-3β; however the fold change was

significantly greater on CM (1.3 (0.9 to 1.6)) compared with CEB (0.8 (0.6 to 0.9) (P= 0.037) (**Figure 3.5B).**

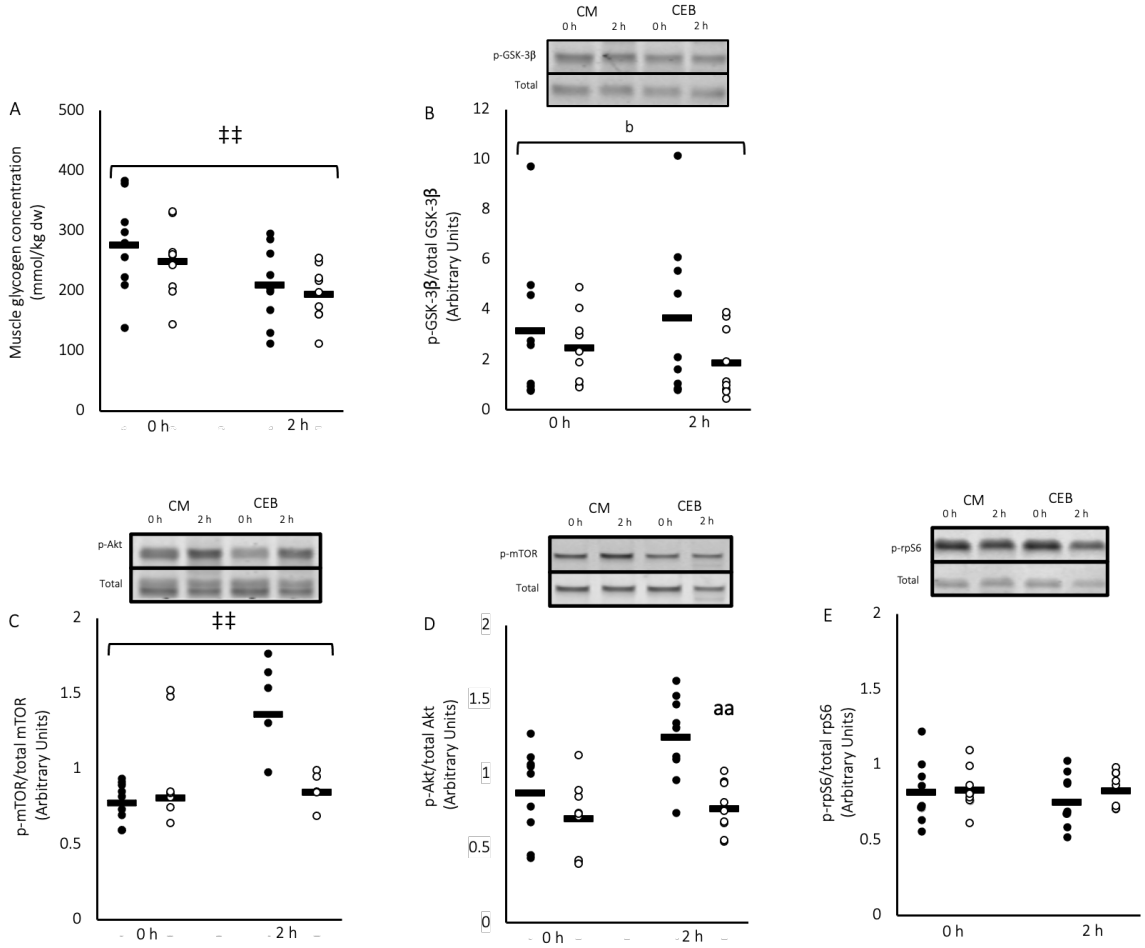
Phosphorylation of muscle signalling proteins

The ratio of phosphorylated mTOR to total mTOR (p-mTOR/TOTAL mTOR) increased after consumption of CM, but not after consumption of CEB (time*trial, P< 0.001) (**Figure 3.5C)**. The overall ratio of phosphorylated Akt to total Akt (p-Akt/Akt) was higher on CM (P< 0.001); however there was no MEOTime and the magnitude of change from 0 h to 2 h recovery did not differ between recovery beverages (**Figure 3.5D**). No main effects or interaction were observed for phosphorylation of rpS6 (**Figure 3.5E).**

Hydration and plasma electrolyte status

There was a MEOTime imposed by the rehydration intervention for P_{Osmol} (P= 0.004), P_V (P< 0.001), rating of thirst (P< 0.001), and TBW (P< 0.001) including extracellular (P< 0.001) and intracellular (P< 0.001) water, with values returning to near baseline values 2 h into the recovery period (**Table 3.1**). P_V was higher in the recovery period on CEB compared with CM (P= 0.015; **Table 3.1**). This was in adjunct with total urine output being higher (P= 0.028) and total fluid retention being lower (P= 0.040) on CEB (393 (286 to 500) ml and 80.2 (75.9 to 84.5) %, respectively) compared with CM (279 (217 to 341) ml and 85.8 (82.5 to 89.0) %, respectively). A MEOTime (P< 0.001) was observed for plasma aldosterone concentration; whereby levels increase in response to exercise and reduced back to baseline during recovery, with no differences associated with consumption of the different recovery beverages (**Table 3.1**). Total fluid intake during the recovery period (CM: 22.9 (20.7 to 25.1) ml/kg BM and CEB: 22.1 (19.6 to 24.6) ml/kg BM) did not differ between trials. A MEOTime was observed for plasma sodium (P= 0.001) and calcium (P= 0.001) concentrations (**Table 3.1**). Plasma calcium concentration decreased in response to exercise (P< 0.01). Both plasma calcium and sodium increased from post-exercise to 2 h and 4 h recovery, with no differences associated with consumption of the different recovery beverages. No main effects or interaction were observed for plasma potassium concentration (**Table 3.1**).

Figure 3.5 Muscle glycogen (A), ratio of phosphorylation of GSK-3β to total GSK-3β (B), ratio of phosphorylated mTOR to total mTOR (C), ratio of phosphorylated Akt to total Akt (D), and ratio of phosphorylated rpS6 to total rpS6 (E) after 2 h HIIT exercise in temperate ambient conditions and consumption of a chocolate dairy milk (CM: ●) or the carbohydrate-electrolyte (CEB: ○) recovery beverage.



Mean and individual responses (n= 9): MEOTrial ‡‡ P< 0.01, [b]P< 0.05 *vs* CM in magnitude, [aa] P< 0.01 *vs* CM

Psychophysiological parameters & performance outcomes

Participants reported greater readiness of invest mental (P= 0.006) and physical effort (P= 0.001) to perform following consumption of CM (6 (5 to 6) and 5 (4 to 6), respectively) compared to CEB (5 (4 to 6) and 4 (3 to 5), respectively). A greater decrease in physical readiness to perform was observed on CEB pre- to post-performance testing (P= 0.014). There were no differences between treatments in pre-exercise nude BM (69.3 (65.8 to 72.8) kg) or TBW (60 (58 to 61) %) the morning after the exercise trial. There were no trial differences in carbohydrate and fat oxidation rates or physiological parameters during the incremental test (Table 3.3). Mean HR (165 (163 to 168) bpm), RPE (15 (15 to 16)) and water intake (430 (328 to 532) ml) did not differ across the distance test between trials. Total distance run over 1 h did not differ between CM (12 (11.2 to 12.9) km) and CEB (11.9 (10.9 to 12.9) km).

Table 3.3 Physiological and performance outcomes graded intensity breath-by-breath testing at 50-80% $\dot{V}O_{2max}$ and 1 h self-paced distance test, following consumption of chocolate dairy milk (CM) and the carbohydrate-electrolyte (CEB) recovery beverages the previous day.

	CM				CEB			
	50% $\dot{V}O_{2max}$	60% $\dot{V}O_{2max}$	70% $\dot{V}O_{2max}$	80% $\dot{V}O_{2max}$	50% $\dot{V}O_{2max}$	60% $\dot{V}O_{2max}$	70% $\dot{V}O_{2max}$	80% $\dot{V}O_{2max}$
RER	0.88 (0.86 to 0.90)	0.91 (0.89 to 0.93)	0.93 (0.91 to 0.94)	0.97 (0.96 to 0.99)	0.88 (0.85 to 0.90)	0.91 (0.88 to 0.94)	0.93 (0.90 to 0.96)	0.97 (0.95 to 0.99)
$\dot{V}O_2$ (ml/kg BM/min)	28.3 (25.6 to 30.9)	33.7 (30.5 to 36.9)	41.2 (38.3 to 44.1)	47.5 (44.2 to 50.8)	27.1 (25.0 to 29.3)	32.8 (30.2 to 35.5)	39.9 (37.3 to 42.6)	46.4 (43.5 to 49.4)
Carbohydrate oxidation (g/min)	1.62 (1.31 to 1.93)	2.22 (1.91 to 2.53)	2.93 (2.57 to 3.29)	4.09 (3.67 to 4.52)	1.54 (1.22 to 1.85)	2.18 (1.77 to 2.59)	2.94 (2.45 to 3.43)	3.99 (3.43 to 4.54)
Fat oxidation (g/min)	0.38 (0.31 to 0.45)	0.34 (0.26 to 0.41)	0.33 (0.27 to 0.40)	0.14 (0.08 to 0.20)	0.38 (0.29 to 0.46)	0.33 (0.22 to 0.45)	0.31 (0.20 to 0.43)	0.15 (0.08 to 0.22)
HR	116 (110 to 122)	133 (124 to 141)	150 142 to 158)	165 (158 to 172)	117 (112 to 123)	133 (124 to 141)	150 (144 to 157)	164 (158 to 171)
RPE	8 (8 to 9)	10 (9 to 11)	12 (12 to 13)	15 (14 to 16)	8 (8 to 9)	10 (9 to 11)	12 (12 to 13)	15 (14 to 15)

Mean (95% CI) (n= 14). RER: respiratory exchange ratio, CHO: carbohydrate, HR: heart rate, RPE: rate of perceived exertion.

Discussion

The current study aimed to investigate the impact of a carbohydrate- and protein-containing flavoured dairy beverage and a non-nitrogenous carbohydrate electrolyte beverage on overall and integrative markers of acute recovery (i.e., EIGS, immune function, muscle glycogen resynthesis, protein synthesis, rehydration, and subsequent performance outcomes the following day), after an exercise stress model known to perturb aspects of physiological and metabolic homeostasis. Carbohydrate malabsorption and GIS was evident during the recovery period on both CM and CEB. A more rapid rate of glucose availability and corresponding acute insulin response were observed on CEB, compared with CM. Post-exercise leukocyte trafficking, depressed neutrophil function in response to bacterial challenge, and modest systemic inflammatory responses were not affected by the different recovery beverages. The greater carbohydrate content of CM did not result in greater muscle glycogen resynthesis in the acute timeframe, but did result in a greater increase in phosphorylation of GSK-3β compared with CEB. Greater phosphorylation of mTOR and absolute Akt protein signalling were observed on CM compared with CEB; but no differences in rpS6 signalling protein was observed between the recovery beverages. Despite lower sodium content, greater fluid retention was observed following consumption of the carbohydrate-protein CM beverage compared to the carbohydrate-only CEB beverage. Although subjects reported an enhanced readiness to invest in physical and mental effort on the following day with CM compared to CEB, this did not translate into differences in performance outcomes on a distance test. This was presumably due to the resumption of normal dietary habits after 2 h recovery and no overall differences in 24 h nutritional intake. These findings build on our current understanding of recovery nutrition to consider acute functionality and patency of the gastrointestinal tract, and restoration of immunocompetency. There were no differences in digestion, absorption, immunomodulation, glycogen resynthesis or performance outcomes between beverages, however the carbohydrate-protein composition of the CM beverage supported greater hydration and expression of cell signalling proteins regulating translation initiation.

Gastrointestinal response to recovery nutrition

It has recently been acknowledged that the epithelial injury, sympathetic drive, and associated GIS, as a result of EIGS, may compromise nutrient ingestion, digestion, and (or) absorption during the exercise recovery period (13,17,64,68,70,163). In the present study, comprehensive markers of gastrointestinal integrity (i.e., I-FABP, LBP, sCD14), function (i.e., breath H_2 concentration) and symptomology were assessed in response to the exercise stress and subsequent nutritional intervention. A substantial increase in post-exercise breath H_2, indicative of carbohydrate malabsorption of clinical significance (223), occurred on both CM and CEB, despite the vastly different nutritional profiles of the recovery beverages (i.e., sucrose, lactose and protein *vs* non-nitrogenous, sucrose and glucose) and evidence of heterogeneous exercise-associated intestinal epithelial injury (overall mean (95% CI): Δ I-FABP 491 (256 to 727) pg/ml) and stress hormone response (Δ cortisol 163 (39 to 288) nmol/L). Previous literature has observed similar peak breath H_2 values (i.e., 10-20 ppm) amongst healthy men and women following consumption of sucrose (<100 g) and lactose (50 g) at rest (224,225), suggesting a timing and bolus volume induced response. A subset of participants in the current study experienced peak breath H_2 values >20 ppm following consumption of one or both beverages. These data suggests a wide individual variability in gastrointestinal absorptive capacity of carbohydrate, possibly linked with: a) intestinal enterocyte carbohydrate transporter saturation capacity of carbohydrate; and (or) b) exercise-associated impairment of carbohydrate transporter translocation and activity effectiveness at the enterocyte brush border, secondary to increased enterocyte damage (i.e., circulatory-gastrointestinal pathway of EIGS) and (or) sympathetic activation (i.e., neuroendocrine-gastrointestinal pathway of EIGS) (11,163). Regarding assessment of protein malabsorption, previous research has suggested protein and amino acid provision immediately after exercise is not malabsorbed to any great extent, even with a wide range of protein provisions (e.g., 0.2 to 0.6 g/kg BM) (112,226). It was not possible within the current protocol to assess protein malabsorption using previously reported methodologies (e.g., -[1-^{13}C] phenylalanine labelled protein ingestion with continuous intravenous L-[ring-$^{2}H_5$] phenylalanine infusion) (70), as these would interfere with the accuracy and reliability of the primary

variables of the current study. Therefore, considering the previous research, reporting (70), and not reporting (112,226) impairment in protein absorption of recovery nutrition provisions, did not use an exercise stress model (e.g., resistance exercise, 90 min cycling at 60% W_{max}, and 60 min running at 70% $\dot{V}O_{2peak}$, all in temperate ambient conditions) sufficient to induce EIGS (64,163); it is still unknown the full impact of prolonged strenuous exercise on recovery beverage protein malabsorption, and whether any reduced protein bioavailability has any influence on cellular recovery responses.

Similar incidences and types of GIS were observed in recovery on CM (76%) and CEB (64%), with overall mild severity reaching a total varying range of 3-51 and 1-46, respectively. In addition, CM resulted in lower appetite only 4 h into recovery, likely attribute to the overall nutrient load (i.e., recovery beverage, plus recovery meal at 2 h post-exercise), inclusive of the protein dose. Previous studies have demonstrated transiently reduced appetite and feeding tolerance, and (or) increased fullness, with no greater gastrointestinal discomfort, after consumption of carbohydrate-protein versus carbohydrate and (or) non-nutritive beverages (64,130,176). Collectively, these results demonstrate that some mild discomfort associated with EIGS may be expected following consumption of both sucrose alone, and sucrose-lactose-protein containing beverages, even for individuals without lactose intolerance or dairy protein allergies. Findings from the current study suggest that individual tolerance (i.e., total volume, nutrient concentration, and intake dosage timing) and intestinal absorptive capacity are more likely determinants of carbohydrate malabsorption, than the broad recovery beverage composition *per se*. Nevertheless, based on the current findings, a pragmatic approach to professional practice would be to initiate recovery nutritional provisions immediately after exercise cessation, and dispense nutrition over a 30 min time frame, in small and frequent doses over the 1-2 h acute recovery time period, as to avoid gastric and intestinal nutrient overload and allow gastrointestinal patency to return as a result of EIGS; noting that habitual dietary patterns should return to normal 2 h into recovery.

Glucose availability to recovery nutrition

Glucose availability appears to be largely influenced by individual tolerance and metabolic status; whereby, appetite, GIS, intake content (i.e., nutrient, volume, and timing), and additional fuel requirements in the post-exercise period (e.g., excess post-exercise O_2 consumption for metabolic re-establishment, tissues repair, immune function, thermoregulation, then surplus for glycogen resynthesis) may influence the overall fuel replacement outcomes. Despite the lower carbohydrate content, CEB resulted in a greater peak and area under the curve (AUC) for blood glucose concentration over the 2 h period after beverage ingestion. It was unfortunate that the insulin response for CEB was not detected due to the sampling time points. Previous studies have reported peak serum insulin concentration to occur 15 to 30 min following consumption of 0.8 g/kg BM of glucose or sucrose, before returning to basal values at 2 h post-consumption (98,99). Whereas, the greater carbohydrate content of CM resulted in a lower blood glucose peak and AUC, likely due to the greater energy content and protein inclusion, known to slow gastrointestinal transit and intestinal absorption activity (227). It is possible that the greater overall energy content in addition to the slowed delivery into circulation resulted in a more sustained insulin response, aligned with previous investigations (95,116). Indeed, protein co-ingestion with carbohydrate has shown to induce a synergistic insulin response, greater than equivalent or lower energy intake of carbohydrate alone (92,97). These two distinct glucose availability trajectories suggest the gastrointestinal tract is a gateway regulatory factor for circulatory nutrient provisions to support the other elements of exercise recovery.

Immune responses to recovery nutrition

A single bout of prolonged strenuous exercise is proposed to promote acute innate immune altering properties (12,207). Restoration of immunocompetency seems necessary to prevent environmental and luminal derived pathogenic agents overriding the body's defences leading to increased risk of infection and (or) delayed recovery (11,18,61). In the current study, an exercise-induced leukocytosis, neutrophilia, and increased neutrophil:lymphocyte ratio was observed. The type of recovery beverage consumed did not influence leukocyte trafficking or prevent a decrease in neutrophil degranulation

(CM: -38% *vs* CEB: -56%). We have previously demonstrated that consumption of a recovery beverage of similar carbohydrate-protein composition, immediately after prolonged strenuous exercise (e.g., 2 h running at ≥70% $\dot{V}O_{2max}$ in temperate ambient conditions) prevented the exercise-associated reduction in *in vitro* bacterially challenged neutrophil functional responses (25,64,138). It is postulated that the 1 h delay in providing the recovery beverage in the current study abolished any immune-enhancing effects of the dairy milk beverage, as previously demonstrated with delayed versus immediate feeding, and provisions of water only (25,64,138). Given that phagocytic immune function is fundamental for clearance of luminal-derived pathogenic agents, and muscle tissue de- and regeneration (207), enhanced immune function associated with immediate provision of a nutritive recovery beverage may be supportive of broader aspects of acute recovery optimisation (Figure 3.4).

In the current study, there was no substantial systemic inflammatory cytokine response, except for the modest post-exercise increase in anti-inflammatory cytokines IL-10 and IL-1ra. These outcomes suggest, 2 h HIIT creates no to minimal consequence to systemic inflammatory status, and that anti-inflammatory cytokine markers (e.g., IL-10 and IL-1ra) appear to be more sensitive to exercise stress. Findings are consistent with previous research using similar exercise stress (209), and same experimental controlled procedures and conditions (13,14,205,210,212,228-230), whereby systemic inflammatory cytokine responses are characteristic of none to small increases in pro-inflammatory cytokine markers (i.e., TNF-α and IL-1β), none to modest increases in systemic response cytokine markers (i.e., IL-6 and IL-8), and modest to large increases in anti-inflammatory cytokine markers (i.e., IL-10 and IL-1ra). The translocation of bacterial endotoxins from the lumen into circulation is reported as a prime contributing factor to the systemic inflammatory response peaking during the exercise recovery period (11,198). Considering there were no substantial increases in plasma sCD14 or LBP concentrations (i.e., indirect markers for luminal translocated bacterial endotoxin), it is not surprising that systemic inflammatory responses were minimal. In addition, inflammatory cytokines were unaltered by the recovery beverage intervention. Previous reports suggest that certain foods have the

propensity to acutely alter systemic cytokine profile in the post-prandial period, however in weight stable individuals, specific foods have not been found to exert significant effects on overall cytokine profiles (231).

Muscle glycogen and recovery nutrition

The availability of stored skeletal muscle glycogen is a major determinant of prolonged exercise performance. A major finding of the current study was that consumption of both CM, with a nutritional composition equivalent to current recovery nutrition guidelines (6), and CEB (suboptimal carbohydrate and non-nitrogenous) failed to achieve a significant increase in muscle glycogen restoration in the acute recovery period (i.e., 2 h post-exercise, 90 min after consumption). This occurred despite the exercise protocol employed was in accordance with previous exercise-induced muscle glycogen depleting protocols (144), and post-exercise circulatory glucose and insulin responses that suggest delivery of carbohydrates into circulation and subsequent uptake into insulin-sensitive tissues. Likewise, these responses suggest that nutrient availability of both beverages was not limited by absorption at the level of the gastrointestinal tract. A heightened insulin response and associated phosphorylation of GSK3-β with carbohydrate-protein provisions on CM suggests enhanced cellular activity towards glycogen disposal. Our failure to detect acute changes in muscle glycogen concentrations as a result of recovery beverage consumption could likely be attributed to: 1) reduced glycogen storage capacity due to compromised skeletal muscle cell structural integrity, 2) reduced translocation of the GLUT-4 transporter to the plasma membrane, and (or) 3) reduced insulin sensitivity and (or) GLUT-4 effectiveness associated with damaged skeletal muscle plasma membrane sustained during eccentric plyometric muscle contractions (232). The current study did not collect samples to measure muscle damage biomarkers (e.g., CK or myoglobin) beyond the 4 h recovery post-exercise period, or to measure muscle glycogen restoration over the full recovery period. Therefore, a potential limitation within the current protocol is the inability to quantify muscle glycogen prior to or 24 h after exercise, and the extent to which muscle damage may have contributed to the impaired glycogen restoration or

the duration of the effect. Additionally, in hindsight, the current study could have integrated a practical measurement test on the 2nd day of testing, such as vertical jump on a force platform or a maximal voluntary contraction test (233), to assess the degree of exercise-induced muscle damage and implications on performance. It is also worth noting that the authors acknowledge the ethical issues, participant burden, and potential confounding factor of performing running HIIT with eccentric plyometric contractions and running performance test after a muscle biopsy sample. The effect of plyometric exercise induced muscle damage on muscle glycogen resynthesis, including the ideal nutrient intake and time course for repletion, warrants further investigation, given that current guidelines regarding dose and timing of carbohydrate intake may not be applicable to sports that involve HIIT interspersed with eccentric exercise (e.g., team sports and combat sports).

Muscle protein and recovery nutrition

The progression of skeletal muscle repair and adaptation following prolonged strenuous exercise, and nutrition support, has only recently begun to be investigated (153). Consistent with previous literature, we found that provision of 0.4 g/kg BM of protein in the CM beverage induced a greater insulinemic effect and enhanced upregulation of protein synthetic pathways, as indicated by greater phosphorylation of mTOR compared to the non-nitrogenous CEB (153). The superior effect of CM on insulin responses and skeletal muscle protein synthesis signalling pathways is likely due to the protein quality, specifically the leucine content, and overall greater carbohydrate content (37). Indeed, phosphorylation of the Akt-mTOR-rpS6 signalling pathway is preceded by nutrient- and contraction-induced activation of the insulin-IGF signalling pathway. Increased Akt-mTOR-S6K phosphorylation has been observed after resistance, endurance, and concurrent exercise protocols (42), with further enhancement associated with post-exercise protein intake (110,111,113). Nonetheless, mTOR has been identified as a key regulator of MPS via mRNA translation initiation (45). Therefore, greater phosphorylation of mTOR and Akt is suggestive of superior effects on anabolic signalling following consumption of the carbohydrate-protein CM beverage, compared to the non-nitrogenous CEB. A

noted limitation within the current study is the low rates of consent to the muscle biopsy procedure by female athletes, possibly due to the high burden and invasive nature of the procedure. Research investigating post-exercise nutritional strategies to maximise MPS is an emerging area of research, and to date has exclusively employed male athletes. Characterisation of sex-based differences in post-endurance exercise MPS and nutrient requirements warrants further investigation (234).

Hydration and recovery nutrition

Exercising in a hypohydrated state has been shown to increase physiological strain, and has potential to decrease physical and mental performance (13,51,235). Despite the prolonged and strenuous nature of the exercise protocol conducted in thermoneutral conditions with minimal water intake, a substantial degree of hypohydration was not observed on either trial. P_{Osmol} and TBW remained within range of euhydration throughout both experimental trials, and were similar to those previously reported after 2 h continuous running at 75% $\dot{V}O_{2max}$ (25,138). As such, these conditions are not conducive to assessing the overall efficacy of a rehydration beverage. Indeed, blood (i.e., P_{Osmol}, plasma electrolyte concentrations, plasma aldosterone), body water (i.e., BM change, TBW), and fluid intake tolerance (i.e., rating of thirst, fluid volume) markers of hydration applied within the current protocol failed to detect significant differences in fluid dynamics associated with each recovery beverage. However, significant differences were detected for urine output, fluid retention and ΔP_V, suggestive of greater osmotic potential exerted by the nutrient density of the CM beverage, beyond the greater sodium content of the CEB. It is theorised that the greater nutrient density on CM: 1) promoted a slowed gastrointestinal transit, 2) a more prolonged and distributed intestinal water absorption, 3) prevented acute circulatory hypervolaemia and subsequent diuresis, and (or) 4) supported circulatory water retention capacity considering no differences in plasma aldosterone concentration were observed. This theory is supported by the observed differences in blood glucose and insulin responses, indicating more rapid absorption of CEB, despite lower carbohydrate content and comparable malabsorption. Indeed, previous studies examining the hydration potential of carbohydrate-protein

beverages after <2% BM loss have reported enhanced fluid retention and (or) more positive fluid balance when intake of the recovery beverage is isovolumetric, and no other food or fluid is consumed (130,132,176). However, when intake is ad libitum and participants have access to food and water, this effect is no longer observed (174,175). These findings are of practical relevance to athletes, such that aggressive hydration strategies with nutritive beverages will have little to no influence on hydration markers when there is sufficient recovery time and access to meals and water (52). Moreover, researchers investigating hydration and fluid dynamics amongst athletes are encouraged to use multiple, validated methods, including blood, body water and feeding tolerance markers for comprehensive global assessment of intake, gastrointestinal transit, and systemic availability, retention and losses.

Practical translation

Many athletes purchase and consume commercial recovery beverages to support recovery outcomes. Flavoured dairy milk beverages in particular have gained popularity within the sporting community due to the close alignment with current recovery nutrition guidelines. Anecdotally, however, some resistance persists due to the belief that dairy products will induce greater gastrointestinal discomfort. Findings from the present study dispel this notion. Based on the presented findings and existing body of literature, athletes are advised to consume small and frequent doses of nutritional composition equivalent to 1.2 g carbohydrate and 0.4 g protein/kg BM over the 1-2 h acute recovery time period, and return to habitual dietary patterns thereafter (6,52,150). It is noted that the nutritional composition of the recovery beverage is inconsequential within the context of overall nutritional intake over a 24 h window, and does not influence subsequent performance 24 h after the initial exercise bout. However, the acute clinical (i.e., minimising gastrointestinal burden, stimulating neutrophil function towards clearance of tissue debris, endotoxins and luminal-derived pathogenic agents, reduced risk of illness and soft tissue injury) and physiological (i.e., maximised nutrient absorption and hydration, substrate provision for repletion of skeletal muscle glycogen, and substrate and anabolic stimulus for skeletal

muscle repair and adaptation) implications of immediate consumption of small and frequent boluses of a dairy milk beverage is likely to provide a cumulative advantage within an extended training regime. Finally, the exercise protocol applied within the present experimental design highlights practical implications for athletes participating in sports that involve HIIT interspersed with eccentric and (or) explosive efforts (i.e., football, soccer, basketball, tennis). The effect of plyometric and eccentric activity on muscle glycogen resynthesis, including the ideal nutrient intake and time course for repletion, warrants further investigation.

Conclusion

The current 2 h HIIT exercise protocol resulted in physiological disturbance (e.g., increased heart rate, RPE, rectal temperature, cortisol, and aldosterone), intestinal injury, GIS, leukocytosis, reduced bacterially-stimulated neutrophil function, modest systemic cytokinaemia (driven by anti-inflammatory cytokines IL-10 and IL-1ra), muscle glycogen depletion, and modest fluid losses without disturbances to plasma electrolyte status. Carbohydrate malabsorption and GIS was evident during the recovery period on both CM and CEB. A more rapid rate of glucose availability and corresponding lower acute insulin response was observed on CEB, compared with CM. Post-exercise leukocyte trafficking, depressed neutrophil function in response to bacterial challenge, and modest systemic inflammatory responses were not affected by the different recovery beverage composition. A greater fold increase in p-GSK-3β/total-GSK-3β was observed on CM compared with CEB; however neither beverage achieved net muscle glycogen re-storage in early recovery. The protein-containing CM resulted in greater phosphorylation of mTOR, but no effects of beverage consumption occurred for Akt or rpS6. Despite lower sodium content, greater fluid retention was observed following consumption of the carbohydrate-protein beverage (CM) compared to carbohydrate only (CEB); indicative of a lower acute P_V hypervolemia, and subsequently lower urine production and output. Physiological and performance outcomes the following day did not differ between trials. Recovery optimisation proposes that recovery aspects are not isolated systems, but rather interconnected; and appear to respond with individual

variation, to the nutrient composition of recovery nutrition. Nevertheless, these findings suggest that small, frequent doses of a flavoured dairy milk beverage is well-tolerated. The carbohydrate and protein composition of CM beverage supported greater hydration and expression of cell signalling proteins regulating translation initiation, suggesting greater overall acute recovery optimisation, however this did not translate to performance benefits the following day.

Contribution to the field

The current study is the first to comprehensively assess the impact of different recovery beverage compositions on overall recovery processes, initiating at the gastrointestinal tract, into systemic circulation, and circulatory to skeletal muscle end points. The concept of recovery optimisation proposes that individual aspects of exercise recovery (i.e., muscle glycogen resynthesis, skeletal muscle repair and adaptation, rehydration, restoration of immune function, and regulation of the gastrointestinal tract) are not isolated systems. Using isovolumetric intake of two commonly consumed exercise recovery beverages, 1) a carbohydrate-protein flavoured dairy milk beverage, and 2) a carbohydrate-electrolyte beverage, we have shown that the different nutritional compositions result in subtly different responses in fluid dynamics and intramuscular cellular signalling towards muscle glycogen disposal and muscle protein synthesis. Reduced gastrointestinal tract functionality and (or) possibly impaired muscle cell structural integrity in response to the 2 h HIIT, appear to promote carbohydrate malabsorption, and may have diminished complete glucose bioavailability and disposal of glycogen in the skeletal muscle, respectively, on both trials. Likewise, delayed intake of recovery nutrition resulted in failure to prevent a reduction in neutrophil functional responses. Despite anecdotal beliefs regarding the propensity for dairy products to induce gastrointestinal symptoms, there were no differences in carbohydrate malabsorption and gastrointestinal symptomatology between the chocolate dairy milk and carbohydrate-electrolyte beverage. Building on existing recovery nutrition guidelines, athletes are advised to initiate recovery nutritional provisions immediately after exercise cessation, in small and frequent doses over the 1-2 h acute recovery time period, and return to normal dietary patterns thereafter.

Chapter Four

Does the nutritional composition of a dairy-based recovery beverage influence post-exercise gastrointestinal and immune status, and subsequent markers of recovery optimisation in response to high intensity interval exercise?

Abstract

This study aimed to determine the effects of flavoured dairy milk-based recovery beverages of different nutrition compositions on markers of gastrointestinal and immune status, and subsequent recovery optimisation markers. After completing 2h high intensity interval running, participants (n=9) consumed a whole food dairy milk recovery beverage (CM, 1.2 g/kg body mass (BM) carbohydrate and 0.4g/kg BM protein) or a dairy milk-based supplement beverage (MBSB, 2.2g/kg BM carbohydrate and 0.8g/kg BM protein) in a randomised crossover design. Venous blood samples, body mass, body water, and breath samples were collected, and GIS were measured, pre- and post-exercise, and during recovery. Muscle biopsies were performed at 0h and 2h of recovery. The following morning, participants returned to the laboratory to assess performance outcomes. In the recovery period, carbohydrate malabsorption (breath H_2 peak: 49 ppm *vs* 24 ppm) occurred on MBSB compared to CM, with a trend towards greater gut discomfort. No difference in gastrointestinal integrity (i.e., I-FABP and sCD14) or immune response (i.e., circulating leukocyte trafficking, bacterially-stimulated neutrophil degranulation, and systemic inflammatory profile) markers were observed between CM and MBSB. Neither trial achieved a positive rate of muscle glycogen resynthesis (-25.8 (35.5) mmol/kg dw/h). Both trials increased phosphorylation of intramuscular signalling proteins. Greater fluid retention (total body water: 86.9% *vs* 81.9%) occurred on MBSB compared to CM. Performance outcomes did not differ between trials. The greater nutrient composition of MBSB induced greater gastrointestinal functional disturbance, did not prevent the post-exercise reduction in neutrophil function, and did not support greater overall acute recovery.

Introduction

Sports focused food and supplement products are commonly used by athletes within elite and recreational level endurance sporting communities (236). Current trends include the popularity of dairy based “whole foods” and “specially formulated supplement” beverages to promote exercise recovery. Flavoured whole food dairy milk beverages are considered a “gold standard” exercise recovery beverage, due to the close alignment of the quality and quantity of their nutrient composition with current exercise recovery nutrition guidelines and recommendations (6,237). However, reconstituted dairy milk-based supplement beverages are favoured by athletes, but generally contain higher energy and nutrient (e.g., carbohydrate and protein) content per volume due to their concentrated formulation (206). According to current exercise recovery nutrition guidelines and recommendations, the immediate post-exercise intake of 1.0 to 1.2 g/kg BM of carbohydrate and 0.2 to 0.4 g/kg BM of protein will support muscle glycogen resynthesis, muscle protein synthesis. In addition, these quantities of post-exercise carbohydrate and protein intake have been shown to support immune status in response to immunodepressive exercise (e.g., 2 h running at ≥70% $\dot{V}O_{2max}$) (13,64,138,139). For example, the consumption of a recovery beverage (i.e., supplementation or whole food source) providing 1.2 g/kg BM of carbohydrate with or without 0.4 g/kg BM of protein, immediately after exercise cessation, has consistently been shown to prevent the post-exercise reduction in bacterial endotoxin challenged (i.e., *E.coli* lipopolysaccharide) neutrophil function *in vitro* (25,64,138). Unlike other immune functional responses (e.g., *in vitro* lymphocyte proliferation and *in vivo* delay type hypersensitivity), bacterially-stimulated neutrophil degranulation (i.e., elastase concentration)has consistently been shown to response to recovery nutrition (25,64,25); and is potentially a fundamental immune function to assist recovery processes (e.g., systemic bacterial and bacterial endotoxin clearance, and soft tissue repair), and required to re-establish gastrointestinal-associated lymphoid structure and function that needed to support the bioavailability of ingested nutrients after exercise (11,18). Fluid intake equivalent to 125% to 150% of the exercise-induced BM loss is recommended for restoration of hydration status to pre-exercise levels, with sodium-containing fluids proposed to drive

thirst and promote extracellular fluid retention (52). In line with these recommendations, flavoured dairy milk beverages contain carbohydrate and protein in an approximate ratio of 3 to 4:1, and a naturally high sodium content (50 to 100 mg/100 ml) (20). A recent systematic literature review found that dairy based exercise recovery beverages may enhance recovery outcomes including muscle glycogen resynthesis, muscle protein synthesis, rehydration, and subsequent endurance exercise performance when compared to non-dairy recovery beverages (206). However, this review did not distinguish the nutritional composition (i.e., nutrient quantity and quality) of beverages, nor differentiate outcomes between the types of dairy products (e.g., dairy milk, dairy based sports beverages, and other variants); and the gastrointestinal tolerance (e.g., regulation of nutrient bioavailability) in response to gastrointestinal perturbing exercise (i.e., exercise-induced gastrointestinal syndrome) (11,163).

Commercially-analogous dairy based exercise recovery beverages exhibit a wide variability of nutritional compositions, sensory profiles, and price (20). For example, standard dairy milk, flavoured dairy milk, and dairy milk based supplementation (e.g., casein and (or) whey mixtures) beverages respectively increase in nutritional density (e.g., 0.6 to 2.4 g kg BM of carbohydrate and 0.2 to 0.8 g/kg BM protein) at an isovolumetric dose; but are equally promoted to act as exercise recovery agent in professional practice, despite nutritional availability may be under or over general recovery nutrition guideline and recommendations (6). Variability in recovery beverage processing (e.g., carbohydrate and protein type, fat and electrolyte content) and nutritive composition (e.g., load, quantity) alters the gastrointestinal transit, digestibility and assimilation of nutrients in whole dairy products compared to isolated nutrient supplements (e.g., whey protein or glucose powders) (238). To date, comparative studies on milk beverages for exercise recovery are generally limited to markers of hydration, GIS, and performance (132,136,176,180). These studies provide some evidence of enhanced fluid retention with a reconstituted dairy based exercise recovery beverage, compared to soy and regular dairy milks (i.e., bovine source) (176). No other differences in fluid retention, GIS, or performance have been observed

between differing dairy based exercise recovery beverages; while, other integrated markers of recovery optimisation (i.e., gastrointestinal integrity and function muscle, immune function and status, glycogen resynthesis, and muscle protein synthesis) are yet to be comprehensively explored.

Given the prevalent use of exercise recovery beverages amongst athletes, there has been encouragement by sport and exercise practitioners (e.g., sport or performance dietitians and nutritionists) for well controlled experimental trials to evaluate the efficacy of these products and develop evidenced based protocols for use (21). Flavoured dairy milk beverages and reconstituted dairy milk-based supplement beverages are the most commonly consumed beverages amongst athletic populations in the post-exercise recovery period, and appear to meet or over-provide post-exercise nutritional requirements (20,206). However, to date, the impact of this nutritional discrepancy provision has not been explore in regards to gastrointestinal and immune status, and subsequent impact on more traditional markers of exercise recovery nutrition (i.e., refuel, repair, and rehydration). With this in mind, the current study aimed to determine the effects of flavoured dairy milk recovery beverages of different energy and nutrient densities (i.e., standard *vs* high nutritional content), but same intake volume, on markers of gastrointestinal and immune status, and subsequent recovery optimisation markers (i.e., muscle glycogen resynthesis, protein synthesis expression, and hydration). It was hypothesised that the higher energy and nutrient density of a dairy milk-based supplement beverage will result in greater muscle glycogen resynthesis, muscle protein synthesis, fluid retention, and enhanced immune functional responses, compared with a standard nutrient content of the whole food dairy milk recovery beverage. In addition, the greater energy and nutrient density of the reconstituted dairy milk-based supplement beverage will result in greater ratings of feeding intolerance and GIS, compared with a standard nutrient content of the whole food dairy milk recovery beverage.

Methods

Participants

Nine amateur recreationally and competitively trained endurance athletes (n= 7 male, n= 2 female) (mean (SD) age 28 (4) years, nude BM 73.3 (14.1) kg, height 1.76 (0.12) m, % body fat 13.6 (7.4) %, $\dot{V}O_{2max}$ 53 (3) ml/kg BM/min, weekly training volume (447 (260) min, and training/competition modality: endurance running, ultra-endurance running, triathlon) volunteered to participate in the study. Participants responded to poster advertisements displayed on social media and in relevant sports clubs in Melbourne, Australia. All participants gave written informed consent. The current study was part of a larger project for which the protocol was prospectively registered with ANZCTR (reference number 375090) and participants were randomly allocated to respective experimental procedures using a computer sequence generator. The study received approval from the local ethics committee (MUHREC: 12799) and conformed to the Helsinki Declaration for Human Research Ethics. All participants confirmed the absence of illness, injury, or disease, including gastrointestinal infections, diseases and (or) disorders. Individuals were excluded if they reported the consumption of potential dietary modifiers of gastrointestinal integrity, were adhering to gastrointestinal-focused dietary regimens within the previous three months, or had consumed non-steroidal anti-inflammatory medications, antibiotic or stool altering medications within one month before the experimental protocol, or if they failed to achieve a $\dot{V}O_{2max}$ greater than 50 ml/kg BM/min.

Preliminary measures

Baseline measurements were recorded one to three weeks prior to the first experimental trial. Height (stadiometer, Holtain Limited, Crosswell, Crymych, United Kingdom) and BM (Seca 515 MBCA, Seca Group, Hamburg, Germany) were recorded. $\dot{V}O_{2max}$ (Vmax Encore Metabolic Cart, Carefusion, San Diego, California, US) was estimated using a continuous incremental exercise test to volitional exhaustion on a motorised treadmill (Forma Run 500, Technogym, Seattle, Washington, US), as previously reported (138). In short, the exercise test began with a treadmill speed of 6 km/h and 1% inclination. Speed was increased by 2 km/h every 3 min until reaching 16 km/h at which point inclination

was increased by 2.5% every 3 min until the participant reached volitional exhaustion and criteria for attaining $\dot{V}O_{2max}$ (i.e., heart rate, rating of perceived exertion, and respiratory exchange ratio). From the $\dot{V}O_2$–work rate relationship, the treadmill speed at 1% inclination corresponding to 50% (6.6 (0.6) km/h), 60% (7.8 (1.0) km/h), 70% (9.4 (1.1) km/h) and 80% (11.6 (1.3) km/h) $\dot{V}O_{2max}$ was extrapolated and verified.

Experimental protocol

Participants were required to consume a standardised diet in accordance with current nutrition guidelines for endurance athletes (6), and low in FODMAPs during the 24 h prior to, and throughout the experimental trials (3018 (1754) ml/day water, 10.6 (3.0) MJ/day, 101 (32) g protein/day, 64 (33) g fat/day, 364 (67) g carbohydrates/day, and 44 (10) g/day fibre). Diets were designed to provide <2 g FODMAPs per meal using a Monash University designed FODMAP specific database (FoodWorks Professional 7, Xyris, Brisbane, Australia) (162). Meal provisions were stratified according to BM, such that participants with greater body mass were provided with additional meal servings and (or) snacks. Participants were asked to refrain from consuming alcohol, and performing strenuous exercise during the 48 h before each experimental trial, and refrain from consuming caffeinated beverages during the 24 h before each experimental trial. Compliance to these instructions was checked via the completion of a food and exercise diary.

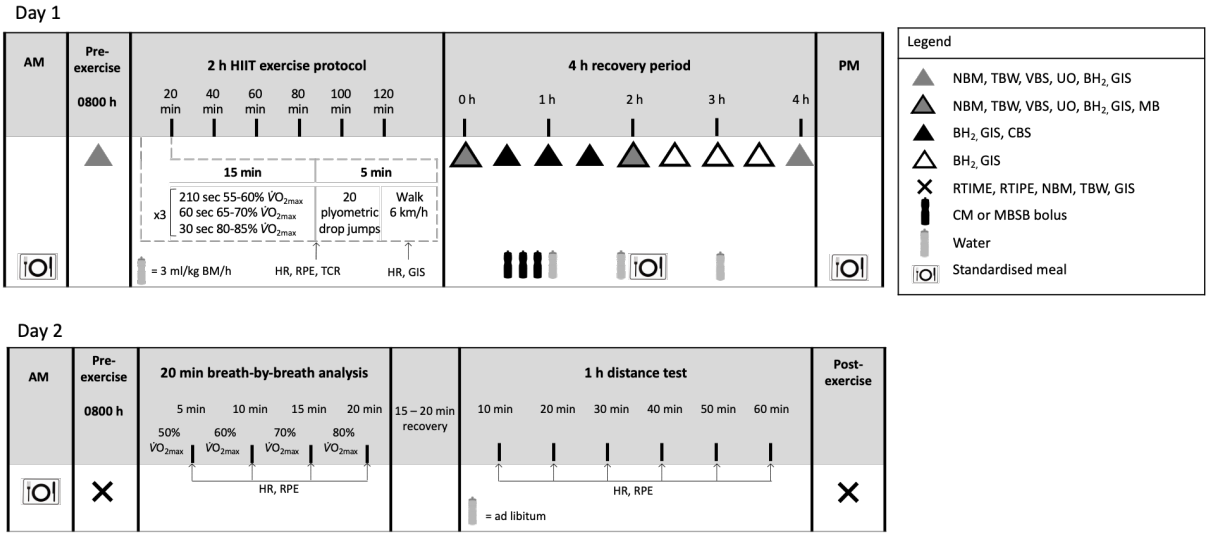
In a randomised order, participants completed two experimental trials separated with at least a 5 day washout period, to accommodate the participants' availability, and providing sufficient time to recovery primary and secondary variables to baseline (206). In the case of the female participants, resting estrogen levels (DKO003/RUO; DiaMetra, Italy) were measured for verification, and did not differ between trials (6.0 (3.0) pg/ml). Participants reported to the laboratory at 0800h after consuming the standardised low FODMAP mixed carbohydrate breakfast (245 (130) ml water, 2.9 (0.9) MJ, 25 (5) g protein, 22 (6) g fat, and 95 (34) g carbohydrates, and 11 (4) g fibre) (162). Before commencing exercise,

participants were asked to void, and pre-exercise nude BM and TBW (Seca 515 mBCA, Seca Group, Hamburg, Germany) were recorded. Participants inserted a thermocouple 12 cm beyond the external anal sphincter to record pre- and post-exercise rectal temperature (Precision Temperature 4600 Thermometer, Alpha Technics, California, USA). Participants provided a breath sample into a 250 ml breath collection bag (Wagner Analysen Technick, Bremen, Germany), and completed an exercise specific modified visual analogue scale GIS assessment tool (162). Blood was collected by venepuncture from an antecubital vein into three separate vacutainers (6 ml 1.5 IU/ml lithium heparin, 4 ml 1.6 mg/ml K_3EDTA, and 5 ml SST; BD, Oxford, UK).

The exercise protocol consisted of a 2 h (initiated at 0900h) HIIT session in 23.4 (0.7) °C ambient temperature and 42 (8) % relative humidity, as described in Figure 4.1. The protocol was designed to provide sufficient exercise stress to perturb key markers of recovery (13,14,64,206). During exercise, participants were provided with water equivalent to 3 ml/kg BM/h (25,138). Heart rate (Polar Electro, Kempele, Finland), rating of perceived exertion (213), and thermal comfort rating (214), were measured at the 15 min mark of each 20 min cycle, as previously described (14). Recovered heart rate and GIS were measured during the final 30 s of the 20 min cycle. Immediately post-exercise, nude BM and rectal temperature were recorded. The recovery period commenced 30 min after the end of the exercise protocol to prepare for muscle biopsy sampling. Participants rested in a supine position in a sterile phlebotomy room for venous blood sampling followed by the first muscle biopsy thereafter. Muscle biopsy samples were taken 0 h and 2 h into the recovery period. TBW was measured immediately after muscle biopsy sampling. Blood samples, nude BM and TBW were collected again at 2 h and 4 h of recovery. Breath samples were collected and GIS recorded every 30 min throughout the recovery period. Total urine output was collected throughout the total recovery period. Weight of urine output was recorded at 2 h and 4 h of recovery. After sampling at 2 h of recovery, participants received a standardised recovery meal (415 (103) ml water, 2.8 (0.7) MJ, 31 (8) g protein, 4 (2) g fat, and 137 (32) g carbohydrates, and 9 (2) g fibre), and were instructed to consume as much as tolerable. The total

weight of the meal consumed was recorded. In addition, participants consumed a standardised low FODMAP evening meal after leaving the laboratory (818 (860) ml water, 2.8 (1.3) MJ, 31 (21) g protein, 14 (12) g fat, and 95 (46) g carbohydrates, and 18 (7) g fibre).

Figure 4.1 Schematic illustration of experimental design.



NBM: nude body mass, TBW: total body water, VBS: venous blood sampling, UO: urine output, BH_2: breath hydrogen, GIS: gastrointestinal symptoms, HR: heart rate, TCR: thermal comfort rating, RPE: rating of perceived exertion, MB: muscle biopsy, CBS: capillary blood sampling, RTIME: readiness to invest mental effort, RTIPE: readiness to invest physical effort, RER: respiratory exchange ratio, CM: chocolate flavoured dairy milk, MBSB: milk-based sports beverage, BM: body mass.

Participants were informed and advised to follow standardised nocturnal habits, previously assessed and reported as a control variable to current physiological and performance markers measured in the current study (196,222,230,239,240). This included sleeping from ~2100-2200h to 0600h (~8-9 h sleep duration). However, objective data of sleep quantity and quality was not measured on this occasion. The following morning, participants returned to the laboratory (0800h) to assess psychophysiological parameters and exercise performance (~0900h). Due to unexpected unavailability, 1 participant did not return for the second day of testing. A standardised low FODMAP mixed carbohydrate breakfast (254 (319) ml water, 2.4 (1.3) MJ, 21 (10) g protein, 19 (9) g fat, and 77 (44) g carbohydrates, and 9 (5) g fibre) was consumed at 0700h. Nude BM, TBW and GIS were recorded on arrival and again after the performance test. Before and after the performance test, participants completed measures of readiness to invest mental and physical effort, rated from 0 to 10 (215). Participants completed a 20

min running exercise bout to measure oxygen uptake and oxidation rates at four submaximal exercise intensities (50%, 60%, 70%, and 80% $\dot{V}O_{2max}$) for 5 min each, before undertaking a 1 h performance test in 23.0 (1.3) °C ambient temperature and 46 (9) % relative humidity, using methods as previously reported (163,222). Participants were instructed to run the maximal distance they are capable of running in 1 h, with the incline set at 1%. During the distance test participants only had information about elapsed time. Total distance, heart rate, rate of perceived exertion, and water intake (provided *ad libitum*) were recorded every 10 min.

Muscle biopsy procedure

Muscle biopsies were performed using a modified 5 mm Bergstrom biopsy needle. Samples were obtained from the vastus lateralis of the ipsilateral leg for the first trial, and contralateral leg for the second. The skin of the lateral aspect of the mid-thigh was washed well (10% Povidone – Iodine solution) then 2 to 3 ml of local anaesthetic (lidocaine 1%) was infiltrated subcutaneously over vastus lateralis to anaesthetise the skin and superficial fascia. After the anaesthetic had taken effect, two 5 mm stab incisions ~15 mm apart were made through skin and fascia, with one incision made for each muscle biopsy sample. Samples were then extracted, immediately submerged in liquid nitrogen, and stored at -80°C prior to further analysis.

Recovery beverages

In a randomised, repeated measures design, participants were provided with 1) commercially available chocolate flavoured dairy milk (CM) or 2) commercially available chocolate flavoured dairy milk-based supplement beverage (MBSB). The commercially available dairy milk and dairy milk-based supplement beverages were kept confidential to comply with product anonymity ethical procedures. For practical relevance, and to primarily control variables from a gastrointestinal and immune recovery perspective, beverages were matched for volume (64,163,206). The beverages were prepared by a third party researcher, and served in opaque bottles, at ~7°C beverage temperature (216), in 3 equal boluses every 10 min, beginning 30 min into the recovery period. The volume of the beverage was calculated to

provide 1.2 g/kg BM of carbohydrate and 0.4 g/kg BM of protein on CM (2715 (514) kJ, 30 (6) g protein, 17 (3) g fat, and 92 (17) g carbohydrate). The MBSB was prepared by dissolving 30 g dry powder in 100 ml water in accordance with manufacturers' instructions, and volume matched to the CM trial (4029 (763) kJ, 63 (12) g protein, 2 (0) g fat, and 170 (32) g carbohydrates). Additional water calculated to provide a total fluid intake of 35 ml/kg BM was provided at hourly intervals. Participants were instructed to drink as much as tolerable. Total fluid intake was recorded hourly. The percentage of ingested fluid retained was calculated from the difference between ingested fluid and urine output, as a fraction of total fluid intake (128).

Sample analysis

Blood glucose concentration, hemoglobin and total and differential leukocyte counts, which included neutrophils, lymphocytes, and monocytes, were determined by HemoCue system (Glucose 201+, Hb201, and WBC DIFF, HemoCue AB, Ängelholm, Sweden) in duplicate from heparin whole blood samples. The CV for blood glucose concentration, hemoglobin, and leukocyte counts were 5.3%, 1.8% and 13.4%, respectively. Hematocrit was determined by capillary method in triplicate from heparin whole blood samples and using a microhaematocrit reader (CV: 0.5%) (ThermoFisher Scientific). Hemoglobin and hematocrit values were used to estimate changes in P_V relative to baseline, and used to correct plasma variables. To determine the blood glucose response to the recovery beverage, immediately before and every 30 min thereafter for 90 min, blood glucose concentration was measured in duplicate using a handheld system from capillary blood samples (CV: 3.7%) (Accu-Chek Proforma, Roche Diagnostics, Indianapolis, Indiana, USA). *In vitro* bacterially-stimulated elastase release was determined using previously described methods (13). The remaining whole blood in the heparin and K_3EDTA vacutainers were centrifuged at 4000 rpm (1500 g) for 10 min within 15 min of sample collection. The whole blood collected in the SST serum tube was allowed to clot for 1 h in ~4°C prior to centrifuging at 4000 rpm (1500 g) for 10 mins. 2 x 50 µl of heparin plasma was used to determine P_{Osmol}, in duplicate (CV: 2.5%), by freezepoint osmometry (Osmomat 030, Gonotec, Berlin, Germany). The

remaining heparin, K_3EDTA and serum plasma was aspirated into the appropriate 1.5 ml micro-storage tubes and frozen at -80°C until analysis. Circulating concentrations of insulin (DKO076; DiaMetra, ItalyRE53171; IBL International, Hamburg, Germany), cortisol (DKO001; DiaMetra, ItalyRE52061; IBL International, Hamburg, Germany), aldosterone (Demeditec Diagnostics GmbH, Kiel, Germany), PMN elastase (BMS269; Affymetrix EBioscience, Vienna, Austria), I-FABP (HK406; Hycult Biotech, Uden, The Netherlands), and sCD14 (HK320; Hycult Biotech), were determined by ELISA. Additionally, systemic cytokine profile (including plasma IL-6, IL-1β, TNF-α, IL-8, IL-10, and IL-1ra concentrations) (HCYTMAG-60K, EMD Millipore, Darmstadt, Germany) were determined by multiplex system. All variables were analysed as per manufacturer's instructions on the same day, with standards and controls on each plate, and each participant assayed on the same plate. The CVs for ELISAs were ≤6.1% and for cytokine profile multiplex was 16.0%. Breath samples (20 ml) were analysed in duplicate (CV: 2.1%) for H_2 content using a gas-sensitive analyser (Breathtracker Digital Microlyzer, Quintron, Milwaukee, Wisconsin, US). Plasma sodium, potassium and calcium concentrations were determined using ion selective electrodes (Cobas c analyser, Roche Diagnostics, Risch-Rotkreuz, Switzerland) and analysed by local pathology services (Cabrini Pathology, Malvern, Victoria, Australia).

Western blot analysis

Approximately 30 mg of skeletal muscle was solubilized in radioimmunoprecipitation buffer (Millipore, Bayswater, Victoria, Australia) with 1 µL/ml protease inhibitor cocktail (Sigma-Aldrich, Castle Hill, New South Wales, Australia) and 10 µl/ml Halt Phosphatase Inhibitor Single-Use Cocktail (Thermo Scientific, Australia, North Ryde, New South Wales, Australia). The concentration of protein per sample was determined by the bicinchoninic acid assay (BCA Protein Assay Kit#23225, Thermo Scientific). 20 µg of skeletal muscle protein lysate was loaded onto into either Bio-Rad precast Criterion TGX Stain-Free 4 to 12% gels (Bio-Rad, Gladesville, New South Wales, Australia). SDS-PAGE was conducted following manufacturer's instructions. Protein was then transferred to PVDF membranes and blocked for 1 h in 5% BSA solution in TBST, (pH 7.6, 20 mmol/L Tris and 150 mmol/L NaCl, 0.1% Tween) at room

temperature. Membranes were then incubated in primary antibodies diluted in 5% BSA/TBST overnight at 4°C. Following washing in TBST, membranes were incubated for 1 h with fluorescent secondary antibodies (mTOR^{Ser2448}, Akt^{Ser473}, rpS6$^{Ser235/236}$, and GSK-3β^{Ser9}) (Anti-Rabbit IgG (H+L) DylightTM 800 Conjugate; Anti-mouse IgG (H+L) DylightTM 680 Conjugate) (Cell Signalling Technologies®, Danvers, Massachusetts, USA) diluted 1:10,000 in TBST. Following 2 further washes in TBST and 1 wash in phosphate buffered saline (PBS) membranes were scanned using the LiCOR® Odyssey CLx® Imaging System (Millennium Science, Mulgrave, Victoria, Australia). All targets were normalized to total protein using either the Bio-Rad stain-free system.

Muscle glycogen analysis

One fraction of muscle sample (20 to 25 mg wet weight) was freeze-dried, after which collagen, blood and other non-muscle material were removed from the muscle fibres. Samples were then pulverized and powdered. Samples were extracted with 0.5 M perchloric acid ($HClO_4$) containing 1 nM EDTA and neutralised using 2.2 M $KHCO_3$. Adenosine triphosphate, phosphocreatine, and creatine was determined from the supernatant by enzymatic spectrophotometric assays (218,219). Muscle glycogen content was determined from 2 aliquots of freeze-dried muscle (2–3 mg) as previously reported (218).

Statistical analysis

Confirmation of adequate statistical power was determined a priori from the applied statistical test, mean, standard deviation, and effect size on markers of 1) gastrointestinal integrity (i.e., plasma I-FABP), function (i.e., breath hydrogen), and GIS (14,13,163,210) ; 2) circulating leukocyte, endotoxin and cytokine profiles (13,25,64,138); 3) total body water, plasma osmolality, plasma volume change (13,64); 4) rate of skeletal muscle glycogen resynthesis (7,74); 5) phosphorylation of intramuscular signalling proteins (111,220); and 6) performance (222,221). Using a standard alpha (0.05) and beta value (0.80), the current participant sample size, within a repeated measures cross-over design, is estimated to provide adequate statistical power (power* 0.80-0.99) for detecting significant between- (trial) and within- (time) group differences (G*Power 3.1, Kiel, Germany). Data in the text and tables

are presented as mean (SD) for descriptive method, and mean and 95% CI for primary variable, as indicated. For clarity, data in figures are presented as mean and SEM, and (or) mean and individual responses, as indicated. Systemic inflammatory cytokine responses are presented as raw values and SIR-profile, as previously reported (208). Both male and female participants with full data sets within each specific variable were used in the data analysis, which is in accordance previously literature demonstrating similar variable outcomes between biological sexes (15,106,129). There were no outliers for female participant data points for any of the primary and secondary outcome measures. All data were checked for normal distribution (Shapiro-Wilks) prior to comparative analysis. Variables with singular data points were examined using paired sample t-tests, or non-parametric Wilcoxon signed-rank test, when appropriate. Variables with multiple data points were examined using a two-way repeated-measures ANOVA. Assumptions of homogeneity and sphericity were checked, and when appropriate adjustments to the degrees of freedom were made using the Greenhouse-Geisser correction method. Main effects were analysed by Tukey's post hoc HSD. Statistics were analysed using SPSS statistical software (V.26.0, Chicago, Illinois, USA) with significance accepted at $P \leq 0.05$.

Results

Physiological strain

A main effect of time (MEOTime) was observed for recovered heart rate (overall mean and 95% CI: 129 (125 to 132) bpm; P= 0.006) and peak (163 (160 to 166) bpm; P= 0.080), rate of perceived exertion (13 (12 to 13); P= 0.001), TCR (9 (9); P< 0.001) and core body temperature (P< 0.001), characterised by an increased physiological strain as the exercise stress progressed. A MEOTime occurred for plasma cortisol concentrations, such that values were significantly lower 4 h into recovery compared to the beginning of the recovery period (P< 0.05), associated with normal daily circadian variation (**Table 4.1**).

Table 4.1 Change in hydration and biomarkers in response to 2 h HIIT exercise (between 60% and 80% $\dot{V}O_{2max}$) in temperate ambient conditions and after consumption of chocolate dairy milk beverage (CM) and the milk-based sports beverage (MBSB).

	CM				MBSB			
	Pre-exercise	0 h recovery	2 h recovery	4 h recovery	Pre-exercise	0 h recovery	2 h recovery	4 h recovery
Total body water (%)	60.7 (58.4 to 63.0)	61.6 (59.3 to 63.9)	60.3† (58.5 to 62.1)	60.7† (58.6 to 62.7)	60.2# (57.9 to 62.6)	61.6 (59.1 to 64.0)	60.4† (58.0 to 62.8)	60.7† (58.5 to 62.9)
(L)	44.5 (39.3 to 49.7)	44.2 (39.1 to 49.4)	44.1 (38.8 to 49.4)	44.8 (39.5 to 50.1)	44.2 (39.2 to 49.2)	44.3 (39.1 to 49.5)	44.3 (39.1 to 49.6)	44.9 (39.7 to 50.0)
Extracellular (%)	24.4 (23.4 to 25.3)	24.2 (23.3 to 25.0)	24.0 (23.1 to 24.9)	24.1 (23.1 to 25.0)	24.1 (23.1 to 25.1)	24.1 (23.1 to 25.1)	23.9 (23.0 to 24.9)	24.0 (23.0 to 25.0)
(L)	17.8 (15.9 to 19.7)	17.3 (15.4 to 19.3)	17.5 (15.5 to 19.5)	17.7 (15.8 to 19.6)	17.6 (15.7 to 19.5)	17.3 (15.3 to 19.2)	17.5 (15.6 to 19.5)	17.7 (15.8 to 19.6)
Plasma osmolality (mOsmol/kg)	295 (289 to 301)	293 (286 to 300)	294 (289 to 299)	294 (288 to 300)	290 (286 to 295)	291 (285 to 297)	292 (287 to 298)	292 (285 to 298)
Δ P_V (%)	----	0.8 (-1.8 to 3.4)	3.5 (-2.6 to 9.5)	1.8 (-1.9 to 5.5)	----	0.9 (-3.7 to 5.5)	0.6 (-3.3 to 2.1)	1.4 (-1.8 to 4.5)
Cortisol (nmol/L)	705 (620 to 790)	883 (570 to 1096)	546 (460 to 632)	367† (265 to 469)	641 (542 to 740)	870 (634 to 1106)	646 (509 to 783)	420† (210 to 630)

I-FABP (pg/ml)	545 (382 to 707)	1793[§] (714 to 2873)	----	----	683 (392 to 975)	1360[§] (973 to 1748)	----	----
sCD14 (µg/ml)	2.37 (2.21 to 2.52)	2.29 (2.12 to 2.46)	----	----	2.40 (2.25 to 2.55)	2.43 (2.18 to 2.68)	----	----
IL-1β (pg/ml)	1.4 (0.5 to 2.3)	1.2 (0.3 to 2.0)	1.7 (0.8 to 2.6)	1.3 (0.3 to 2.2)	1.0 (0.3 to 1.7)	1.1 (0.4 to 1.8)	1.3 (0.3 to 2.2)	2.0[##] (0.7 to 3.3)
TNF-α (pg/ml)	6.9 (5.3 to 8.5)	7.0 (5.3 to 8.7)	7.8 (5.6 to 10.0)	5.9 (4.4 to 7.5)	6.5 (4.8 to 8.2)	7.3 (5.5 to 9.1)	6.7 (4.8 to 8.6)	6.8 (4.5 to 9.0)
IL-6 (pg/ml)	18.4 (<0.1[c] to 38.3)	15.6 (<0.1[c] to 31.9)	19.6 (<0.1[c] to 41.8)	17.3 (<0.1[c] to 36.0)	14.7 (<0.1[c] to 29.2)	16.0 (<0.1[c] to 31.5)	16.1 (<0.1[c] to 34.1)	20.8 (<0.1[c] to 46.1)
IL-8 (pg/ml)	11.3 (0.7 to 21.9)	10.0 (0.8 to 19.3)	11.2 (0.5 to 21.8)	10.2 (0.1 to 20.4)	9.3 (1.2 to 17.4)	10.0 (1.8 to 18.2)	9.1 (0.5 to 17.8)	11.4 (<0.1[c] to 23.9)
IL-10 (pg/ml)	8.6 (4.1 to 13.1)	18.2 (5.6 to 30.9)	9.7 (3.8 to 15.6)	7.6 (2.8 to 12.5)	7.3 (3.2 to 11.5)	16.5 (5.7 to 27.3)	7.2 (2.9 to 11.5)	8.1 (2.8 to 13.4)
IL-1rα (pg/ml)	17.7 (9.0 to 26.4)	22.7 (11.2 to 34.1)	26.7 (14.2 to 39.1)	23.0 (14.4 to 31.5)	15.9 (9.5 to 22.4)	22.4 (13.1 to 31.7)	23.5 (13.9 to 33.0)	27.2 (10.6 to 43.8)
Aldosterone (nmol/L)	165 (118 to 212)	436[§] (288 to 584)	178 (136 to 221)	127 (101 to 152)	180 (104 to 256)	446[§] (306 to 586)	178 (116 to 239)	147 (124 to 169)

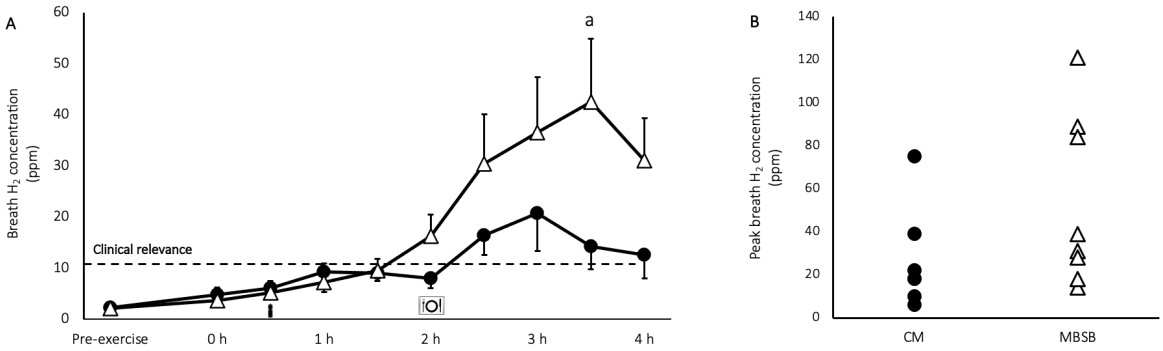
Serum sodium (mmol/L)	141 (139 to 143)	143 (139 to 147)	137 (137 to 155)	143 (137 to 149)	141 (140 to 142)	142 (136 to 149)	142 (136 to 149)	144 (138 to 150)
Serum potassium (mmol/L)	4.7 (4.3 to 5.0)	4.6 (4.3 to 4.8)	4.5 (4.2 to 4.7)	4.5 (4.1 to 4.9)	4.6 (4.2 to 5.1)	4.4 (4.1 to 4.7)	4.4 (4.2 to 4.6)	4.5 (4.4 to 4.7)
Serum calcium (mmol/L)	2.32 (2.26 to 2.38)	2.29 (2.22 to 2.36)	2.43 (2.30 to 2.56)	2.40 (2.28 to 2.51)	2.34 (2.29 to 2.39)	2.29 (2.17 to 2.41)	2.37 (2.27 to 2.47)	2.39 (2.30 to 2.47)

Mean (95% CI) (n= 9); MEOTime †† P< 0.01 *vs* 0 h recovery, MEOTime §§ P< 0.01 and § P< 0.05 *vs* pre-exercise, [aa] P< 0.01 *vs* CM, [c] under detectable lowest standard.

Gastrointestinal integrity, function and symptoms

An increase in I-FABP occurred in response to the exercise stress (P= 0.008), with no trial differences (742 (466 to 1019) pg/ml). No significant effects or interaction were observed for plasma sCD14 concentration (**Table 4.1**). A trial*time interaction was observed for breath H_2, with significantly greater concentrations observed on MBSB at 3.5 h into recovery (P< 0.05; **Figure 4.2A**). Peak breath H_2 (P= 0.014) was significantly greater on MBSB (49 (24 to 74) ppm) than CM (24 (10 to 38) ppm). Both trials reached peak breath H_2 of clinical significance (i.e., >10 ppm) between 3 and 3.5 h into recovery (**Figure 4.2B**). A corresponding MEOTime occurred for lower GIS 3.5 h (P< 0.01) and 4 h (P< 0.05) into recovery. There was a trend towards greater total gut discomfort on MBSB (P= 0.053). No significant main effects or interactions were observed for upper-GIS, nausea or total-GIS (**Table 4.2).**

Figure 4.2 Breath hydrogen response (A) and individual peak breath hydrogen (B) after 2 h HIIT exercise in temperate ambient conditions and consumption of a chocolate dairy milk (CM: ●) or the milk-based sports beverage (MBSB: △).



Mean ± SEM (n= 9): a P< 0.05 *vs* CM.

Table 4.2 Incidence of gastrointestinal symptoms and severity of gut discomfort, total, upper-, and lower-gastrointestinal symptoms in response to 2 h HIIT exercise (between 60% and 80% $\dot{V}O_{2max}$) in temperate ambient conditions and after consumption of chocolate dairy milk (CM) and the milk-based sports beverage (MBSB).

	CM					MBSB				
	Exercise		Recovery			Exercise		Recovery		
	Incidence % (severe)	Severity	Incidence % (severe)	Severity Acute (0-2 h)	Severity Total (0-4 h)	Incidence % (severe)	Severity	Incidence % (severe)	Severity Acute (0-2 h)	Severity Total (0-4 h)
Gut discomfort	NA	7 (1-27)	NA	4 (1-19)	10 (2-41)	NA	6 (3-17)	NA	6 (3-16)	19 (1-48)
Total GIS[a]	89 (56)	11 (1-52)	89 (67)	4 (1-19)	12 (2-59)	67 (56)	8 (3-28)	89 (78)	8 (3-29)	28 (1-91)
Upper GIS[b]	78 (33)	5 (1-19)	56 (22)	3 (1-19)	5 (1-32)	56 (22)	3 (2-12)	56 (33)	3 (1-21)	7 (1-39)
Belching	67 (22)	2 (1-9)	11 (0)	0 (1-1)	0 (1-1)	22 (0)	1 (1-5)	22 (11)	1 (1-5)	1 (1-7)
Heartburn	33 (22)	2 (1-10)	11 (0)	0 ----	0 (3-3)	22 (22)	2 (9-12)	11 (0)	0 (1-1)	0 (2-2)
Bloating	0 (0)	0 ----	33 (22)	2 (5-8)	4 (3-27)	0 (0)	0 ----	44 (33)	3 (1-16)	6 (1-27)
Stomach pain	11 (11)	1 (7-7)	11 (11)	0 ----	1 (5-5)	11 (0)	0 (3-3)	11 (11)	0 ----	1 (5-5)

Urge to regurgitate	0 (0)	0 ----	0 (0)	0 ----	0 ----	0 (0)	0 ----	0 (0)	0 ----	0 ----
Regurgitation	0 (0)	0 ----	0 (0)	0 ----	0 ----	0 (0)	0 ----	0 (0)	0 ----	0 ----
Lower GIS[b]	33 (22)	1 (2-6)	67 (44)	0 (2-2)	6 (2-23)	33 (22)	2 (2-9)	67 (44)	1 (6-7)	18 (1-65)
Flatulence	22 (0)	1 (2-3)	22 (22)	0 (2-2)	1 (5-6)	22 (11)	1 (2-9)	22 (0)	0 (1-1)	0 (1-1)
Lower bloating	11 (0)	0 (2-2)	56 (22)	0 ----	2 (1-6)	11 (0)	1 (3-6)	44 (33)	1 (3-6)	4 (1-15)
Urge to defecate	11 (0)	0 (3-3)	11 (0)	0 ----	0 (3-3)	11 (0)	0 (4-4)	44 (33)	0 ----	5 (2-25)
Intestinal pain	11 (0)	0 3-3	0 (0)	0 ----	0 ----	0 (0)	0 ----	11 (0)	0 (3-3)	0 (3-3)
Abnormal defecation[c]	0 (0)	0 ----	22 (22)	0 ----	2 (10-10)	0 (0)	0 ----	33 (33)	0 ----	8 (10-40)
Others										
Nausea	11 (11)	1 (5-5)	11 (0)	0 ----	0 4-4	0 (0)	0 ----	0 (0)	0 ----	0 ----
Dizziness	44 (22)	2 (2-8)	33 (11)	1 (2-7)	1 (2-9)	33 (11)	2 (2-16)	44 (33)	3 (3-13)	3 (3-13)
Stitch[d]	11 (11)	2 (19-19)	0 (0)	0 ----	0 ----	11 (0)	0 (3-3)	11 (0)	0 (2-2)	0 (2-2)

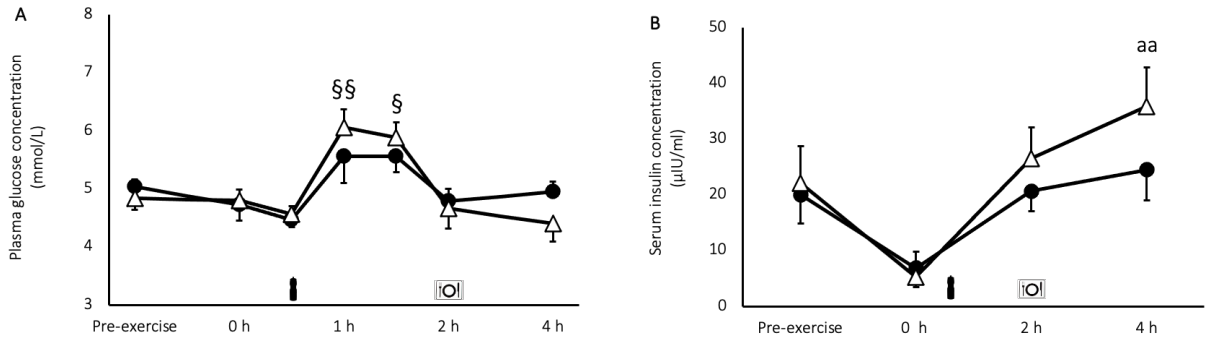
Feeding Tolerance											
Appetite	NA	11 (1-33)	NA	19 (2-44)	25 (2-68)		NA	11 (1-24)	NA	20 (12-50)	24 (12-65)
Thirst	NA	24 (1-46)	NA	7 (1-16)	24 (7-42)		NA	23 (6-35)	NA	18 (3-29)	26 (3-48)

Values are presented as means and range of participant reporting GIS incidence (n = 9). GIS incidence during exercise and recovery are presented as percentage of total participants reporting GIS ≥1 on the mVAS. GIS severity during exercise and recovery are presented as mean summative accumulation of mVAS rating scale of measured time periods and individual range of participant reporting GIS incidence (162,212). [a] Summative accumulation of upper-, lower-, and other gastrointestinal symptoms, [b] summative accumulation of upper- or lower- gastrointestinal symptoms, [c] abnormal defecation including loose watery stools, diarrhoea and blood in stools, and [d] acute transient abdominal pain. NA: not applicable. Wilcoxon signed-rank tests showed no differences between CM and MBSB for GIS. Hedge's g measurement of effect size for GIS and feeding tolerance severity between CM and MBSB was determined as >0.50 and >0.80 for medium and large effects, respectively; however, no medium or large effects size value were detected between CM and MBSB.

Glucose availability & insulin response

There was a MEOTime for blood glucose responses, whereby blood glucose increased from the beginning to 1 h (P< 0.01) and 1.5 h (P< 0.05) into recovery (**Figure 4.3A**). A trial*time interaction occurred for serum insulin (P< 0.01), whereby concentrations were significantly greater 4 h into recovery on the MBSB trial (35.9 (22.2 to 49.5) µIU/ml) compared with CM (24.5 (13.8 to 35.2) µIU/ml; **Figure 4.3B**).

Figure 4.3 Blood glucose (A) and serum insulin (B) concentrations after 2 h HIIT exercise protocol in temperate ambient conditions and consumption of a chocolate dairy milk (CM: ●) or milk-based sports beverage (MBSB: △).

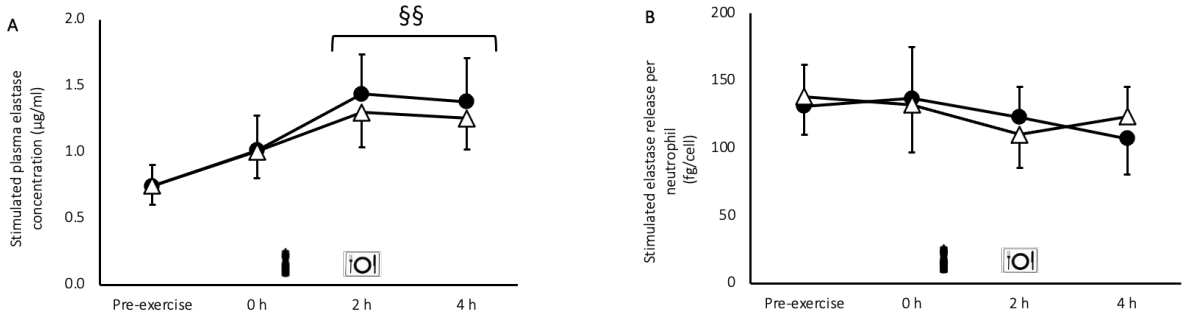


Mean ± SEM (n= 9): [aa] P< 0.01 *vs* CM. MEOTime [§§] P< 0.01 *vs* pre-exercise and [§] P< 0.05 *vs* pre-exercise.

Immune responses

An exercise-induced leukocytosis (MEOTime) (10.6 (3.0) x10^9/L; P= 0.001), neutrophilia (7.0 (2.3) x10^9/L; P< 0.001), lymphocytosis (3.4 (1.0) x10^9/L; P= 0.002) and monocytosis (0.7 (0.3) x10^9/L; P= 0.005) occurred in response to the exercise stress. A trial*time interaction was observed for neutrophil counts (P= 0.005), whereby a significantly greater neutrophil count was observed on CM (6.7 (2.6) x10^9/L) at 4 h of the recovery period compared with MBSB (5.3 (1.6) x10^9/L) (P< 0.01). No main effects or interaction were observed for unstimulated plasma elastase concentration (71 (47 to 96) ng/ml). A MEOTime was observed for total bacterially-stimulated plasma elastase concentration (P= 0.001), such that values increased during recovery (**Figure 4.4A**). Bacterially-stimulated elastase release per neutrophil *in vitro* did not differ between CM and MBSB (125 (107 to 144) fg/cell). Neutrophil function decreased by 4 h of recovery during the CM (-18%) trial and 2 h of recovery during the MBSB (-20%) trial (**Figure 4.4B**), however this change was not significant.

Figure 4.4 Total (A) and per cell (B) bacterially-stimulated neutrophil elastase release after 2 h HIIT exercise in temperate ambient conditions and consumption of a chocolate dairy milk (CM: ●) or the milk-based sports beverage (MBSB: △).



Mean ± SEM (n= 9): MEOTime §§ P< 0.01 *vs* pre-exercise.

Systemic inflammatory cytokine profile

A trial*time interaction occurred for IL-1β concentrations, whereby values were significantly higher on MBSB 4 h into recovery (2.0 (0.6 to 3.4) pg/ml) compared to CM (1.3 (0.2 to 2.3) pg/ml) (P< 0.01). No significant effects or interaction were observed for plasma TNF-α, IL-6, IL-8, IL-10, and IL-1ra concentrations. Exercise-induced SIR-profile (CM: 22 (12 to 32) arb.unit and MBSB: 38 (7 to 69) arb.unit) and recovery beverage post-prandial SIR-profile (CM: 4 (-20 to 29) arb.unit and MBSB: 6 (-26 to 38) arb.unit) did not differ between trials (Table 4.1).

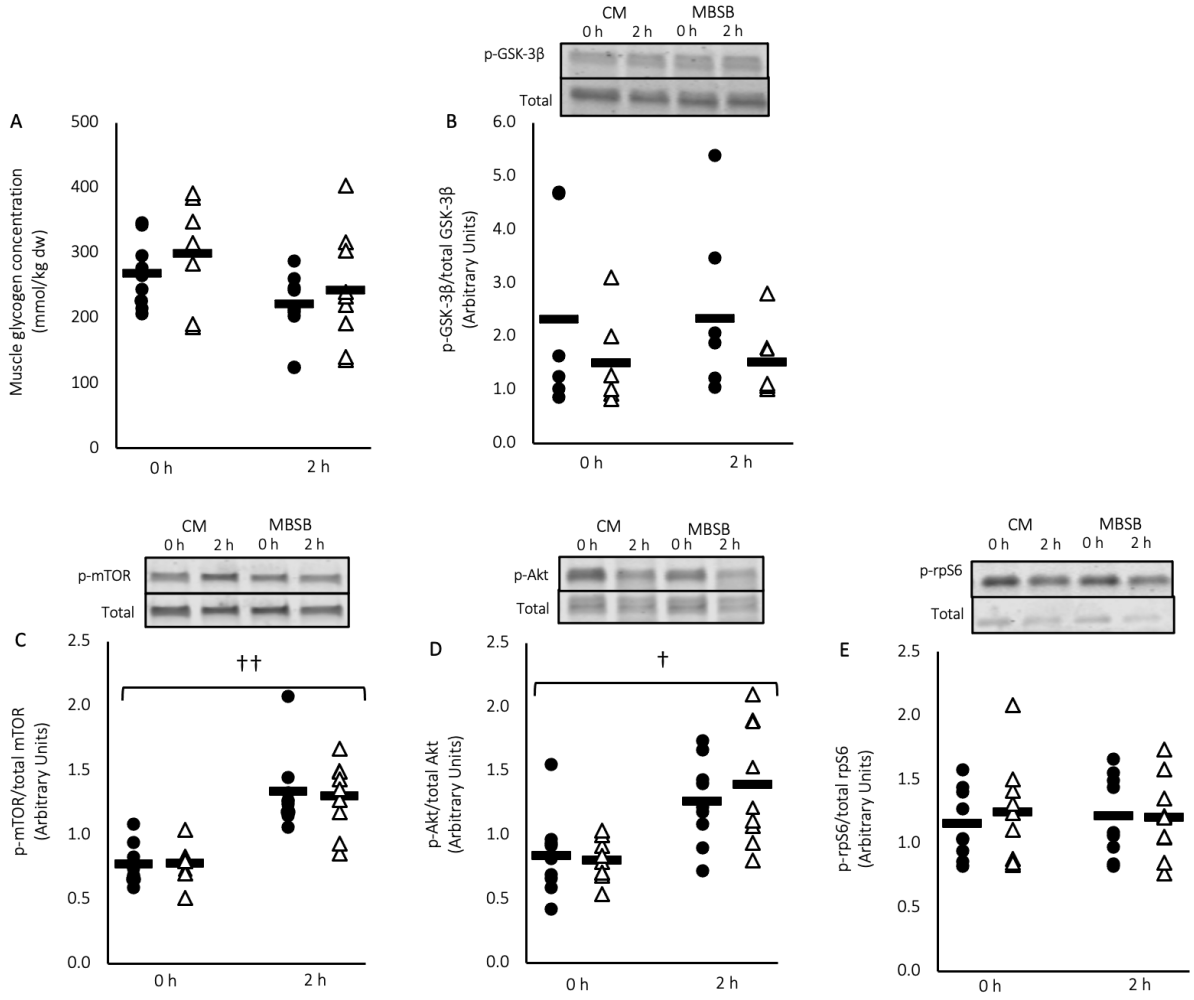
Muscle glycogen resynthesis

No significant main effects or interaction were observed for muscle glycogen concentration or rate of resynthesis. Likewise, no main effects or interaction occurred for the ratio of phosphorylated GSK-3β to total GSK-3β, or fold change of this ratio from the beginning to the 2 h into the recovery period (Figure 4.5A & B).

Phosphorylation of muscle signalling proteins

A MEOTime was observed for the ratio of phosphorylated mTOR to total mTOR (p-mTOR/total mTOR) (P< 0.001) and phosphorylated Akt to total Akt (p-Akt/total Akt) (P= 0.011), whereby phosphorylation of both proteins increased 2 h into the recovery period (Figure 4.5 C & D). No time or trial interactions were observed for the ratio of phosphorylated rpS6 to total rpS6 (Figure 4.5E).

Figure 4.5 Muscle glycogen content (A), phosphorylated GSK3-β to total GSK3-β (B), ratio of phosphorylated mTOR to total mTOR (C), phosphorylated Akt to total Akt (D), and phosphorylated rpS6 to total rpS6 (E) after 2 h HIIT exercise in temperate ambient conditions and consumption of a chocolate dairy milk (CM: ●) or milk-based sports beverage (MBSB:△).



Mean and individual responses (n= 9): MEOTime †† P< 0.01 *vs* 0 h recovery, † P< 0.05 *vs* 0 h recovery.

Hydration status

A significant difference in resting TBW was observed (CM: 61 (58 to 63) % *vs* MBSB: 60 (58 to 63) %; P= 0.020), however all participants commenced within range of euhydration (i.e., >55%). Exercise-induced BM loss did not differ between CM and MBSB (1.7 (1.4 to 2.0) %). A MEOTime was observed for plasma aldosterone concentrations pre- to post-exercise (P< 0.05). Total fluid intake during the recovery period did not differ between trials (CM: 24.6 (21.2 to 28.0) ml/kg BM and MBSB: 24.6 (22.9 to 26.4) ml/kg BM). Greater fluid retention (CM: 81.9% *vs* MBSB: 86.9%; P= 0.028) with a corresponding lower urine output (CM: 415 (264 to 567) ml *vs* MBSB: 284 (190 to 379) ml; P= 0.015) was observed on MBSB. No

significant main effects or interaction were observed for P_{Osmol}, P_V change or plasma electrolyte concentrations (Table 4.1).

Psychophysiological parameters & subsequent performance

Prior to the performance tests, participants reported greater readiness to invest physical effort on MBSB (P= 0.001). Readiness to invest physical effort decreased on both trials from pre- to post-exercise (P= 0.021). No main effects or interaction were observed for readiness to invest mental effort. There were no trial differences in carbohydrate and fat oxidation rates or physiological parameters at any stage of the incremental test (Table 4.3). There were no differences in distance covered during the 1 h running performance test (10.5 (9.7 to 11.2) km), and no significant differences in heart rate (171 (168 to 174) bpm) or rate of perceived exertion (16 (15 to 16) throughout the distance test on CM and MBSB.

Table 4.3 Physiological and performance outcomes graded intensity breath-by-breath testing at 50-80% $\dot{V}O_{2max}$ and 1 h self-paced distance test, following consumption of chocolate dairy milk (CM) and the milk-based sports beverage (MBSB) the previous day.

	CM				MBSB			
	50% $\dot{V}O_{2max}$	60% $\dot{V}O_{2max}$	70% $\dot{V}O_{2max}$	80% $\dot{V}O_{2max}$	50% $\dot{V}O_{2max}$	60% $\dot{V}O_{2max}$	70% $\dot{V}O_{2max}$	80% $\dot{V}O_{2max}$
RER	0.905 (0.882 to 0.927)	0.936 (0.904 to 0.968)	0.938 (0.909 to 0.967)	0.965 (0.930 to 0.999)	0.911 (0.898 to 0.924)	0.928 (0.917 to 0.940)	0.935 (0.912 to 0.957)	0.963 (0.933 to 0.993)
$\dot{V}O_2$ (ml/kg BM/min)	24.4 (21.6 to 27.2)	31.7 (29.5 to 34.0)	37.7 (34.2 to 41.2)	45.3 (41.8 to 48.9)	24.2 (21.1 to 27.3)	32.2 (30.4 to 34.2)	38.6 (36.4 to 40.7)	45.2 (42.5 to 47.9)
Carbohydrate oxidation (g/min)	1.6 (1.2 to 2.0)	2.4 (1.8 to 2.9)	2.9 (2.2 to 3.5)	3.8 (3.0 to 4.6)	1.6 (1.3 to 1.9)	2.3 (2.0 to 2.6)	2.9 (2.4 to 3.3)	3.7 (3.2 to 4.3)
Fat oxidation (g/min)	0.3 (0.2 to 0.3)	0.3 (0.1 to 0.4)	0.3 (0.2 to 0.4)	0.2 (0.1 to 0.4)	0.3 (0.2 to 0.3)	0.3 (0.2 to 0.4)	0.3 (0.2 to 0.4)	0.2 (0.1 to 0.4)
HR (bpm)	120 (110 to 130)	142 (135 to 149)	156 (146 to 166)	172 (163 to 181)	117 (106 to 128)	139 (130 to 149)	158 (149 to 166)	173 (167 to 179)
RPE (6-20)	9 (8-10)	10 (9-12)	13 (11-14)	15 (14-17)	9 (8-10)	11 (10-11)	12 (11-13)	15 (13-16)

Mean (95% CI) (n= 8). RER: respiratory exchange ratio, HR: heart rate, and RPE: rating of perceived exertion.

Discussion

The current study aimed to determine the effects of flavoured dairy milk-based recovery beverages of different nutrition compositions, but same intake volume, on markers of gastrointestinal and immune status, and subsequent recovery optimisation markers. In accordance with our hypothesis, significantly greater carbohydrate malabsorption was observed after consumption of the MBSB, accompanied by a trend towards greater gut discomfort. However, the greater nutritional content on MBSB did not influence any immune markers during the acute recovery period. Both beverages increased phosphorylation of mTOR and Akt; however, contrary to our hypothesis, there were no differences between trials. Additionally, neither beverage was associated with measurable increases in skeletal muscle glycogen stores within 90 min of consumption. In accordance with the hypothesis, greater fluid retention was observed with the MBSB beverage, however there were no trial differences in TBW or plasma hydration markers. The observed differences in the assimilation of recovery nutrition and acute recovery outcomes did not result in differences in substrate oxidation or performance the following day, suggesting overall dietary intake over the post-exercise period to the following day supersedes any impact of recovery optimisation markers in response to an acute exercise recovery beverage. From a professional practice perspective, considering the current study is the first to assess the impact of dairy milk recovery beverages with differing nutritional content (i.e., whole food standard recovery nutrition recommendation *vs* volume matched higher nutrient density of reconstitute recovery supplement) on gastrointestinal and immune status, the finding suggest whole food dairy milk poses a lower gastrointestinal intolerance, despite equal outcomes for other recovery optimisation markers.

Gastrointestinal responses and acute exercise recovery beverage

It is recommended and is a common practice for athletes to consume food(s) and fluid(s) in the early stages after exercise cessation (i.e., training and (or) competition). The diverse availability of manufactured recovery products, especially beverages, either as whole-food (e.g., dairy milk) or supplementation (e.g., dairy based supplement powder) form, appears to promote diverse nutrient density intakes, representing an ideal to over-load of nutrient provision. Considering exercise stress

compromises gastrointestinal functional activities (i.e., motility, digestion, and absorption) and prompts GIS, as a result of exercise-induced gastrointestinal syndrome (11), it has been previously unknown how the provisions of differing nutrient density recovery beverages impact on post-exercise gastrointestinal status. Therefore, the nutritional quantity and quality provided during the exercise recovery period may affect how an athlete tolerates the recovery nutrition provided (13,64,163). Participants in the current study did not present lactose intolerance or dairy protein allergies, yet a clinically significant rise in breath H_2 was observed 1.5 h after consumption of both beverages, with a significantly greater peak observed after consumption of the MBSB (**Figure 4.2**) (223). **Figure 4.2B** highlights the large individual variation, as the participants recording the highest peak breath hydrogen concentrations (84–121 ppm) were those who experienced diarrhoea on the MBSB trial, two of whom also experienced diarrhoea on the CM trial. The remaining participants experienced mild or no lower-GIS on either trial, despite clinically significant malabsorption. Asymptomatic malabsorption (i.e., >10 to 20 ppm) has been observed amongst healthy individuals (i.e., no known gastrointestinal disease or disorder), following consumption of lactose (50 g), and sucrose (<100 g), at rest (224,225). It is possible that the observed carbohydrate malabsorption in the current study was exacerbated by neuroendocrine and circulatory derived exercise induced gastrointestinal syndrome, leading to oversaturation of epithelial transporters (11,68,164). The current study provides evidence of individual, nutritive, and exercise-induced factors may separately and interactively contribute to malabsorption of dairy based exercise recovery beverages. However, further research is required to clarify underlying mechanisms and possible strategies for remediation.

Immune responses and acute exercise recovery beverage

Athletes commonly cite using sports food products to prevent microorganism-borne illness and infection (5). Dairy milk beverages have been reported to prevent the exercise-induced immunodepression commonly observed after strenuous exercise, and interestingly one recent study showed an acute boost in immune functional responses (i.e., 85% increase in *in vitro* bacterially-stimulated neutrophil elastase release) in the exercise recovery period; although dairy milk beverages

are not promoted as immunostimulants (13). This benefit appears to be specific to dairy beverages, as opposed to non-dairy beverages with equivalent or similar nutritional composition. This phenomenon has been attributed to the potential combination of insulinaemic and calcaemic effect of dairy milk, on priming neutrophil functional responses (e.g., suppressing desensitisation, enhancing phagocytosis and elastase release efficiency) (13,64,158,159,241). The current study failed to demonstrate such an immunostimulation, or even prevent the commonly observed exercise-induced depression (19% reduction in the current study) of *E.coli* LPS challenged neutrophil elastase release with either beverage. However, it is important to highlight that the reported reduction in *in vitro* bacterially-stimulated neutrophil elastase release did not reach significance compared with pre-exercise baseline level. This may be likely due to the 2 h HIIT exercise model providing a low exercise stress load than previous exercise models reporting ~30% reduction in neutrophil function with continuous running at ≥70% $\dot{V}O_{2max}$ in temperate ambient conditions (13,25,64,138). Nevertheless, the absence of a preventative effect is likely associated with delay in delivery of the beverage, as previously observed (138). More research is needed to determine if immediate provision of carbohydrate and protein greater than 1.2 g/kg BM and 0.4 g/kg BM, respectively, and the associated heightened insulin response, will further enhance immune functional responses, and whether this enhancement is at all beneficial (e.g., reduced microorganism-borne illness or infection risk, intestinal originated bacterial endotoxin systemic translocation clearance, and (or) exercise associated tissue adaptions) from a translational perspective, considering the *in vitro* methodologies used in the current study and the interaction of immune response components *in vivo* (242).

It is well established that exercise stress has the capability of inducing a systemic inflammatory response (198), which appears to be proportional to the exertional load and exacerbated by external factors (e.g., environmental conditions- heat) (14,210). Evidence from the recent scientific literature suggests that exercise stress up to 3 h in duration, with or without additional heat stress, promotes modest increases in systemic inflammatory profile, characteristic of none to small increases in pro-inflammatory cytokine

markers (i.e., TNF-α and IL-1β), none to modest increases in systemic response cytokine markers (i.e., IL-6 and IL-8), and modest to large increases in anti-inflammatory cytokine markers (i.e., IL-10 and IL-1ra) (13,205,212,230,228,229). For example, 2-3 h running at ~60-70% $\dot{V}O_{2max}$ consistently results in <10 pg/ml pre- to peak post-exercise (0-4 h measurement period) increase in plasma pro-inflammatory and response cytokine concentrations. However, substantially greater (e.g., >20 pg/ml) systemic anti-inflammatory cytokine responses are seen, after such exercise loads, suggesting anti-inflammatory cytokine markers (e.g., IL-10 and IL-1ra) are more sensitive to exercise induced changes. Nevertheless, it is important to note that the magnitude of systemic inflammatory cytokine responses to such exercise loads are of little clinical significance (e.g., comparable to cytokine profiles associated with clinical sepsis) (243,244), and only with an extreme exercise load (e.g., ultra-endurance exercise) does systemic inflammatory cytokine responses appear to reach clinical relevance (205,229). In the current study, there was no substantial systemic inflammatory cytokine response, suggesting 2 h HIIT creates no to minimal consequence to systemic inflammatory status. It is reported that the translocation of bacterial endotoxins from the lumen into circulation contributed to the systemic inflammatory response peaking at the cessation of exercise (11,198). In the current study there was no substantial evidence of intestinal epithelial injury or increases in circulating sCD14 or LBP (i.e., indirect markers for luminal translocated bacterial endotoxin). Therefore, it is not surprising that systemic inflammatory responses were minimal. It is also suggested that certain foods have the propensity to acutely alter systemic cytokine profile in the post-prandial period (231). In the current study, inflammatory cytokines were unaltered by the recovery beverage intervention, with the exception of IL-1β, which was significantly greater in MBSB at 4 h recovery compared to CM. It is unclear why this response was seen following consumption of the MBSB, but links to some inflammatory aspect within product development and provisions should not be overlooked. It should also be noted that the magnitude of difference in IL-1β between MBSB and CM (i.e., 0.7 pg/ml) appears to be of little clinical relevance, as indicated by the lack of trial differences in the SIR-profile.

Muscle glycogen resynthesis and acute exercise recovery beverage

Replenishment of skeletal muscle glycogen stores through high carbohydrate intake is a primary goal of acute recovery nutrition, especially if consecutive bouts of strenuous exercise bouts are programmed in the short term (e.g., same or following day) (6), which is dependant of gastrointestinal patency (e.g., intake behaviour linked to tolerance and GIS, ingested food-fluid motility, digestion and absorption) and potentially immune responses associated with systemic pathogenic (e.g., luminal originated bacteria and (or) bacterial endotoxin) and tissue injury (e.g., cell damage debris) clearance (11,198). In the current study, an intake of 1.2 and 2.2 g/kg BM of carbohydrate on CM and MBSB trials, respectively, failed to initiate muscle glycogen replenishment processes 90 min after consumption. Blood glucose and insulin responses on both trials indicate glucose absorption, systemic availability, and uptake by insulin-sensitive tissues (**Figure 4.3**). However, phosphorylation of GSK-3β remained unchanged, suggesting a limitation of glucose uptake at the level of skeletal muscle, and not at the gastrointestinal level. Therefore, impaired glucose uptake at the sarcolemma, and disposal of glycogen within the skeletal muscle cell are likely to have limited resynthesis of skeletal muscle glycogen. Skeletal muscle-damaging eccentric exercise protocols have provided evidence of impaired rates of muscle glycogen resynthesis, associated with compromised cell structural integrity, reduced translocation of GLUT-4 to the skeletal muscle plasma membrane, and reduced insulin sensitivity (232). Given the sampling time-frame in the current study, it is unclear if greater carbohydrate intakes on the MBSB enhanced glucose disposal and glycogen synthesis beyond 2 h post-exercise. Further research is required to verify these findings and examine possible mechanisms.

Protein synthesis expression to acute exercise recovery beverage

Following prolonged, strenuous exercise, provision of adequate protein is required to generate a net positive protein balance (115), which is also dependant on gastrointestinal patency and potentially immune responses. In the current study, CM (30 (6) g protein (derived from fresh cow's milk protein) and MBSB (63 (13) g protein (derived from non-fat milk solids and whey protein concentrate)) equally increased phosphorylation of mTOR and Akt. Findings from the current study are supported by previous

literature demonstrating maximal myofibrillar fractional synthetic rate following endurance exercise occurred with 23 to 30 g milk protein intake, with no further benefit at an intake >45 g (112,113). Whey protein derived from dairy milk has been identified as a superior protein for stimulating muscle protein synthesis owing to the rapid digestibility and absorption kinetics. Digestion and absorption of protein within the whole milk matrix and with carbohydrate co-ingestion is delayed compared to isolated whey, casein or soy protein, however this does not appear to diminish rates of muscle protein synthesis provided adequate overall protein (i.e., >23 g) is consumed (245,246). Quantification of myofibrillar and mitochondrial fractional synthetic rate, and the corresponding aminoacidaemia and leucinaemia, following consumption of dry and liquid dairy proteins is warranted to expand on these findings.

Hydration status to exercise recovery beverage

Considering hydration status (e.g., hypohydration *vs* euhydration) has been shown to impact gastrointestinal patency and immune functional responses (i.e., *E.coli* LPS challenged neutrophil elastase release) (13), it seems plausible that an enhanced rehydration beverage would also benefit gastrointestinal and immune status. Beverages with a greater nutrient density have been shown to aid fluid retention via delaying gastric emptying (i.e., avoiding excessive water dumping along the intestine), enhancing intestinal water absorption and circulatory water retention through the osmotic properties of present nutrients (165,247,248). Indeed, flavoured and unflavoured dairy milk products have been shown to enhance fluid retention compared to equal intakes of carbohydrate-electrolyte beverages with a lower energy density, and equal or greater sodium concentrations (130,132,133,176). For example, following mildly dehydrating exercise (i.e., <1.8% BM loss), 150% replacement of BM losses with skim milk (i.e., 5 g/100ml of carbohydrate and 3-4 g/100g of protein, and 17-58 mmol/L of sodium) resulted in a more positive net fluid balance, and corresponding lower urine output and greater P_{Osmol}, compared to isovolumetric intake of water or carbohydrate-electrolyte beverage (i.e., 4-6 g/100ml of carbohydrate and 21-23 mmol/L of sodium) (130,132,133). In the current study, greater fluid retention was observed after consumption of the reconstituted MBSB, compared to CM. Globally, these findings suggest that the nutrient density of the beverage exerts a greater osmotic effect beyond

that of sodium concentration (123). However, faecal losses occurred in the CM (n= 2) and MBSB (n= 5) trials, with participants reporting diarrhea on both trials. Estimated faecal losses per individual were greater on MBSB (705 (271) g), compared to CM (486 (79) g), however it was not possible to quantify faecal water losses *per se*. Therefore, it is likely that the equation described for fluid retention will produce an overestimate of the true value. Importantly, exercise-induced fluid losses were mild (i.e., <2%), and euhydration was maintained throughout the prescribed exercise protocol. These findings would suggest that when mild fluid losses occur, there is a threshold to the concentration and volume of nutritive beverages that can be consumed before detrimental outcomes and excessive fluid losses occur, even for lactose tolerant individuals.

Conclusion

Consumption of a flavoured whole food dairy milk and a flavoured reconstituted dairy milk-based supplement beverage both increased activation of anabolic signalling (i.e., p-mTOR and p-Akt) and restored hydration status. Neither beverage increased muscle glycogen stores, nor immune functional responses (i.e., *in vitro* bacterially-stimulated neutrophil elastase release), likely associated with impaired skeletal muscle cell structural integrity and delayed feeding, respectively. Both beverages resulted in clinically significant malabsorption of the recovery beverage, however the degree of malabsorption was significantly greater after consumption of the MBSB, and was associated with a trend towards significantly greater gut discomfort. The observed differences in the assimilation of recovery nutrition and acute recovery outcomes did not translate to differences in performance outcomes the following day. Flavoured whole food dairy milk therefore provides a cheaper and more practical alternative to specially formulated reconstituted dairy milk-based supplementation, and poses a lower risk of nutrient malabsorption and associated GIS.

Chapter Five

The effect of an acute *'train-low'* nutritional protocol on markers of recovery optimisation in endurance trained male athletes.

Abstract

This study aimed to determine the effects of an acute *'train-low'* nutritional protocol on markers of recovery optimisation compared to standard recovery nutrition intake. After completing 2h high intensity interval running, 8 male endurance athletes (mean (SD): age 25 (7) years, nude BM 71.7 (5.3) kg, height 1.76 (0.06) m, $\dot{V}O_{2max}$ 54 (4) ml/kg BM/min) consumed a standard (CM: 1.2g/kg BM of carbohydrate and 0.4g/kg BM of protein) and a modified lower-carbohydrate (L-CHO: isovolumetric with 0.35g/kg BM of carbohydrate and 0.5g/kg BM of protein) dairy milk beverage in a double-blind randomised-crossover design. Venous blood samples, nude BM, body water, breath samples, and GIS were collected before exercise and during recovery. Muscle biopsies were performed at 0h and 2h of recovery. Participants returned to the laboratory the following morning to assess performance outcomes. The exercise protocol resulted in depletion of muscle glycogen stores (250mmol/kg dw) and mild fluid losses (BM loss: 1.8%). Neither recovery beverage replenished muscle glycogen stores (279mmol/kg dw) or prevented a decrease in bacterially-stimulated neutrophil function (-21%). Both recovery beverages increased phosphorylation of mTOR (main effect of time $P<0.001$) and returned hydration status to baseline. A greater fold increase in p-GSK-3β/total-GSK-3β occurred on CM ($P=0.012$). Blood glucose ($P=0.005$) and insulin ($P=0.012$) responses were significantly greater on CM (618mmol/L/2h and 3507µIU/ml/2h, respectively) compared to L-CHO (559mmol/L/2h and 1147µIU/ml/2h, respectively). Rates of fat oxidation were greater on CM the following day, but performance was not affected. A *'train-low'* nutritional protocol is suitable for acute recovery processes and will shift substrate utilisation towards fat oxidation, without compromising absolute exercise performance within 24 h.

Introduction

Carbohydrate availability can alter physiological and metabolic activity underpinning recovery outcomes, which includes training adaptations (149). Muscle glycogen plays a key regulatory role for skeletal muscle fuel utilisation, enzyme activity, cellular signalling events, and gene expression (249). Previously defined *'train-low, compete-high'* training protocols exploit these diverse roles by reducing carbohydrate availability during and (or) after specific training sessions that deplete muscle glycogen content (e.g., ~2 h HIIT) to enhance training adaptations (e.g., altered fuel kinetics during aerobic exercise- increase fat and reduced carbohydrate oxidation at relative exercise intensities), while maintaining high carbohydrate availability during competition (149,206). When repeated over an extended training regimen, *'train-low'* protocols can increase mitochondrial enzyme activity and concentration, and improve endurance performance through altered fuel kinetics, albeit in sub-elite athletes (19). The compact *'sleep-low, train-low'* model, which involves overnight restriction of carbohydrate after glycogen-depleting exercise, followed by a fasted training session the subsequent morning in a glycogen depleted state, has been reported to stimulate adaptive responses as described above, to a greater degree compared with relative exercise with replete glycogen stores (19).

It is generally accepted that training intensity is likely to be acutely reduced in sessions undertaken with low muscle glycogen concentrations due to reduced substrate availability needed for high intensity and anaerobic contractibility of myofibres (250). When continuously repeated in an extended training program, reduced overall training impulse will negate the potential benefits of a *'train-low'* protocol. Moreover, carbohydrate availability indirectly influences hydration status, net protein balance, immune function, and gastrointestinal function, all of which have implications for recovery optimisation (206); but in the context of gastrointestinal and immune status have generally been neglected in recovery research (61). For example, hydration status may be acutely influenced by endogenous (e.g., muscle glycogen molecules bound to water) and exogenous (e.g., delayed gastric emptying, enhanced intestinal water and sodium absorption) carbohydrate availability (251). Moreover, the concomitant

rise in circulating insulin level following carbohydrate consumption post-exercise may prevent exercise-induced immunodepression and muscle protein breakdown (25,252). Carbohydrate provisions and subsequent digestion and absorption activity along the gastrointestinal tract after stress exposure (e.g., EIGS that compromises gastrointestinal epithelial integrity and functional responses may prompt patency and essential function for regulation of nutrient bioavailability (11).

Current recovery nutrition guidelines offer prescriptive values for the intakes of carbohydrate, protein, and water to support muscle and liver glycogen replenishment, skeletal muscle protein synthesis, and rehydration, respectively (6). The quantity and quality of nutrients (i.e., carbohydrates, protein and electrolytes) provided by flavoured dairy milk beverages align closely with current recovery nutrition guidelines, and have been suggested to provide the 'gold standard' of exercise recovery nutrition (20). However, the ideal nutritional intake during *'train-low'* exercise protocols has not yet been established. With this in mind, the study aimed to determine the effects of a *'train-low'* nutritional protocol, using a lower carbohydrate dairy-based recovery beverage, on markers of recovery optimisation (i.e., skeletal muscle glycogen resynthesis, expression of cell signalling proteins regulating translation initiation, rehydration, immune function, and gastrointestinal integrity and functional status), and subsequent impact on exercise performance the following day, compared to a carbohydrate protocol, using a standard dairy recovery beverage containing a higher carbohydrate content. It was hypothesised that the *'train-low'* nutritional protocol would result in a diminished rate of glycogen resynthesis, rehydration properties, immune functional responses, and subsequent diminished endurance performance the following day, compared with standard carbohydrate intake; but would not differ in protein synthesis expression markers, and may limit the gastrointestinal burden (e.g., carbohydrate malabsorption and GIS) in response to nutrient intake into an exercise-associated compromised gastrointestinal tract.

Method

Subjects

Eight endurance trained male athletes (mean (SD): age 25 (7) years, nude BM 71.7 (5.3) kg, height 1.76 (0.06) m, % body fat 14.2 (4.1) %, $\dot{V}O_{2max}$ 54 (4) ml/kg BM/min, weekly training volume 309 (70) min, and modality: endurance running, ultra-endurance running, field team sports including HIIT) volunteered to participate in the study. All participants gave written informed consent. The study protocol was part of a larger study prospectively registered with ANZCTR (reference number 375090), received ethical approval (MUHREC: 12799) and conformed to the Helsinki Declaration for Human Research Ethics. All participants confirmed being free from illness, disease (including gastrointestinal infections, diseases and (or) disorders, or dietary intolerances) and injury. Individuals were excluded if they confirmed having consumed potential dietary modifiers of gastrointestinal integrity, were adhering to gastrointestinal-focused dietary regimes within the previous three months, or consumed non-steroidal anti-inflammatory medications, antibiotic and (or) stool altering medications within one month before the experimental protocol, or if they failed to achieve a $\dot{V}O_{2max}$ greater than 50 ml/kg BM/min. Baseline measurements were recorded one to three weeks prior to the first experimental trial. Height and BM were recorded (Seca 515 MBCA, Seca Group, Germany). $\dot{V}O_{2max}$ (Vmax Encore Metabolic Cart, Carefusion, US) was estimated using a continuous incremental exercise test to volitional exhaustion on a motorised treadmill (MyRun Technogym; Technogym, Italy), as previously reported (25).

Design

In a randomised order, participants completed two experimental trials separated by a washout period of at least 5 days (i.e., n= 7 14-day washout and n= 1 5-day washout) to accommodate participant availability. Participants were provided with standardised low FODMAP meals the day before and throughout the experimental trials, in accordance with their randomised trial allocation (i.e., low carbohydrate and higher carbohydrate availability trials), with energy content targeted at meeting daily energy requirements and FODMAP content targeted to avoid artefact outcomes on EIGS markers and

GIS (6,212). Compliance was assessed using a food-fluid dietary log and compliance checklist (Table 5.1). Participants were asked to refrain from alcohol consumption and strenuous exercise for 48 h, and from consuming caffeinated beverages for 24 h, before each experimental trial.

Table 5.1 Dietary intake throughout experimental trial under CM and L-CHO conditions.

	CM						L-CHO					
	Water (L)	Energy (MJ)	Protein (g)	Fat (g)	CHO (g)	Fibre (g)	Water (L)	Energy (MJ)	Protein (g)	Fat (g)	CHO (g)	Fibre (g)
24 h prior	2.9 (2.4 to 3.3)	10.5 (8.1 to 13.0)	92 (70 to 113)	56 (46 to 67)	386 (286 to 485)	46 (34 to 58)	2.8 (2.3 to 3.3)	9.6 (8.6 to 10.7)	82 (72 to 92)	49 (40 to 59)	359 (322 to 396)	43 (37 to 49)
Breakfast Day 1	0.4 (0.2 to 0.5)	2.5 (1.8 to 3.3)	20 (14 to 25)	19 (14 to 25)	85 (59 to 111)	13 (8 to 17)	0.4 (0.2 to 0.6)	2.3 (1.6 to 3.1)	18 (13 to 24)	18 (12 to 23)	77 (48 to 106)	12 (7 to 17)
Recovery beverage	0.7 (0.7 to 0.8)	2.5 (2.4 to 2.7)	28 (26 to 29)	16 (15 to 17)	85 (81 to 90)	----	0.8 (0.8 to 0.9)	1.4^{aa} (1.3 to 1.5)	34^{aa} (32 to 35)	13^{aa} (13 to 14)	25^{aa} (24 to 27)	----
Lunch	0.4 (0.3 to 0.4)	3.0 (2.6 to 3.3)	30 (26 to 33)	9 (7 to 12)	124 (110 to 138)	7 (6 to 9)	0.2^{a} (0.2 to 0.2)	2.6 (2.4 to 2.8)	31 (29 to 33)	51^{a} (48 to 54)	8^{a} (7 to 8)	5 (5 to 6)
Dinner	1.1 (0.6 to 1.6)	2.5 (1.7 to 3.3)	22 (15 to 29)	13 (8 to 17)	85 (53 to 117)	19 (13 to 25)	1.0 (0.5 to 1.5)	2.3 (1.9 to 2.7)	47^{aa} (41 to 52)	28^{aa} (19 to 37)	25^{aa} (16 to 35)	13 (10 to 16)
Breakfast Day 2	0.5 (0.4 to 0.7)	2.3 (1.6 to 3.1)	18 (12 to 24)	18 (12 to 24)	78 (52 to 103)	11 (7 to 15)	0.6 (0.4 to 0.8)	2.0 (1.6 to 2.3)	13 (11 to 16)	40^{a} (33 to 47)	13^{a} (11 to 16)	8 (6 to 9)

Mean (95% CI) (*n*= 8); ^{aa} P< 0.01 and ^{a} P< 0.05 *vs* CM.

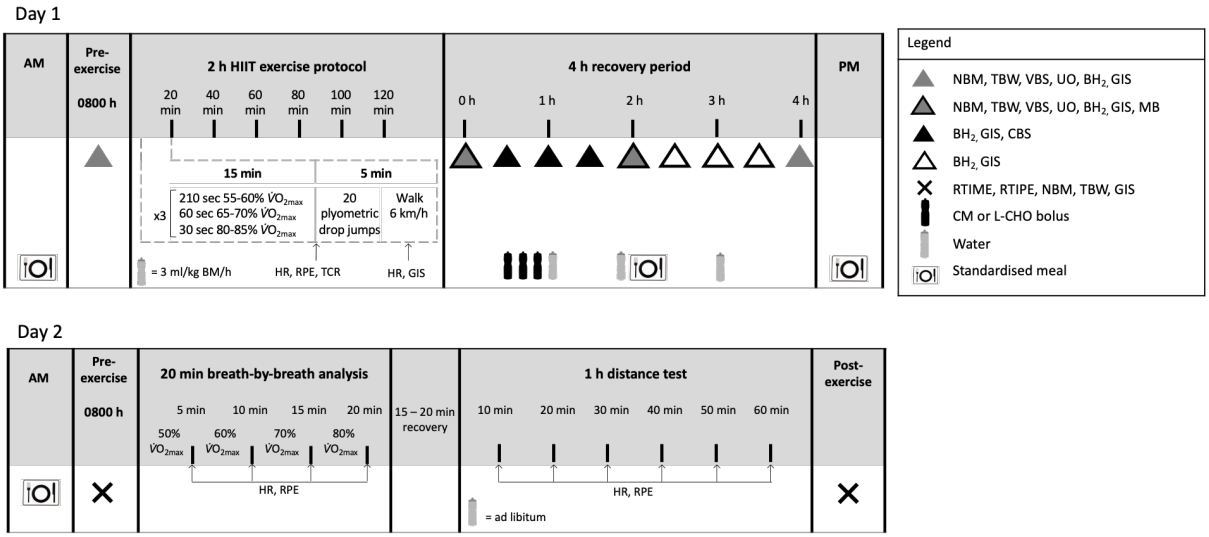
Methodology

Participants reported to the laboratory at 0800h. Pre-exercise nude BM and TBW (Seca 515 MBCA, Seca Group, Germany) were recorded, after voiding. To monitor thermoregulatory strain, participants inserted a thermocouple 12 cm beyond the external anal sphincter to record pre- and post-exercise rectal temperature (Precision Temperature 4600 Thermometer, Alpha Technics, USA). Participants provided a breath sample into a 250 ml breath collection bag, and completed an exercise-specific mVAS GIS assessment tool (162). Blood was collected by venepuncture from an antecubital vein into three separate vacutainers (6 ml 1.5 IU/ml lithium heparin, 4 ml 1.6 mg/ml K_3EDTA, and 5 ml SST).

The exercise protocol consisted of 2 h (initiated at ~0900h) HIIT session conducted in 23.5 (0.9) ^{o}C ambient temperature and 42 (8) % RH on a motorised treadmill (**Figure 5.1**). The protocol involved 3 rounds of running for 3.5 min at 55-60% $\dot{V}O_{2max}$, 1 min running at 65-70% $\dot{V}O_{2max}$ and 30 sec running at 75-80% $\dot{V}O_{2max}$, followed by 20 plyometric drop jumps of alternating legs. Participants then returned to the treadmill to walk until the 20 min cycle was completed. This was repeated 6 times. Adapted from previous research, the protocol was designed to provide sufficient exercise stress to deplete muscle glycogen (144), disturb recovery markers measured (i.e., gastrointestinal, immune, skeletal muscle protein, and hydration) (13,14,25,138,206,210), provide a challenge base for the recovery nutrition intervention (64,206), and to ensure practical relevance to both recreationally (amateur) and elite competitively trained individual and group athletes who may be applying *'train low'* exercise models (19), and exposed to such an exercise stress variations (e.g., team-based sports, endurance and ultra-endurance trail and adventure) (253). The recovery period commenced 30 min post-exercise to allow muscle biopsy preparation and skeletal muscle sampling. After sampling at 2 h of recovery, a third-party researcher provided participants with a meal respective to the trial. Before leaving the laboratory (approximately 1600h), a third-party researcher provided participants with a standardised evening and breakfast meal according to respective trial (i.e., low carbohydrate and higher carbohydrate availability; **Table 5.1**). Participants returned to the laboratory the following morning (approximately 0800h). Nude

BM, TBW and GIS were recorded on arrival and after the distance test. Before and after the distance test, participants completed measures of readiness to invest mental and physical effort on a rating scale from 0-10, with higher ratings indicating greater readiness to invest effort (215). A 20 min run was completed to measure oxygen uptake and oxidation rates at four submaximal exercise intensities (50%, 60%, 70%, and 80% $\dot{V}O_{2max}$) for 5 min each, in 22.6 (1.7) ^{o}C ambient temperature and 37 (6) % RH. Participants then performance a self-paced distance test and were instructed to run the maximal distance possible in 1 h, with the incline set at 1%. To prevent expectation bias, the participant information sheet did not inform participants if or under which recovery feeding interventions they were expected to perform better.

Figure 5.1 Schematic illustration of the experimental design.



NBM: nude body mass, TBW: total body water, VBS: venous blood sampling, UO: urine output, BH_2: breath hydrogen, GIS: gastrointestinal symptoms, HR: heart rate, TCR: thermal comfort rating, RPE: rating of perceived exertion, MB: muscle biopsy, CBS: capillary blood sampling, RTIME: readiness to invest mental effort, RTIPE: readiness to invest physical effort, RER: respiratory exchange ratio, CM: standard carbohydrate conditions, L-CHO: low carbohydrate conditions, BM: body mass.

Participants were provided with a commercially available dairy milk (CM; per 100 ml: 281 kJ, 9.4 g carbohydrate, 3.1 g protein, 1.8 g fat, 42 mg sodium) or a modified lower carbohydrate dairy milk beverage (L-CHO; per 100 ml: 183 kJ, 3.4 g carbohydrate, 4.3 g protein, 1.4 g fat, 87 mg sodium) in a double-blind randomised crossover protocol. Both beverages were chocolate flavoured, prepared by a third-party researcher and served in opaque bottles, at ~7°C beverage temperature (216). The

beverages consumed in 3 equal boluses every 10 min, beginning 30 min into the recovery period. The volume was calculated to provide 1.2 g/kg BM of carbohydrate and 0.4 g/kg BM of protein on CM in 9.5% carbohydrate *w/v*, and volume matched for L-CHO (0.35 g carbohydrate/kg BM and 0.5 g protein/kg BM). Participants completed an alimentary sensory assessment tool, adapted from the mVAS (162), whereby '0' represents none to '10' representing complete oral sensory appreciation and pleasure for taste, flavour, texture, and after-taste. Additional water calculated to provide a total fluid intake of 35 ml/kg BM was provided at hourly intervals. Participants were instructed to drink as much as tolerable and total fluid intake was recorded hourly. The percentage of ingested fluid retained was calculated from the difference between ingested fluid and urine output, as a fraction of total fluid intake (128). Outcome measures collected up until 2 h recovery are attributed to differences in the recovery beverage nutrition, and thereafter are described in relation to the overall train-low nutrition protocol.

Blood glucose concentration, haemoglobin, haematocrit, total and differential leukocyte counts, were determined as previously described (234). The CV for blood glucose concentration, haemoglobin, haematocrit, and leukocyte counts were 5.6%, 2.4%, 0.6%, and 12.3%, respectively. Haemoglobin and haematocrit values were used to estimate changes in P_V relative to baseline, and used to correct plasma variables. To determine the blood glucose response to the recovery beverage, immediately before and every 30 min thereafter for 2 h, blood glucose concentration was measured from capillary blood samples (CV 4.0%). The remaining whole blood processing and analysis (i.e., insulin, cortisol, aldosterone, PMN elastase, I-FABP and sCD14 ELISA, and multiplex system for systemic inflammatory profile) as previously reported (13,64,208). The CVs for ELISAs were ≤6.1% and for cytokine profile multiplex was 16.0%. Breath samples (20 ml) were analysed (CV 2.0%) for H_2 content using a gas-sensitive analyser (Breathtracker Digital Microlyzer, Quintron, USA). Plasma sodium, potassium, and calcium concentrations were determined using ion selective electrodes (Cobas c 501, Roche Diagnostics, Switzerland) and analysed by local pathology services (Cabrini Pathology, Australia). P_{Osmol} was determined by freezepoint osmometry (Osmomat 030, Gonotec, Germany) (CV 2.5%).

Muscle biopsy procedure

Muscle biopsies were performed using a modified 5 mm Bergstrom biopsy needle. Samples were obtained from the vastus lateralis of the ipsilateral leg for the first trial, and contralateral leg for the second. The skin of the lateral aspect of the mid-thigh was washed well (10% Povidone – Iodine solution) then 2 to 3 ml of local anaesthetic (lidocaine 1%) was infiltrated subcutaneously over vastus lateralis to anaesthetise the skin and superficial fascia. After the anaesthetic had taken effect, two 5 mm stab incisions ~15 mm apart were made through skin and fascia, with one incision made for each muscle biopsy sample. Samples were then extracted, immediately submerged in liquid nitrogen, and stored at -80°C prior to further analysis.

Western blot analysis

Approximately 30 mg of skeletal muscle was solubilized in radioimmunoprecipitation buffer (Millipore, Bayswater, Victoria, Australia) with 1 µL/ml protease inhibitor cocktail (Sigma-Aldrich, Castle Hill, New South Wales, Australia) and 10 µl/ml Halt Phosphatase Inhibitor Single-Use Cocktail (Thermo Scientific, Australia, North Ryde, New South Wales, Australia). The concentration of protein per sample was determined by the bicinchoninic acid assay (BCA Protein Assay Kit#23225, Thermo Scientific). 20 µg of skeletal muscle protein lysate was loaded onto into either Bio-Rad precast Criterion TGX Stain-Free 4 to 12% gels (Bio-Rad, Gladesville, New South Wales, Australia). SDS-PAGE was conducted following manufacturer's instructions. Protein was then transferred to PVDF membranes and blocked for 1 h in 5% BSA solution in TBST, (pH 7.6, 20 mmol/L Tris and 150 mmol/L NaCl, 0.1% Tween) at room temperature. Membranes were then incubated in primary antibodies diluted in 5% BSA/TBST overnight at 4°C. Following washing in TBST, membranes were incubated for 1 h with fluorescent secondary antibodies (mTOR^{Ser2448}, Akt^{Ser473}, rpS6$^{Ser235/236}$, and GSK-3β^{Ser9}) (Anti-Rabbit IgG (H+L) DylightTM 800 Conjugate; Anti-mouse IgG (H+L) DylightTM 680 Conjugate) (Cell Signalling Technologies®, Danvers, Massachusetts, USA) diluted 1:10,000 in TBST. Following 2 further washes in TBST and 1 wash in phosphate buffered saline (PBS) membranes were scanned using the LiCOR® Odyssey CLx® Imaging

System (Millennium Science, Mulgrave, Victoria, Australia). All targets were normalized to total protein using either the Bio-Rad stain-free system.

Muscle glycogen analysis

One fraction of muscle sample (20 to 25 mg wet weight) was freeze-dried, after which collagen, blood and other non-muscle material were removed from the muscle fibres. Samples were then pulverized and powdered. Samples were extracted with 0.5 M perchloric acid ($HClO_4$) containing 1 nM EDTA and neutralised using 2.2 M $KHCO_3$. Adenosine triphosphate, phosphocreatine, and creatine was determined from the supernatant by enzymatic spectrophotometric assays (218,219). Muscle glycogen content was determined from 2 aliquots of freeze-dried muscle (2–3 mg) as previously reported (218).

Statistical analysis

Confirmation of adequate statistical power was determined a priori from the applied statistical test, mean, standard deviation, and effect size on markers of 1) gastrointestinal integrity (i.e., plasma I-FABP), function (i.e., breath hydrogen), and GIS (14,13,163,210) ; 2) circulating leukocyte, endotoxin and cytokine profiles (13,25,64,138); 3) total body water, plasma osmolality, plasma volume change (13,64); 4) rate of skeletal muscle glycogen resynthesis (7,74); 5) phosphorylation of intramuscular signalling proteins (111,220); and 6) performance (222,221). Using a standard alpha (0.05) and beta value (0.80), the current sample size, within a cross-over design, is estimated to provide adequate statistical precision to detect significant trial differences (G*Power 3.1, Kiel). Data in the text and tables are presented as mean (SD) for descriptive method, and mean and 95% CI for primary variable, as indicated. For clarity, data in figures are presented as mean and SEM, and (or) mean and individual responses, as indicated. GIS incident is reported as %, while GIS severity is report as mean and range of participant reporting incidence Systemic inflammatory cytokine responses are presented as individual inflammatory cytokine values and SIR-profile, as previously reported (208). Only participants with full data sets within each specific variable were used in the data analysis. All data were checked for normal distribution using Shapiro-Wilks test of normality. Variables with singular data points were examined

using paired sample t-tests, or non-parametric Wilcoxon signed-rank test, when appropriate. Variables with multiple data points were examined using a two-way repeated-measures ANOVA. Assumptions of homogeneity and sphericity were checked, and when appropriate adjustments to the degrees of freedom were made using the Greenhouse-Geisser correction method. Main effects were analysed by Tukey's post hoc HSD. Statistics were analysed using SPSS statistical software (V.26.0, Chicago, Illinois, USA) with significance accepted at $P \leq 0.05$.

Results

Physiological strain

During exercise, there was a MEOTime for peak ($P < 0.001$) and recovered ($P < 0.001$) HR, RPE ($P < 0.001$), TCR ($P = 0.001$) and core body temperature ($P = 0.003$), as all markers increased with increasing physiological strain. A MEOTrial was observed for TCR ($P = 0.039$), whereby ratings were higher on L-CHO (9 (8 to 9)) compared to CM (8 (8 to 8)). Exercise-induced BM loss did not differ between CM and L-CHO (**Table 5.2**).

Blood glucose and insulin responses

Significantly greater blood glucose values were observed on CM at 60 ($P < 0.01$) and 90 min ($P < 0.05$) recovery (**Figure 5.2A**). A MEOTrial occurred for serum insulin, such that values were greater on CM ($P = 0.028$) (**Figure 5.2B**). Blood glucose ($P = 0.005$) and insulin ($P = 0.012$) area under the curve was significantly greater on CM (618 (585 to 652) mmol/L/2 h and 3507 (1864 to 5151) µIU/ml/2 h, respectively) compared to L-CHO (559 (526 to 591) mmol/L/2 h and 1147 (458 to 1837) µIU/ml/2 h, respectively).

Table 5.2 Change in hydration and biomarkers in response to 2 h HIIT exercise (between 60% and 80% $\dot{V}O_{2max}$) in temperate ambient conditions and after consumption of regular carbohydrate (CM) and the low carbohydrate (L-CHO) recovery beverages and meals.

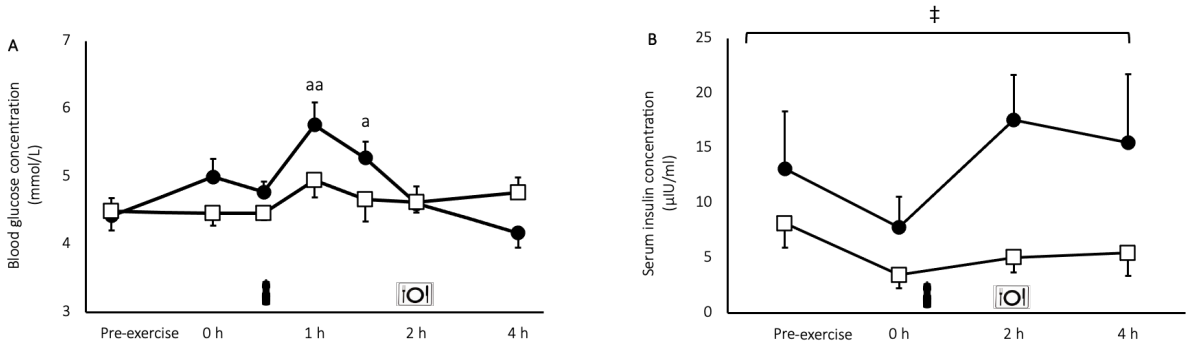
	CM				L-CHO			
	Pre-exercise	0 h	2 h	4 h	Pre-exercise	0 h	2 h	4 h
Total body water (%)	61.4 (59.9 to 62.8)	62.8[§§] (61.0 to 64.5)	61.7 (59.9 to 63.5)	61.8 (59.9 to 63.7)	61.9 (60.2 to 63.6)	63.1[§§] (61.2 to 65.1)	62.5 (60.4 to 64.5)	62.6 (60.6 to 64.7)
(L)	43.2 (40.8 to 45.6)	43.3 (41.1 to 45.5)	43.3 (40.9 to 45.7)	43.8 (41.3 to 46.2)	43.6 (41.2 to 46.0)	43.4 (41.0 to 45.9)	43.5 (41.0 to 46.0)	43.9 (41.3 to 46.5)
Extracellular (%)	23.9 (22.9 to 24.6)	23.7 (22.9 to 24.6)	23.5 (22.7 to 24.4)	23.5 (22.5 to 24.4)	24.1 (23.1 to 25.0)	23.9 (23.0 to 24.9)	23.8 (22.8 to 24.8)	24.0 (23.0 to 25.0)
(L)	16.7 (15.7 to 17.8)	16.4 (15.4 to 17.4)	16.5 (15.5 to 17.5)	16.6 (15.6 to 17.7)	17.0 (15.8 to 18.1)	16.6 (15.4 to 17.7)	16.8 (15.6 to 18.0)	17.1 (15.9 to 18.2)
Plasma osmolality (mOsmol/kg)	294 (289 to 299)	294 (290 to 298)	293 (290 to 297)	291 (287 to 296)	293 (287 to 298)	290[aa] (286 to 295)	290[a] (284 to 295)	292 (285 to 298)
Δ P_V (%)	----	-3.1 (-7.9 to 1.6)	-0.5[†] (-4.4 to 3.4)	0.4 (-4.3 to 5.2)	----	-0.2 (-3.6 to 3.3)	6.9[†] (2.0 to 11.0)	5.0 (0.5 to 9.5)
Cortisol (nmol/L)	787 (637 to 936)	698 (525 to 871)	503 (402 to 604)	369† (297 to 441)	716 (554 to 877)	1056 [aa] (620 to 1493)	534 (376 to 695)	309† (244 to 373)

I-FABP (pg/ml)	463 (247 to 397)	817[§§] (672 to 962)	----	----	357 (218 to 496)	1085[§§] (729 to 1440)	----	----
sCD14 (µg/ml)	2.0 (1.4 to 2.6)	2.2 (1.6 to 2.9)	----	----	2.4 (2.2 to 2.6)	2.4 (2.2 to 2.6)	----	----
IL-1β (pg/ml)	1.4 (<0.1[c] to 3.0)	1.5 (<0.1[c] to 3.3)	1.7 (<0.1[c] to 3.9)	1.8 (<0.1[c] to 3.6)	1.6 (0.3 to 2.8)	1.3 (<0.1[c] to 2.7)	1.4 (<0.1[c] to 3.1)	1.4[a] (<0.1[c] to 2.8)
TNF-α (pg/ml)	7.5 (5.3 to 9.7)	8.2 (6.2 to 10.1)	8.7 (5.9 to 11.6)	10.2 (5.6 to 14.8)	8.0 (5.8 to 10.2)	7.0 (5.3 to 8.8)	8.1 (5.6 to 10.6)	6.9 (4.8 to 9.1)
IL-6 (pg/ml)	41.5 (<0.1[c] to 119.1)	46.1 (<0.1[c] to 133.5)	39.1 (<0.1[c] to 113.6)	45.6 (<0.1[c] to 133.0)	34.8 (<0.1[c] to 98.7)	34.7 (<0.1[c] to 98.9)	34.9 (<0.1[c] to 99.1)	36.1 (<0.1[c] to 103.6)
IL-8 (pg/ml)	15.1 (<0.1[c] to 39.7)	16.0 (<0.1[c] to 41.9)	15.2 (<0.1[c] to 40.6)	17.9 (<0.1[c] to 47.6)	13.0 (<0.1[c] to 32.7)	13.1 (<0.1[c] to 33.8)	12.8 (<0.1[c] to 32.8)	12.5 (<0.1[c] to 34.0)
IL-10 (pg/ml)	6.2 (3.1 to 9.3)	14.7 (7.9 to 21.6)	6.9 (3.4 to 10.4)	8.8 (3.8 to 13.8)	5.8 (2.9 to 8.8)	16.7 (7.6 to 25.7)	7.5 (5.2 to 9.9)	4.4 (2.3 to 6.4)
IL-1rα (pg/ml)	26.5 (10.6 to 42.4)	32.0 (14.4 to 49.6)	31.2 (14.4 to 50.6)	31.4 (12.3 to 50.6)	23.9 (11.3 to 36.5)	33.4 (15.6 to 51.1)	38.6 (15.0 to 62.1)	31.0 (14.9 to 47.1)
Stimulated elastase release (µg/ml)	1.7[‡] (0.5 to 3.0)	2.2 (1.5 to 2.9)	2.4 (1.6 to 3.1)	1.7 (1.0 to 2.5)	1.3[‡] (0.6 to 2.0)	2.2 (1.4 to 3.0)	2.7 (1.9 to 3.4)	2.3 (1.2 to 3.3)

Stimulated elastase release per neutrophil (fg/cell)	182 (155 to 209)	248 (161 to 336)	211 (121 to 301)	143 (97 to 189)		193 (147 to 238)	237 (139 to 225)	213 (141 to 284)	153 (118 to 187)
Aldosterone (nmol/L)	178 (93 to 262)	439§§ (252 to 626)	160†† (101 to 218)	146†† (95 to 196)		142 (88 to 196)	385§§ (229 to 541)	106†† (64 to 149)	88†† (53 to 122)
Serum sodium (mmol/L)	142 (141 to 144)	146 (137 to 154)	139†† (135 to 144)	138†† (133 to 143)		141 (140 to 142)	143 (137 to 148)	131†† (123 to 138)	134†† (128 to 139)
Serum potassium (mmol/L)	4.4 (4.2 to 4.6)	4.6 (4.2 to 5.0)	4.2 (4.0 to 4.5)	4.3 (3.9 to 4.7)		4.4 (4.1 to 4.7)	4.3 (3.9 to 4.6)	3.9 (3.5 to 4.3)	4.3 (4.0 to 4.7)
Serum calcium (mmol/L)‡	2.41 (2.32 to 2.49)	2.41 (2.27 to 2.55)	2.35§ (2.29 to 2.41)	2.35 (2.28 to 2.42)		2.38 (2.34 to 2.41)	2.33 (2.24 to 2.43)	2.20§ (2.08 to 2.32)	2.23 (2.12 to 2.35)

Mean (95% CI) (*n*= 8); MEOTime †† *P*< 0.01 and † P< 0.05 *vs* 0 h recovery, MEOTime §§ P< 0.01 and § P< 0.05 *vs* pre-exercise, MEOTime ⁑ P< 0.05 *vs* peak, [aa] P< 0.01 and [a] P< 0.05 *vs* CM, MEOTrial ‡ P< 0.05, [c] under detectable lowest standard.

Figure 5.2 Blood glucose (A) and serum insulin (B) concentrations after 2 h HIIT exercise protocol in temperate ambient conditions and a regular carbohydrate dietary intervention (CM: ●) or low carbohydrate dietary intervention (L-CHO: □).



Mean ± SEM (n= 8): MEOTrial ‡ P< 0.05, MEOTime [aa] P< 0.01 and [a] P< 0.05 *vs* CM.

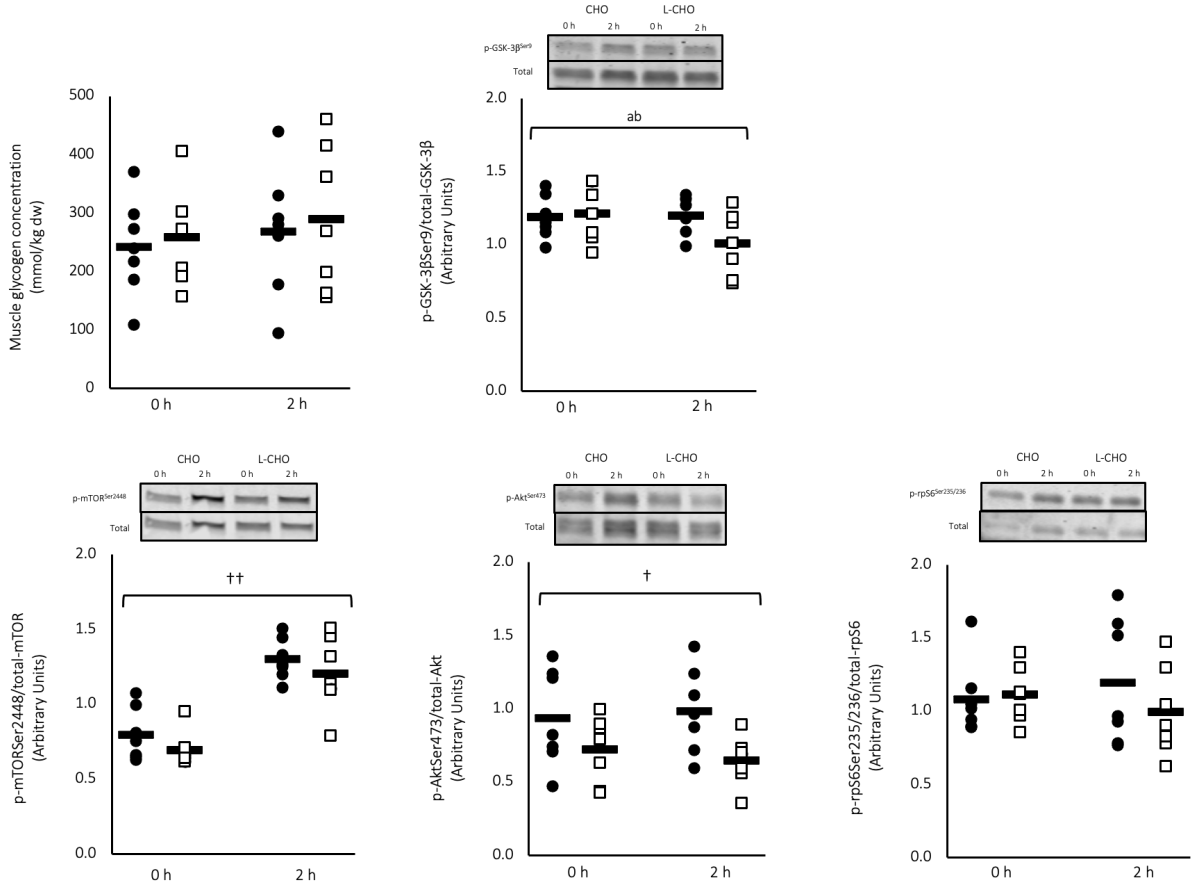
Muscle glycogen concentration

A trial*time interaction (P= 0.011) and significant difference in fold change (P= 0.012) was observed for ratio of phosphorylated of GSK-3β to total GSK-3β, whereby the ratio was unchanged from 0 h to 2 h recovery on CM (1.01 (0.99 to 1.04) fold change), but decreased on the L-CHO trial (0.83 (0.79 to 0.87) fold change) (**Figure 5.3B**). Neither trial significantly increased muscle glycogen from 0 h (250 (208 to 292) mmol/kg dw) to 2 h recovery (279 (219 to 338) mmol/kg dw (**Figure 5.3A).**

Phosphorylation of muscle signalling proteins

A greater ratio of phosphorylated Akt to total Akt was observed on CM (P= 0.027), however no trial differences were observed in the fold change from 0 h to 2 h recovery (**Figure 5.3D**). The ratio of phosphorylated to total mTOR increased from 0 h to 2 h recovery on both trials (P< 0.001; **Figure 5.3C**). No time or trial interactions were observed for the ratio of phosphorylated to total rpS6 (**Figure 5.3D**).

Figure 5.3 Muscle glycogen content (A), phosphorylated GSK3-β to total GSK3-β (B), ratio of phosphorylated mTOR to total mTOR (C), phosphorylated Akt to total Akt (D), and phosphorylated rpS6 to total rpS6 (E) after 2 h HIIT exercise in temperate ambient conditions and a regular carbohydrate dietary intervention (CM: ●) or low carbohydrate dietary intervention (L-CHO: □).



Mean and individual responses (n= 7): MEOTime †† P< 0.01 and † P< 0.05 *vs* 0 h recovery, [ab] P< 0.05 *vs* CM fold change.

Hydration status

P_{Osmol} was greater at 0 h (P< 0.01) and 2 h recovery (P< 0.05) on CM compared to L-CHO. P_V increased from 0 h to 2 h recovery (P< 0.05; **Table 5.2**). Total fluid intake during the recovery period was greater on L-CHO (27 (24 to 31) ml/kg BM) compared with CM (23 (20 to 27) ml/kg BM; P= 0.017). Plasma aldosterone values increased pre- to post-exercise (P< 0.05) and returned to baseline 2 h recovery (**Table 5.2**). Fluid retention (77 (71 to 83) %) did not differ between trials.

Electrolyte status

Plasma calcium concentrations were higher on CM compared to L-CHO (P= 0.033). Plasma sodium concentrations were significantly greater at 0 h recovery compared to 2 h and 4 h recovery on both trials (P< 0.01). No time or trial interactions were observed for plasma potassium (**Table 5.2**).

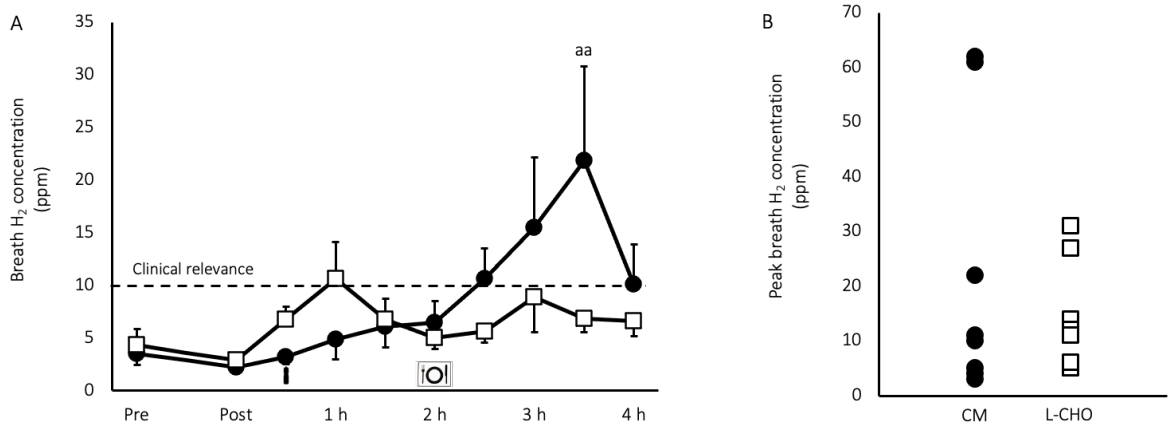
Immune function

An exercise-induced leukocytosis (10.6 (9.5 to 11.7) x10^9/L; P< 0.001), neutrophilia (7.0 (5.9 to 8.2) x10^9/L; P= 0.001), lymphocytosis (2.9 (2.6 to 3.2) x10^9/L; P= 0.011), monocytosis (0.7 (0.6 to 0.8) x10^9/L; P= 0.006), and increased neutrophil:lymphocyte ratio (3.0 (2.4 to 3.6); P< 0.001) were observed in the recovery period. No main effects or interaction were observed for unstimulated elastase, stimulated elastase or stimulated elastase release per neutrophil (Table 5.2). However, bacterially-stimulated total elastase release peaked post-exercise significantly greater than pre-exercise values in both trials (P= 0.025). Bacterially-stimulated neutrophil degranulation declined during recovery on both trials (-21%).

Gastrointestinal integrity and symptoms

There were no trial differences in intestinal injury as indicated by a rise in plasma I-FABP in response to exercise stress (Table 5.2). No significant main effects or interaction were observed for plasma sCD14 concentrations (Table 5.2). Breath H_2 concentrations were significantly greater on CM (22 (4 to 39) ppm) compared to L-CHO (7 (4 to 10) ppm; P< 0.01) at 3.5 h into recovery (Figure 5.4). The exercise load on both trials results in >60% incidence of GIS, which was sustained in recovery. During the recovery period, there were no trial differences in the severity of reported gastrointestinal symptoms (Table 5.3).

Figure 5.4 Breath hydrogen response (A) and individual peak breath hydrogen (B) after 2 h HIIT exercise in temperate ambient conditions and a regular carbohydrate dietary intervention (CM: ●) or low carbohydrate dietary intervention (L-CHO: □).



Mean ± SEM (*n*= 8): MEOTime aa P< 0.05 *vs* 0 h recovery.

Table 5.3 Incidence of gastrointestinal symptoms and severity of gut discomfort, total, upper-, and lower-gastrointestinal symptoms in response to 2 h HIIT exercise (between 60% and 80% $\dot{V}O_{2max}$) in temperate ambient conditions and after consumption of regular carbohydrate (CM) and the low carbohydrate (L-CHO) recovery beverages and meals.

	CM					L-CHO				
	Exercise		Recovery			Exercise		Recovery		
	Incidence % (severe)	Severity	Incidence % (severe)	Severity Acute (0-2 h)	Severity Total (0-4 h)	Incidence % (severe)	Severity	Incidence % (severe)	Severity Acute (0-2 h)	Severity Total (0-4 h)
Gut discomfort	NA	4 (5-14)	NA	2 (2-5)	3 (2-7)	NA	5 (5-19)	NA	2 (2-11)	3 (5-11)
Total GIS[a]	63 (50)	6 (5-27)	63 (25)	2 (2-5)	3 (2-11)	75 (50)	11 (2-65)	63 (38)	2 (2-11)	3 (5-11)
Upper GIS[b]	13 (0)	0 (3-3)	25 (0)	1 (2-3)	1 (2-3)	25 (25)	3 (5-20)	38 (38)	1 (2-6)	2 (5-7)
Belching	0 (0)	0 ----	0 (0)	0 ----	0 ----	13 (13)	0 (5-5)	11 (0)	0 (2-2)	0 (2-2)
Heartburn	13 (0)	2 (2-2)	0 (0)	0 ----	0 ----	13 (13)	1 (11-11)	11 (0)	0 ----	0 (3-3)
Bloating	0 (0)	0 ----	25 (0)	1 (2-3)	1 (2-3)	0 (0)	0 ----	25 (25)	1 (6-6)	2 (6-7)
Stomach pain	13 (0)	0 (1-1)	0 (0)	0 ----	0 ----	13 (13)	1 (9-9)	0 (0)	0 ----	0 ----
Urge to regurgitate	0 (0)	0 ----	0 (0)	0 ----	0 ----	0 (0)	0 ----	0 (0)	0 ----	0 ----

Regurgitation	0 (0)	0 ----	0 (0)	0 ----	0 ----	0 (0)	0 ----	0 (0)	0 ----	0 ----
Lower GIS[b]	38 (13)	2 (3-10)	25 (13)	0 (1-1)	1 (4-5)	25 (13)	4 (3-27)	13 (0)	0 ----	0 (1-1)
Flatulence	13 (0)	0 (0-1)	13 (0)	0 ----	0 (3-3)	13 (0)	0 (1-1)	0 (0)	0 ----	0 ----
Lower bloating	38 (0)	1 (3-3)	13 (0)	0 ----	1 (4-4)	13 (13)	1 (1-10_	13 (0)	0 ----	0 (1-1)
Urge to defecate	13 (0)	0 (3-3)	13 (13)	0 (1-1)	1 (5-5)	0 (0)	0 ----	0 (0)	0 ----	0 ----
Intestinal pain	13 (0)	0 (4-4)	0 (0)	0 ----	0 ----	25 (13)	1 (3-17)	0 (0)	0 ----	0 ----
Abnormal defecation[c]	0 (0)	0 ----	0 (0)	0 ----	0 ----	0 (0)	0 ----	0 (0)	0 ----	0 ----
Others										
Nausea	13 (0)	0 ----	0 (0)	0 ----	0 ----	13 (0)	0 (1-1)	0 (0)	0 ----	0 ----
Dizziness	50 (38)	4 (2-13)	38 (0)	1 (2-3)	1 (2-3)	50 (25)	4 (9-18)	25 (13)	1 (5-5)	1 (5-5)
Stitch[d]	0 (0)	0 ----	0 (0)	0 ----	0 ----	25 (0)	1 (404)	0 (0)	0 ----	0 ----
Feeding Tolerance										

Appetite	88 (75)	12 (2-29)	100 (88)	16 (4-28)	21 (4-39)	75 (63)	13 (8-43)	88 (88)	19 (12-48)	27 (18-71)
Thirst	100 (100)	19 (10-25)	100 (100)	13 (2-22)	16 (6-28)	100 (100)	21 (9-44)	100 (88)	15 (4-38)	22 (4-62)

Values are presented as means and range of participant reporting GIS incidence (n = 8). GIS incidence during exercise and recovery are presented as percentage of total participants reporting GIS ≥1 on the mVAS. GIS severity during exercise and recovery are presented as mean summative accumulation of mVAS rating scale of measured time periods and individual range of participant reporting GIS incidence (162,212). Summative accumulation of upper-, lower-, and other gastrointestinal symptoms,[a] summative accumulation of upper- or lower- gastrointestinal symptoms,[b] abnormal defecation including loose watery stools, diarrhoea and blood in stools,[c] and acute transient abdominal pain.[d] NA: not applicable. Wilcoxon signed-rank tests showed no differences between CM and L-CHO for GIS. Hedge's *g* measurement of effect size for GIS and feeding tolerance severity between CM and L-CHO was determined as >0.50 and >0.80 for medium and large effects, respectively; however, no medium or large effects size value were detected between CM and L-CHO.

Systemic inflammatory profile

IL-1β concentration was significantly higher on CM at 4 h recovery compared to L-CHO (P< 0.05; Table 5.2). IL-1rα concentrations were significantly greater at 2 h recovery compared to pre-exercise, and IL-10 concentrations increased at 0 h recovery then returned to baseline (Table 5.2). No time or trial interactions were observed for IL-6, IL-8 or TNF-α. No difference in exercise-induced SIR-profile (CM: 33 (10 to 56) arb.unit and L-CHO: 31 (15 to 48) arb.unit) and recovery beverage post-prandial SIR-profile (CM: 13 (-1 to 26) arb.unit and L-CHO: 12 (4 to 21) arb.unit) was observed between trials.

Recovery beverage sensory & alimentary profiles

Significant differences in taste (CM: 9 (8 to 10) out of 10 and L-CHO: 6 (5 to 7) out of 10; P< 0.001), flavour (CM: 9 (8 to 9) out of 10 and L-CHO: 6 (5 to 7) out of 10; P= 0.002), texture (CM: 8 (8 to 9) out of 10 and L-CHO: 6 (4 to 7) out of 10; P= 0.009), and aftertaste (CM: 9 (8 to 9) out of 10 and L-CHO: 7 (6 to 7) out of 10; P< 0.001) were noted between the two beverages. Significant differences in taste (CM: 9 (8 to 10), and L-CHO: 6 (5 to 7); P< 0.001), flavour (CM: 9 (8 to 9), and L-CHO: 6 (5 to 7); P= 0.002), texture (CM: 8 (8 to 9), and L-CHO: 6 (4 to 7); P= 0.009), and after taste (CM: 9 (8 to 9), and L-CHO: 7 (6 to 7); P< 0.001) were noted between the two recovery beverages. CM showed greater appreciation and oral sensory pleasure for taste, flavour, texture, and aftertaste than L-CHO.

Psychophysiological parameters & subsequent performance

There were no trial differences in nude BM (69.4 (66.9 to 72.0) kg), TBW (61.3 (60.0 to 62.5) %) and ECW (23.6 (23.0 to 24.2) %) the following morning. Participants reported greater readiness to invest mental (8 (7 to 9); P= 0.016) and physical (7 (5 to 9); P= 0.009) effort on CM, compared to L-CHO (7 (5 to 8) and 6 (5 to 8), respectively). A MEOTrial was observed for HR (P= 0.039), RER (P< 0.001), and carbohydrate (P= 0.002) and fat (P< 0.001) oxidation rates, characterised by a greater propensity for fat oxidation on L-CHO (Table 5.4). No trial differences were observed for $\dot{V}O_2$ or RPE at any intensity. There were no differences in the distance run (11.0 (10.0 to 11.9) km, HR (173 (170 to 177) bpm) or RPE (16 (1 to 16) during the 1 h performance test.

Table 5.4 Physiological and performance outcomes graded intensity breath-by-breath testing at 50-80% V̇O2max following consumption of regular carbohydrate (CM) and the low carbohydrate (L-CHO) recovery beverages and meals.

	CM				L-CHO			
	50% $\dot{V}O_{2max}$	60% $\dot{V}O_{2max}$	70% $\dot{V}O_{2max}$	80% $\dot{V}O_{2max}$	50% $\dot{V}O_{2max}$	60% $\dot{V}O_{2max}$	70% $\dot{V}O_{2max}$	80% $\dot{V}O_{2max}$
RER‡‡	0.897 (0.873 to 0.922)	0.926 (0.905 to 0.948)	0.936 (0.918 to 0.954)	0.995 (0.961 to 1.028)	0.812 (0.789 to 0.835)	0.860 (0.841 to 0.879)	0.885 (0.861 to 0.908)	0.924 (0.886 to 0.963)
$\dot{V}O_2$ (ml/kg/min)	25.9 (21.8 to 29.9)	33.0 (29.8 to 36.1)	38.6 (34.9 to 42.2)	45.5 (42.8 to 48.3)	27.4 (24.0 to 30.8)	34.8 (30.5 to 39.2)	41.1 (37.0 to 45.2)	49.2 (44.1 to 54.3)
CHO oxidation‡‡ (g/min)	1.6 (1.3 to 1.8)	2.3 (2.0 to 2.7)	2.9 (2.5 to 3.2)	4.2 (3.7 to 4.7)	0.9 (0.6 to 1.2)	1.6 (1.1 to 2.1)	2.2 (1.7 to 2.8)	3.3 (2.3 to 4.3)
Fat oxidation‡‡ (g/min)	0.3 (0.2 to 0.4)	0.3 (0.2 to 0.4)	0.3 (0.2 to 0.4)	0.1 (0.1 to 0.2)	0.6 (0.6 to 0.7)	0.6 (0.5 to 0.7)	0.6 (0.5 to 0.7)	0.4 (0.2 to 0.6)
HR‡‡	120 (105 to 134)	139 (126 to 152)	155 (141 to 169)	172 (159 to 185)	125 (111 to 139)	147 (134 to 159)	161 (146 to 176)	171 (157 to 184)
RPE	8 (7 to 9)	10 (9 to 11)	12 (10 to 13)	14 (12 to 15)	8 (7 to 9)	10 (8 to 12)	12 (11 to 14)	14 (12 to 16)

Mean (95% CI) (*n*= 8); MEOTrial ‡‡ P< 0.01

Discussion

The study aimed to determine the effects of a *'train-low'* nutritional protocol, using a lower carbohydrate recovery beverage, on markers of recovery optimisation, and subsequent exercise performance the following day, compared to a regular carbohydrate protocol using a recovery beverage containing a higher carbohydrate content. In line with our hypothesis, there were no trial differences in phosphorylation of mTOR-Akt-rpS6 signalling proteins. Contrary to our hypothesis, neither beverage increased muscle glycogen concentration in the acute recovery period (i.e., 2 h) or prevented the decrease in neutrophil functional responses in early recovery. Likewise, there were no trial differences in rehydration status, and despite differences in carbohydrate absorption, there were no differences in GIS. The low carbohydrate trial resulted in increased oxidation rates of fats, however there was no trial difference in endurance performance. Collectively, these outcomes suggest that reduced carbohydrate intake (i.e., <40 g per meal) does not impair acute (i.e., <24 h) recovery outcomes.

Replenishment of muscle glycogen stores has customarily been a primary focus of exercise recovery nutrition (6). In the current study, neither beverage increased muscle glycogen concentrations within 90 min after consumption, which is in contrast to previous reported research using 90 min intermittent cycling exercise protocol (98,99). While this was the intended and expected outcome from the low-carbohydrate beverage, these results are surprising given the standard carbohydrate beverage provided carbohydrate equivalent to current recovery nutrition guidelines (i.e., 1.2 g/kg BM) (6). It is possible that the eccentric-plyometric contractions induced muscle damage such that the impaired structural integrity of the myofibre prevented muscle glycogen disposal within the sampling timeframe (232). Indeed, the observed blood glucose and insulin responses, and greater GSK-3β activity on the CM trial indicate enhanced activity towards muscle glycogen disposal. A previous study that applied a modified *'train-low'* protocol and comparable relative intake of carbohydrate (i.e., <1.0 g/kg BM/24 h), observed mean muscle glycogen stores <300 mmol/kg dw prior to a *'train-low'* session performed 2

days after the initial glycogen-depleting exercise session (254). Therefore, although it was impractical and ethically challenging to perform muscle biopsies before the performance test in the current study, it is likely that replenishment of muscle glycogen was prevented under L-CHO conditions, but occurred under the CM trial, albeit with some attenuation associated to the plyometric-induced muscle damage (232).

Exercising with low carbohydrate availability has been shown to alter substrate utilisation and signalling mechanisms favouring fat oxidation, but may acutely reduce absolute training intensity (255). Although endogenous carbohydrate availability on day 2 of the experimental trial was not measured, we observed marked differences in fuel utilisation across intensities, suggesting a potential difference in muscle glycogen availability between CM and L-CHO. Interestingly, despite greater readiness to invest mental and physical effort on CM, and altered substrate oxidation rates, there were no overall differences in performance outcomes (e.g., 1 h distance test (mean and 95% CI): CM (11.1 (10.0 to 12.2) km) and L-CHO (10.7 (9.2 to 12.3) km). It is however important to note that the relative distance covered is indicative of recreational competitive athletes. Performance outcome results may have differed in an elite athlete population, whereby sustained exercise intensities during the performance test is expected to be higher and more reliant of endogenous carbohydrate availability. Previous studies that have observed reduced absolute exercise intensity with low carbohydrate availability are under conditions of reduced absolute energy intake and (or) where greater exercise intensities reliant on anaerobic oxidation must be reached (254). It is plausible that despite increased physiological strain, the L-CHO trial did not impair absolute exercise performance, as the 1 h distance run (i.e., <75% $\dot{V}O_{2max}$) may not be limited by lower glycogen availability. Alternatively, such findings could be the result of skeletal muscle glycogen stores remaining below resting levels on both trials due to the eccentric contraction induced muscle damage (232), and that the differing substrate utilisation was mediated by greater exogenous carbohydrate availability on CM (256). These findings have practical implications for athletes

undertaking mixed intensity glycogen depleting exercise before quality sessions and (or) repeated competitive events.

Adequate intake of leucine-rich protein is required to support myofibrillar, and possibly support mitochondrial protein synthesis after prolonged strenuous exercise, although further research using prolonged endurance, intermittent and (or) concurrent exercise models is required to substantiate the latter (153). In the current study, both the L-CHO (0.5 g PRO/kg BM) and the CM beverage (0.4 g PRO/kg BM) provide some evidence of enhanced cell signalling markers regulating translation initiation, as indicated by increased phosphorylation of mTOR 2 h into the recovery period. Moreover, minimal carbohydrate intake (i.e., >30 g) during the acute post-exercise period appears to be sufficient to induce insulin-mediated suppression of muscle protein breakdown (257). In the absence of muscle biopsy data from the subsequent day, the impact of 24 h carbohydrate restriction on Akt-mTOR-rpS6 signalling within the current protocol is unknown. Although not measured in the current study, previous research has observed that performing endurance exercise with low carbohydrate availability has a dual effect on net protein balance, characterised by increased breakdown and decreased synthesis, despite greater overall protein intake in matched (252), and reduced energetic conditions (254). Despite these acute observations, there is no evidence that chronic *'train-low'* interventions lead to substantial muscle wasting (147).

Endurance exercise is known to depress immune function and induce gastrointestinal injury, which may impact the assimilation of recovery nutrition (11,13,64). In the current study, modest exercise-induced immune and gastrointestinal perturbations were observed in response to the 2 h HIIT exercise protocol. Carbohydrate malabsorption occurred on the CM trial; however, similar breath H_2 concentrations have been detected amongst healthy individuals following consumption of sucrose and lactose at rest (224,225). The mild carbohydrate malabsorption did not translate to GIS, which is in accordance with previous findings (13,64), but contrary to other reports (163). These discrepancies are likely associated

carbohydrate intake timing, dosage, type, and individual variation. Both nutrition interventions failed to prevent the commonly observed post-exercise decrease in neutrophil functional response. These results are likely associated with the delay in beverage provision, as immediate post-exercise recovery beverage provisions prevent the neutrophil functional depression, linked to insulinotropic mechanisms (25,64). Whether a low-carbohydrate dairy milk beverage with equivalent or greater protein content (i.e., ≥0.5 g protein/kg BM), consumed immediately after exercise would prevent post-exercise immunodepression still requires substantiation.

Strengths and limitations

Despite a plethora of elegantly conducted recovery nutrition research, primarily focusing on individual aspects of recovery (i.e., muscle glycogen resynthesis, skeletal muscle protein resynthesis, and rehydration) the current study is the first to globally assess recovery outcomes within the context of a *'train-low'* nutritional protocol. This was achieved by implementing an exercise stress (i.e., 2 h HIIT) known to disturb all recovery markers, thereby ensuring practical relevance and broadening the scope of practical application to endurance and team sport athletes (253). We subsequently compared recovery outcomes using multiple, validated methods, in response to a low carbohydrate dairy milk recovery beverage and low carbohydrate meal provisions, compared to a higher carbohydrate dairy milk recovery beverage and higher carbohydrate meal provisions. A noted limitation is that it was not feasible (i.e., ethically, participant burden, and procedure would impact primary and secondary outcomes) to collect muscle biopsy samples before, 4 h after and 24 h after the exercise stress, to determine pre-exercise levels and the longer recovery period. Additionally, markers of EIMD (i.e., 24-48 h post-exercise) were not determined. In hindsight, blood samples collected at these time-points would provide greater insight into the time course of muscle glycogen resynthesis beyond 2 h recovery and any differences between trials. While EIMD potentially influenced recovery of skeletal muscle glycogen and (or) muscle protein synthesis, gastrointestinal function and patency, immune function, and hydration recovery outcomes are sound responses not confounded by any potential muscle

damage. Moreover, efforts were made to provide double-blinding throughout the experimental procedures, but it was not possible to blind participants to sensation differences between the recovery beverages and meals (i.e., flavour, taste, and texture), due to the vast contrast in beverage and meal nutritional composition. Therefore, risk of performance bias may exist, mainly on performance test result and (or) subjective outcomes (i.e., GIS, RPE, RTIME, RTIPE). However, participants were unaware of the nature and purpose of the interventions, despite having clear knowledge of the experimental procedures in accordance with ethical procedures (e.g., participant explanatory statement). We also acknowledge the limitations in the application of these findings directly to elite level athletes, particularly associated with the performance test. Nevertheless, these findings have meaningful practical relevance amongst competitive sub-elite and recreational masses. Acute recovery outcomes assessed in the current study likely translate to elite level cohorts, as they are in accordance with recovery nutrition guidelines and recommendation for the elite population (6,149). Finally, meal provisions were matched for energy, protein and fibre. However, participants were instructed to "eat as much as tolerable". With the exception of protein intake during dinner the evening of day 1, no statistical differences were detected. It is possible that differences in nutrient composition impacted on appetite and tolerance. The authors have differentiated between outcomes associated with the recovery beverage alone (i.e., gastrointestinal and immune status, hydration, blood glucose and serum insulin, and intra-muscular marker responses within 2 h recovery) and outcomes associated with overall dietary manipulation (i.e., RTIME, RTIPE fuel kinetics, and performance test) for clarity.

Practical applications

Considering recovery nutrition is a main part of athletes' dietary practices, consumption of a lower-carbohydrate or a standard dairy milk recovery beverage prompts instigation of physiological recovery processes. Therefore, both dairy milk recovery formulations appear suitable for acute recovery, and tolerable from a gastrointestinal perspective. Restoration of immune functional responses appears to be time dependant of nutrient availability post-exercise, with a delayed feeding not preventing the

exercise-associated immunodepression (i.e., neutrophil function), irrespective of nutrient content of the recovery beverage. Findings of an absent muscle glycogen resynthesis process in both recovery beverages suggests the exercise stress (e.g., 2 h HIIT) plays a role in the magnitude or nutritional recovery processes. Finally, in conjunction with appropriate dietary intervention (i.e., energy matched low carbohydrate dietary provisions), a *'train-low'* protocol does not substantially impair endurance running performance outcomes (i.e., a 1 h distance test) the following day in recreationally competitive athletes and their respective sustainable exercise intensities, despite shifts towards fuel oxidation kinetic adaptations (e.g., favouring increase fat oxidation at relative exercise intensities).

Conclusion

After 2 h of HIIT, consumption of a lower-carbohydrate or a regular carbohydrate dairy milk recovery beverage increased activation of protein synthesis expression markers and restored hydration status. Neither beverage presents characteristics of initiating muscle glycogen storage replenishment in the acute phase or recovery (2 h) despite showing expression of glycogen resynthesis markers, or prevented the post-exercise depression in neutrophil function. Substantial carbohydrate malabsorption occurs after consumption of the regular dairy milk recovery beverage, however this was not associated with greater GIS incidence or severity. Rates of total fat oxidation were greater on the lower-carbohydrate beverage, however performance was not affected. Acutely, when adequate overall energy intake and water is provided, consumption of a lower-carbohydrate recovery beverage did not negatively impact on recovery outcomes.

Chapter Six

General Discussion

The overall aim of the studies undertaken for this thesis was to compare different, but commonly consumed, recovery beverages of varying nutritional composition on integrative markers of exercise recovery following a 2 h high intensity exercise. This was achieved by firstly conducting a systematic literature review in two parts; the first to identify the ideal quantity of nutrients to support recovery optimisation following prolonged strenuous exercise, and the second to determine the effectiveness of dairy milk beverages for recovery optimisation following prolonged strenuous exercise. A normative methodological approach to exercise recovery research was established and applied to three randomised cross-over trials to investigate the effectiveness of a flavoured dairy milk beverage on markers of recovery optimisation, and subsequent performance, compared to a) a non-nitrogenous carbohydrate-electrolyte beverage, b) a high nutrient density reconstituted milk-based sports beverage and c) an low carbohydrate dairy milk-based sports beverage. Provision of recovery beverages was isovolumetric in each study, and provided in small, frequent doses (i.e., 3 isovolumetric doses over a 30 min period). It was hypothesised that a) the greater energy and nutrient density of a dairy based supplement beverage would result in greater muscle glycogen resynthesis, muscle protein synthesis, fluid retention, and enhanced immune functional responses, compared with a dairy milk beverage. In addition, the greater energy and nutrient density would result in greater ratings of feeding intolerance and GIS; b) the greater energy and nutrient density of a dairy based supplement beverage would result in greater muscle glycogen resynthesis, muscle protein synthesis, fluid retention, and enhanced immune functional responses, compared with a dairy milk beverage. In addition, the greater energy and nutrient density would result in greater ratings of feeding intolerance and GIS; and c) the low-carbohydrate nutritional protocol would result in a diminished rate of glycogen resynthesis, rehydration properties, immune functional responses, and subsequent endurance performance the following day, compared with standard carbohydrate intake; but would not differ in protein synthesis expression

markers, and may limit the gastrointestinal burden in response to nutrient intake into an exercise-associated compromised gastrointestinal tract.

In accordance with our hypothesis, the CM achieved greater fluid retention and increased signalling of mTOR-rpS6-Akt pathway, however both beverages induced clinically relevant carbohydrate malabsorption associated with mild gastrointestinal discomfort, and neither beverage increased muscle glycogen stores within 90 min of consumption, or prevented a decrease in *in vitro* neutrophil function. Secondly, in accordance with our hypothesis, the greater nutrient density of the MBSB induced greater carbohydrate malabsorption and gastrointestinal discomfort, however there were no observed differences in TBW or plasma hydration markers, or intramuscular mTOR-rpS6-Akt signalling. Again, neither beverage increased muscle glycogen stores within 90 min of consumption, or prevented a decrease in *in vitro* neutrophil function. Finally, as hypothesised, there were no trial differences in phosphorylation of mTOR-Akt-rpS6 signalling proteins and the low carbohydrate trial resulted in increased oxidation rates of fats, however there were no trial differences in endurance performance. Contrary to our hypothesis, there were no trial differences in rehydration status, and despite differences in carbohydrate absorption, there were no differences in GIS. Neither beverage increased muscle glycogen concentration in the acute recovery period or prevented the decrease in *in vitro* neutrophil functional responses.

The current studies are the first to look at recovery nutrition as a whole, from a sports dietetic practical perspective. Based on the systematic literature reviews conducted in this thesis, the major methodological issues identified within the existing body of literature include i) failure to consider gastrointestinal function and patency in the regulation of bioavailability and bioaccessiblity as a prerequisite to overall recovery; ii) failure to apply multiple, validated markers to globally assess outcomes associated with recovery; iiI) failure to apply quality controls, including blinding, specification of randomisation techniques, and prospective trial registration; iv) selection bias towards trained male

athletes aged <30 years. The standardised methodological approach applied within this thesis was designed to comprehensively assess gastrointestinal patency and function, innate immune markers that are responsive to nutrition interventions, skeletal muscle glycogen resynthesis, intramuscular anabolic signalling, hydration status and subsequent performance within 24 h.

Gastrointestinal integrity and function

It is now widely accepted that following prolonged endurance and (or) HIIT exercise, increased sympathetic drive and (or) reduced splanchnic output can perturb the patency and functionality of the gastrointestinal tract. EIGS, characterised by reduced tolerance to food and fluid, upper and low GIS, epithelial injury, reduced gastric motility, enzymatic release, and transporter activity, and (or) translocation of lumen-derived endotoxins and pathogenic agents, may impair nutrient bioavailability and bioaccessibility during the acute recovery period (11). A peak breath H_2 response of clinical relevance occurred following consumption of the CM (i.e., carbohydrate and protein; **Chapters 3-5**), CEB (i.e., non-nitrogenous and lower carbohydrate content; **Chapter 3**) and L-CHO (reduced carbohydrate content; **Chapter 5**) beverages. Conversely, the MBSB (i.e., isovolumetric with high nutrient density; **Chapter 4**) induced significantly greater malabsorption than CM, with an associated trend towards greater gastrointestinal discomfort. Given gastrointestinal injury was mild during all trials, it was proposed that the concentration of the MBSB lead to oversaturation of active and (or) passive transporters. Notably, there was large individual variation on each trial with regards to carbohydrate malabsorption and to a lesser degree, symptomology. Findings from the current study are supported by previous literature that demonstrate sub-clinical to mild carbohydrate malabsorption amongst healthy adults, following consumption of comparable quantities of lactose and sucrose at rest. To date, only one has also examined the effect of prolonged strenuous exercise on nutrient assimilation. Costa et al., 2019 also demonstrated that following 2 h running exercise at 70% $\dot{V}O_{2max}$ in temperate ambient conditions, clinically relevant carbohydrate malabsorption occurred 3 h after consumption of a comparable CM beverage, as well as isovolumetric consumption of plain water (13). Authors

attributed the rise in breath H_2 to malabsorption of the pre-exercise meal, associated with EIGS including delayed gastrointestinal transit, epithelial injury and impaired active and (or) passive transported release.

The studies presented in this thesis are the first to examine the effect of EIGS on subsequent nutrient malabsorption and symptomology between commonly consumed recovery nutrition beverages. From a practical perspective, these findings suggest that there is a threshold to the concentration and (or) volume of nutritive beverages that can be consumed before athletes experience severe GIS associated with clinically relevant malabsorption, even for lactose tolerant individuals. Based on the findings within this thesis, consumption 1.2 g carbohydrate and 0.4 g protein/kg BM induced carbohydrate malabsorption at the threshold of clinical relevance. High nutrient density beverages may appeal to individuals who experience low tolerance to food and fluid following training or competitive events, but this strategy may in fact present a greater risk of impaired subsequent performance due to severe GIS, and compromised recovery due to suboptimal delivery of nutrients to target organs and (or) systems. Based on the findings presented in this thesis in addition current nutrition guidelines, it is recommended that athletes consume small, frequent boluses (i.e., over a 30-60 min period) of solutions with tolerable carbohydrate content (6,11). Indeed, individual susceptibility to EIGS, food and fluid tolerance, and absorptive capacity varies greatly, therefore practitioners are encouraged to assess individual tolerance prior to making recommendations, and (or) conduct clinical assessment of individuals presenting with EIGS.

Immune function

A single bout of prolonged strenuous exercise is proposed to promote acute innate immune altering properties, including leukocytosis and lymphopenia, reduced phagocytic capacity, and increased systemic inflammatory profile. It has been suggested that exercise-induced immunodepression may increase the athlete's risk of contracting pathogen-borne illness, soft tissue injury and (or) burdening

training load. A functioning immune system is also important for regeneration of damaged epithelial gastrointestinal and skeletal muscle tissue, and clearance of endotoxins. Exercise-induced immune-depression was observed across all trials, and the type of recovery beverage consumed did not influence leukocyte trafficking, prevent a decrease in neutrophil degranulation, or influence the SIR profile. Failure to prevent further decline in immune function has been attributed to the 60 min delay in consumption of the recovery beverage after exercise. Indeed, our research team has previously demonstrated that immediate post-exercise consumption of a dairy milk recovery beverage of comparable nutritional composition boosted functional immune responses (64). It is theorised that the insulinotropic and hypercalcemic effect of the beverage consumed immediately post-exercise increased neutrophil chemotaxis, phagocytosis, and bactericidal capacity, and the effectiveness of neutrophil phagocytosis and degranulation processes, respectively (64). A limitation within the experimental protocol applied within this thesis was the use of an *in vitro* model to establish changes in neutrophil function, and the subsequent translational relevance. Previously used *in vivo* methods (i.e., immune responses assessed using hypersensitivity challenge with diphenylcyclopropenone antigen) to assess cell-mediated responses have not been identified to response to nutrition interventions. Therefore, the use of *in vitro* bacterial challenge is the most appropriate, validated method identified to assess innate immune function. Findings within this thesis highlight the importance of timing in the practical application of recovery nutrition strategies. Considering the existing body of literature and current recovery nutrition guidelines, it is recommended that consumption of a nutritive beverage, specifically a flavoured dairy milk beverage, immediately post-exercise in small, frequent doses, is ideal to prevent further decline in exercise-induced immunodepression and minimise gastrointestinal load.

Muscle glycogen resynthesis

Replenishment of skeletal muscle glycogen stores through high carbohydrate intake is a primary goal of acute recovery nutrition. In particular, athletes undertaking consecutive bouts of strenuous exercise

within the short term (e.g., same or following day) are encouraged to prioritise timely carbohydrate intake (6). The ideal quantity, quality, and timing of carbohydrate intake to maximally replenish endogenous glycogen stores has been extensively researched. However, the current body of research has neglected to consider gastrointestinal patency (e.g., food and fluid tolerance, upper and lower GIS, gastrointestinal motility, digestion, and absorption) and potentially immune responses associated with systemic pathogenic (e.g., luminal originated bacteria and (or) bacterial endotoxin) and tissue injury (e.g., gastrointestinal epithelial and skeletal muscle tissue cell damage debris) clearance (163,198). Moreover, the vast majority of studies upon which current nutrition guidelines are based, employ prolonged, moderate to high intensity cycling protocols, and therefore limit practical application to exercise modalities known induce greater perturbations to global markers of recovery outcomes (i.e., gastrointestinal epithelial and skeletal tissue muscle cell damage), such as prolonged running and intermittent sports (29).

A major finding from this thesis was that following 2 h HIIT exercise to reduce muscle glycogen concentration (overall mean (SD); 286 (108) mmol/kg dw), recovery beverages containing a) 1.2 g carbohydrate/kg BM (Chapter 3-5) b) 0.75 g carbohydrate/kg BM (Chapter 3) c) 2.2 g carbohydrate/kg BM (Chapter 4) and d) 0.35 g carbohydrate/kg BM (Chapter 5) did not increase muscle glycogen concentration within 90 min following consumption. Based on previous studies that have observed impaired muscle glycogen repletion following eccentric exercise (232,258,259), it was theorised that impaired GLUT-4 translocation and (or) insulin signalling associated with eccentric contraction induced muscle damage prohibited glucose uptake and subsequent glycogen disposal within the muscle cell. The observed blood glucose, insulin and GSK-3β responses support this theory. Blood glucose and insulin responses with 1.2 g, 0.75 g and 2.2 g carbohydrate/kg BM indicate glucose absorption by the gastrointestinal tract, systemic availability, and uptake by insulin-sensitive tissues. Phosphorylation of GSK-3β remained unchanged or increased slightly, suggesting glucose uptake was limited at the level of skeletal muscle sarcolemma. Contrary to the findings presented in this thesis, it was initially thought

that impaired glycogen resynthesis was distinctively observed 24-48 h after the injury, and not during the early recovery phase (i.e., 4-6 h post-exercise) (256,258). However, a more recent study by Zendher et al., demonstrated impaired repletion of muscle glycogen stores 2 h after mixed steady state and eccentric exercise, despite consumption of 112 g carbohydrate (approximately 1.6 g/kg BM) immediately post-exercise (260). The major differences between the present study and Zendher et al., compared to earlier studies, is the timing and amount of carbohydrate consumed during recovery. Immediately post-exercise, participants in studies conducted by Widrick et al., and Doyle et al., consumed 4.25 g/kg BM over 2 h, and 6.4 g/kg BM over 4 h, respectively (256,258). Both the present study and Zendher et al. al provided a single dose of carbohydrate (1.2 and 1.6 g/kg BM, respectively). Moreover, in the current study, feeding was delayed by 60 min as it was impossible to provide the recovery beverages immediately post-exercise, given the nature of data collection and time required for preparation and sampling of muscle.

Glucose uptake is mediated by insulin- and contraction-induced translocation of GLUT-4 to the sarcolemma, supposedly by independent signalling pathways that contribute to the biphasic nature of muscle glycogen resynthesis (29). During the initial, rapid phase of muscle glycogen resynthesis, contraction-induced translocation of the GLUT-4 transporter occurs independent of insulin signalling. During the latter phase, following continuous concentric muscle contractions, insulin sensitivity of the muscle increases, and stimulates GLUT-4 translocation. Conversely, 24-48 h after eccentric exercise protocols, insulin sensitivity, GLUT-4 concentration of the sarcolemma, and insulin-stimulated glucose uptake all appear to be diminished (232,261-264). A recent murine study has provided evidence that following high-intensity eccentric contractions, insulin-stimulated glucose uptake, but not contraction-induced uptake, is inhibited 30 min, and 130-150 min post-exercise (265). Since the mechanisms of glucose uptake and skeletal muscle glycogen resynthesis were not a focal point of this thesis, GLUT-4 transporter concentration was not measured. However, considering the existing body of research, it is theorised that having withheld carbohydrate during this initial period of contraction-induced GLUT-4

glucose uptake, successful replenishment of glycogen stores would be predominantly dependent on insulin-stimulated pathways for GLUT-4 translocation, which, as demonstrated by Anderson et al., appear to be impaired for up to 2 h following intense eccentric muscular contractions (265). In line with this theory, early studies by Widrick et al., and Doyle et al., successfully demonstrated muscle glycogen repletion during the early phases of recovery, by capitalising on the insulin-independent phase of glucose uptake via frequent, high carbohydrate feedings (256,258).

A noted limitation within the current study is that muscle samples were not obtained at rest, 4 h recovery or the following morning. However, the studies within this thesis focused on acute recovery (i.e., 2 h), as from a practical perspective athletes would normally consume a recovery beverage after exercise and a meal 2-4 h into recovery. Therefore, a muscle biopsy taken at 4 h recovery would have been contaminated with a recovery meal input. With the samples taken in the experimental protocol, the acute rate of muscle glycogen resynthesis in response to each recovery beverage was successfully established. Second, due to various constraints (e.g., predominantly participant burden and invasiveness within the context of the experimental design) this was not feasible in the current protocol. It is acknowledged that previous studies have similarly examined rates of muscle glycogen resynthesis within the early (0-2 h post-exercise) recovery period with and without examining later time points, so we follow these previous studies as methodological guidance (86,98,99).

These findings are of practical relevance, especially for athletes participating in sports that involve HIIT interspersed with eccentric and (or) explosive efforts (i.e., football, soccer, basketball, tennis). Once again, athletes are encouraged to prioritise high carbohydrate intake (i.e., 1.2 g carbohydrate/kg BM) in small, frequent doses immediately post-exercise. The effect of plyometric and eccentric activity on muscle glycogen resynthesis, including the ideal nutrient intake and time course for repletion, warrants further investigation.

Muscle protein synthesis

Both MPS and MPB increase in response to prolonged, strenuous and (or) intermittent exercise. Consumption of adequate, high quality protein during the acute recovery period is required to create a positive NPB, towards muscle fibre accretion. Phosphorylation of proteins mTOR, Akt and rpS6 within the total protein pool were measured to evaluate the effect of each beverage on promoting MPS. Given the role of protein as both a mechanistic stimulator and nitrogenous substrate for muscle protein synthesis, greater upregulation of anabolic pathways in the presence of increased circulating amino acids was expected (37,146,187). Dairy beverages containing a) CM: 0.4 g protein/kg BM (**Chapters 3-5**) b) MBSB: 0.8 g protein/kg BM (**Chapter 4**), and c) L-CHO: 0.4 g protein/kg BM (**Chapter 5**), increased phosphorylation of mTOR within 90 min of consumption. Increased phosphorylation of Akt was observed following consumption of the MBSB (i.e., 0.8 g protein/kg BM; **Chapter 4**) and for some, but not all, cohorts consuming the CM beverage (i.e., 0.4 g/kg BM; **Chapters 3-5**). Indeed, phosphorylation of Akt has been shown to peak 30-60 min after endurance exercise (42). As such, the peak activation of Akt may have been captured for some, but not all individuals.

As acknowledged in previous chapters, it was not possible to perform continuous isotopic tracer infusions for quantification of MPS. Although fluctuations in the concentration of phosphorylated signalling proteins have not been consistently correlated to changes in fractional synthetic rate and long-term functional outcomes, such changes can provide an indication of acute muscle protein anabolism in response to exercise and nutrition intervention (220). Indeed, findings from the original research studies align with the systematic literature review (**Chapter 2, Part I**) of this thesis, showing in a step-wise manner that consumption of a nitrogenous beverage containing 0.2-0.4 g protein/kg BM will stimulate mixed and myofibrillar muscle protein synthesis to a greater extent than a non-nitrogenous beverage, but this effect plateaus at intakes greater than 0.5 g protein/kg BM (94,110-116).

Hydration

It has been consistently shown that following prolonged strenuous and (or) intermittent exercise with none or minimal water provision, mild dehydration ensues as indicated by body water losses of ≤3%. Replacement of fluid in excess of what is lost is essential to allow for ongoing urine losses to excrete waste, maintenance of blood volume, and to prevent decrements in subsequent performance. In the studies presented within this thesis, euhydration was maintained throughout all experimental trials, as indicated by TBW and P_{Osmol}. In line with these findings, other research teams have observed that when a prescribed volume is given, dairy milk beverages provide superior hydration potential to carbohydrate-electrolyte beverages. This appears to be proportional to the energy density of the beverage, as opposed to the sodium and (or) overall electrolyte content. However, when intake is *ad libitum* and participants have access to food and water, there is no discernible difference in the hydration status 4 h after consumption of dairy and non-dairy recovery beverages.

Despite each beverage restoring hydration status to pre-exercise levels, subtle differences in the fluid dynamics were observed during each experimental trial. Most notably, the CM and MBSB beverages were delivered into circulation more slowly, as evidenced by a drop in P_V during the second half of the recovery period during the CEB and L-CHO trials. As discussed in previous chapters, it appears that the greater energy density of the CM and MBSB beverages delayed gastric emptying, preventing a rapid hypervolemia, decreased P_{Osmol}, and prevented subsequent diuresis. Although not reflected in other markers of hydration (i.e., P_V change, P_{Osmol}, BM change or fluid retention), faecal losses were significantly greater on MBSB, presumably associated with significant carbohydrate malabsorption inducing an intestinal osmotic gradient and subsequent loose and (or) diarrhoea bowel movements (Chapter 4). A noted oversight within the current study design is that urine and faecal losses were not diligently measured at regular intervals. However, the use of multiple, validated plasma and body water markers of hydration collected at each timepoint has successfully monitored hydration status throughout the experimental protocol thus answering the research questions. It is concluded that when

adequate food and water is unavailable, BM losses are significant (i.e., ≥3%) and (or) there is <4 h prior to subsequent training and (or) competitive events, small and frequent boluses of a chocolate flavoured dairy milk beverage offers the best option to rapidly achieve and sustain euhydration. In accordance with current rehydration guidelines, it is contended in each experimental chapter of this thesis that exercise-induced fluid losses ≤2% BM loss is insufficient to warrant aggressive hydration strategies, provided athletes have adequate time for recovery (i.e., >4 h) and access to food and water.

Substrate oxidation & performance

In recent years there has been increasing interest in the manipulation of the substrate storage profile of the skeletal muscle between training sessions and (or) competitive events to influence substrate utilisation and performance. A final major finding within this thesis was the significant shifts in substrate oxidation following consumption of the L-CHO (i.e., 0.3 g CHO/kg BM) and withholding of carbohydrates during subsequent meals (i.e., <0.4 g CHO/kg BM/meal; **Chapter 5**). The application of train-low compete-high protocols has seen high uptake by competitive athletes seeking strategies to enhance training adaptations towards a competitive edge. However, the ideal protocols, including timing and amount of carbohydrate intake relative to training, remain elusive. Although it was not possible to determine muscle glycogen content prior to assessment of oxidation rates and performance, these findings provide evidence of manipulation of substrate utilisation across intensities, without significant impairment in performance (up to 80% $\dot{V}O_{2max}$). These findings need to be verified at greater intensities and amongst elite level athletes. Moreover, the different nutritional compositions of the dairy milk beverage, carbohydrate electrolyte beverage and milk-based sports beverage did not significantly alter substrate utilisation or performance outcomes the following morning (**Chapters 3 & 4**). Readiness to invest physical effort was greater with the dairy milk beverage, compared to the carbohydrate-electrolyte and low-carbohydrate beverages, but not the milk-based sports beverage, and was not associated with differences in exercise performance. Moreover, readiness to invest physical effort varied between chapters for the dairy milk beverage. Physical and psychological readiness is sensitive

to confounding factors (i.e., lifestyle stressors) (215), and should be used in conjunction with validated, objective exercise performance measures. Differences in the nutritional profile of each recovery beverage is inconsequential within the context of overall nutritional intake over a 24 h window, for a single subsequent exercise bout. However, as previously discussed, the acute clinical (i.e., minimising gastrointestinal burden, stimulating neutrophil function towards clearance of tissue debris, endotoxins and luminal-derived pathogenic agents, reduced risk of illness and soft tissue injury) and physiological (i.e., maximised nutrient absorption and hydration, substrate provision for repletion of skeletal muscle glycogen, and substrate and anabolic stimulus for skeletal muscle repair and adaptation) implications of immediate consumption of small and frequent boluses of a dairy milk beverage is likely to provide a cumulative advantage within an extended training regimen.

Overall strengths and limitations

The studies within this thesis are the first to globally assess outcomes of recovery optimisation using four commonly consumed recovery beverages. These studies provide a comprehensive breakdown of a variety of recovery beverages to which athletes are commonly exposed; namely protein, nutrient density, and carbohydrate variations. A novel aspect of the studies within this thesis is the assessment of gastrointestinal patency and function as a primary outcome. Previous literature has assessed gastrointestinal symptoms and (or) tolerance secondary to other aspects of recovery (i.e., muscle glycogen resynthesis and (or) hydration status). The studies within this thesis are the first to examine intestinal injury, carbohydrate malabsorption and gastrointestinal symptomology as a prerequisite to assimilation of recovery nutrition targeted at other aspects of recovery. Moreover, each of the variables examined within the standardised methodology used established and validated measures. Although preceding studies have been indispensable in formulating current recovery nutrition guidelines, such studies have examined recovery outcomes in isolation, failed to apply multiple, validated outcome measures and (or) have introduced risk of bias (i.e., selecting, performance, detection and (or) reporting bias). Another strength of the standardised experimental protocol is the exercise stress was designed

to challenge and disturb all recovery markers, thereby ensuring practical relevance and broadening the scope of practical application.

The focus of this thesis was to examine the effects of various recovery beverages on acute recovery markers (i.e., 2 h). As previously discussed, a noted limitation within the experimental protocol is that it was impossible to collect muscle biopsy samples before, 4 h after and 24 h after the exercise stress. It is acknowledged that samples collected at these timepoints would provide valuable insight into the time course of muscle glycogen resynthesis beyond 2 h recovery, however it was not ethical nor practical to collect samples at these timepoints. Moreover, these samples would be contaminated by the consumption of one or more recovery meals, and would not reflect the efficacy of the recovery beverages specifically. Indeed, it is likely that no differences were detected during the performance test as it was impacted by total diet. This could be resolved by conducting the performance test 2-3 h after the exercise stress, however it would be ethically impossible to conduct a performance test on a treadmill in such close temporal proximity to two repeated muscle biopsies. It would be possible to examine recovery optimisation and performance outcomes during the acute recovery period using C MRS technology, however this technology is expensive and not accessible to many sports and exercise laboratories. Another limitation within the current thesis is the imbalance between male and female participant numbers. The authors experienced difficulty recruiting female athletes to take part in the current study to completion, presumably due to the high burden and invasive nature of the experimental protocol. Despite lower female participant numbers, power calculations indicated n= 8 was sufficient to detect differences of a magnitude of practical and clinical relevance for the investigated markers.

Milk as a recovery beverage; practical implications

The chocolate flavoured dairy milk beverage and low-carbohydrate milk-based beverage achieved most of the intended outcomes for recovery optimisation. Based on the evidence presented in this thesis, the low carbohydrate milk-based beverage achieved the same overall acute recovery outcomes as the

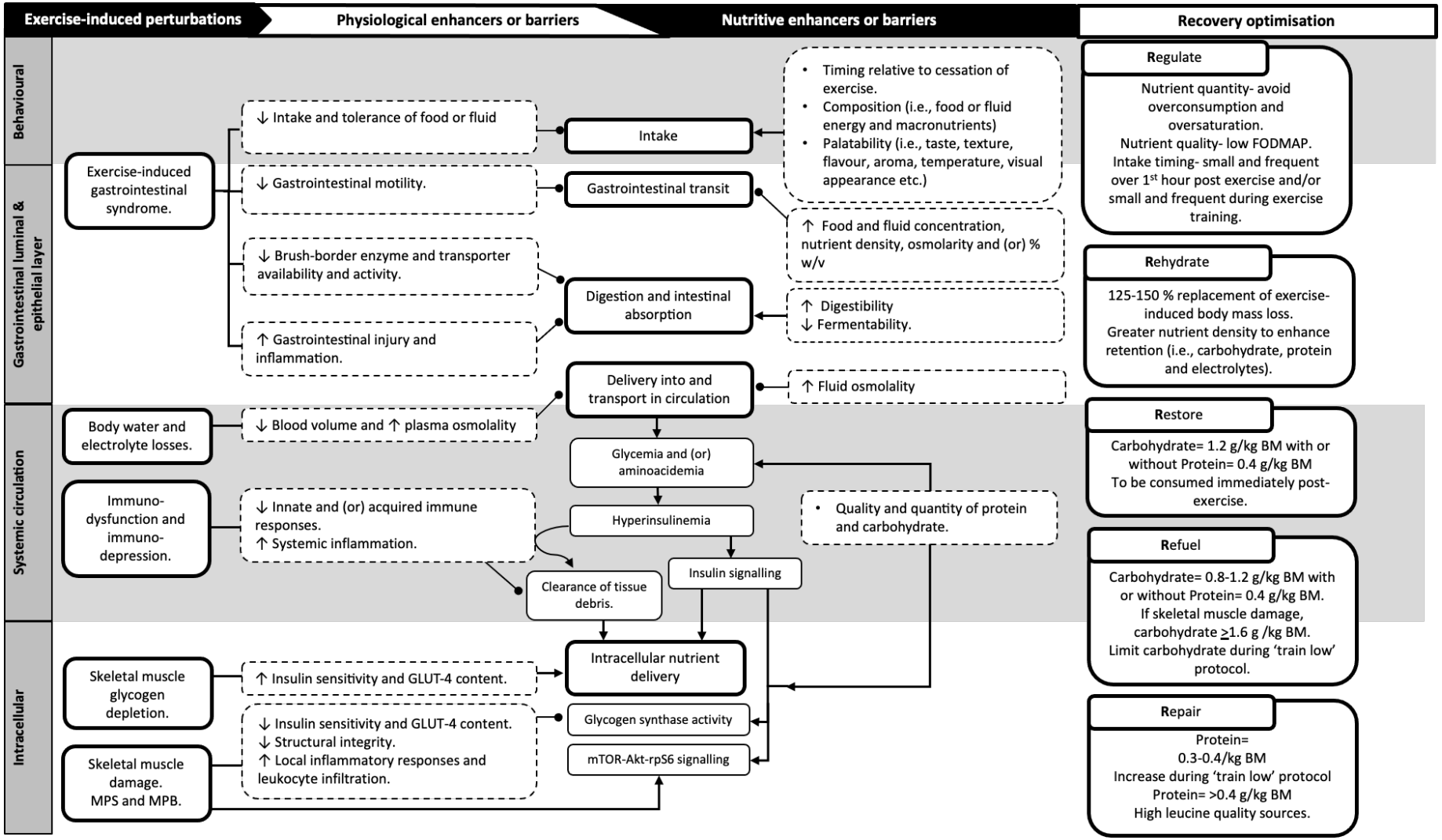
regular carbohydrate milk beverage, and may also support training adaptation via shifting substrate utilisation towards greater fat oxidation. With regards to the chocolate flavoured dairy milk beverage, failure to increase muscle glycogen concentration and prevent a decrease in neutrophil function is likely attributed to limitations associated with the exercise stress and timing of ingestion, as opposed to inadequate nutrition provided by the beverage *per se*. As previously discussed, following continuous concentric exercise models, provision of 1.2 g carbohydrate per kg BM immediately post-exercise has been shown to increase muscle glycogen concentration and prevent a decrease in neutrophil function. Isovolumetric intake of the reconstituted milk-based sports beverage similarly failed to 'refuel' and 'restore', and also resulted in significantly greater carbohydrate malabsorption, which in some cases was associated with severe gastrointestinal symptoms and greater estimated faecal fluid losses. The greater nutrient concentration of the milk-based sports beverage does not provide any physiological benefits over the flavoured dairy milk beverage.

Directions for future research

The major directions for future research investigating recovery optimisation include i) verification of the prevention of *in vitro* neutrophil functional decline with immediate consumption of a flavoured dairy milk beverage containing 1.2 g carbohydrate and 0.4 g protein/kg BM in response to prolonged continuous, HIIT and (or) exertional heat strain exercise model; ii) verification of rates of skeletal muscle glycogen resynthesis in response to 2 h HIIT and consumption of 1.2 g carbohydrate/kg BM with or without 0.4 g protein/kg BM; iii) investigation into the time course and (or) underlying mechanisms that prevent skeletal muscle glycogen disposal following eccentric and (or) plyometric exercise; iv) quantification of myofibrillar, mitochondrial and mixed muscle protein synthesis following mixed continuous concentric and eccentric muscle contractions and variable protein quantity and quality intake; v) quantitative and qualitative assessment of exercise-induced muscle damage during the acute and extended (i.e., 24-72 h) recovery period, with and without protein supplementation; vi) investigation into long-term implications of chronic (i.e., $\geq$4 weeks) *'train-low'* protocols on skeletal muscle mass, neutrophil function and (or) susceptibility to pathogenic invasion; vii) characterisation of

the influence of training status and biological sex on exercise-induced muscle damage and subsequent protein synthesis and skeletal muscle glycogen resynthesis.

Figure 6.1 Depiction of inter-related exercise-induced, physiological and nutritive factors that influence recovery optimisation outcomes and requirements.



↑: Increase, ↓: decrease, ⟶: enhance, ⟶●: inhibit, MPS: muscle protein synthesis, MPB: muscle protein breakdown, GLUT-4: glucose transporter 4, mTOR: mammalian target of rapamycin, Akt: protein kinase B, rpS6: ribosomal protein S6, FODMAP: fermentable oligo-, di- and monosaccharides and polyols, BM: body mass

Conclusion

Together the studies within this thesis highlight that exercise-induced perturbations are dependent on individual and external factors, and that assimilation of recovery nutrition may be inhibited by physiological (i.e., gastrointestinal or skeletal muscle injury), and nutritive (i.e., nutritional composition, concentration, timing of intake) factors. Therefore, strategies to overcome these barriers for optimal nutrient assimilation will most likely need to target physiological (i.e., gastrointestinal and muscular training) and nutritional (i.e., composition, timing, type) interventions. Recovery beverages and sports foods are designed to provide a convenient source of nutrition, and are only effective when used appropriately in relation to the exercise stress and individual nutritional requirements. These findings and the use of recovery beverage are of importance to athletes, coaches, and sports dietitians.

The major conclusion from this thesis are:

1) Provision of the recommended 1.2 g carbohydrate/kg BM 60 min after 2 h HIIT failed to increase muscle glycogen stores within 90 min of consumption, possibly associated with impaired insulin signalling and (or) GLUT-4 translocation due to plyometric induced muscle damage. These findings question the appropriateness of current recovery nutrition guidelines for athletes involved in high intensity intermittent and (or) eccentric sports.

2) Provision of the recommended 1.2 g carbohydrate and 0.4 g protein/kg BM 60 min after 2 h HIIT, and all comparator beverages, failed to prevent a decrease in neutrophil function, most likely associated with the delay in feeding and associated hyperinsulinemia and calcemia.

3) The 2 h HIIT exercise stress resulted in mild intestinal injury that resulted in clinically significant malabsorption of 0.75 g sucrose/kg BM and 1.2 g carbohydrate (combined lactose and

sucrose)/kg BM, similar to carbohydrate malabsorption observed amongst healthy adults following consumption of 75 g sucrose and 50 g lactose at rest.

4) Consumption of 2.2 g carbohydrate (combined lactose and sucrose)/kg BM resulted in significantly greater carbohydrate malabsorption compared to 1.2 g lactose and sucrose/kg BM, and was associated with increased gastrointestinal discomfort.

5) The low carbohydrate milk-based beverage achieved the same overall acute recovery outcomes as the regular carbohydrate milk beverage, but resulted in increased fat oxidation across intensities (50-80 % $\dot{V}O_{2max}$) the following morning without compromising performance amongst recreational level athletes.

Chapter Seven Works Cited

1. Hulteen RM, Smith JJ, Morgan PJ, Barnett LM, Hallal PC, Colyvas K, et al. Global participation in sport and leisure-time physical activities: A systematic review and meta-analysis. Preventative Medicine. 2017 Feb; 1(95): p. 14-25.
2. Anderson JJ. The State of Running. [Online].; 2020 [cited 2020 Aug 14. Available from: https://runrepeat.com/state-of-running.
3. Bailey RL, Gahche JJ, Lentino CV, Dwyer JT, Engel JS, Thomas PR, et al. Dietary supplement use in the United States, 2003–2006. The Journal of Nutrition. 2011 Feb; 141(2): p. 161-6.
4. Garthe I, Maughan RJ. Athletes and supplements: prevalence and perspectives. International Journal of Sports Nutrition and Exercise and Metabolism. 2018 Mar; 28(2): p. 126-38.
5. Maughan RJ, Depiesse F, Geyer H. The use of dietary supplements by athletes. Journal of Sports Sciences. 2007 Dec; 25(S1): p. S103-13.
6. Thomas DT, Erdman KA, Burke LM. American Journal of Sports Medicine Joint Position Statement. Nutrition and athletic performance. Medicine and Science in Sports and Exercise. 2016; 48(3): p. 543-568.
7. Alghannam AF, Jedrzejewski D, Bilzon J, Thompson D, Tsintzas K, Betts JA. Influence of post-exercise carbohydrate-protein ingestion on muscle glycogen metabolism in recovery and subsequent running exercise. International Journal of Sport Nutrition and Exercise Metabolism. 2016 Dec; 26(6): p. 572-80.
8. Wong SH, WIlliams C. Influence of different amounts of carbohydrate on endurance running capacity following short term recovery. International Journal of Sports Medicine. 2000 Aug; 21(6): p. 444-52.
9. Magalhães J, Rebelo A, Oliveira E, Silva JR, Marques F, Ascensão A. Impact of Loughborough Intermittent Shuttle Test versus soccer match on physiological, biochemical and neuromuscular parameters. European Journal of Applied Physiology. 2010 Jan; 108(1): p. 39.
10. Miyama MO, Nosaka KA. Influence of surface on muscle damage and soreness induced by consecutive drop jumps. Journal of Strength and Conditioning Research. 2004 May; 18(2): p. 206-11.
11. Costa RJ, Snipe RM, Kitic CM, Gibson PR. Systematic review: exercise-induced gastrointestinal syndrome—implications for health and intestinal disease. Alimentary Pharmacology and Therapeutics. 2017 Aug; 46(3): p. 246-65.
12. Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop N, et al. Position statement part one: immune function and exercise. 2011.
13. Costa RJ, Camões-Costa V, Snipe RM, Dixon D, Russo I, Huschtscha Z. Impact of exercise-induced hypohydration on gastrointestinal integrity, function, symptoms, and systemic endotoxin and inflammatory profile. Journal of Applied Physiology. 2019 May; 126(5): p. 1281-91.
14. Snipe RM, Khoo A, Kitic CM, Gibson PR, Costa RJ. The impact of exertional-heat stress on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profile. European Journal of Applied Physiology. 2018 Feb; 118(2): p. 389-400.
15. Snipe RM, Costa RJ. Does biological sex impact intestinal epithelial injury, small intestine permeability, gastrointestinal symptoms and systemic cytokine profile in response to exertional-heat stress? Journal of Sports Sciences. 2018 Dec; 36(24): p. 2817-35.
16. Tidball JG, Villalta SA. Regulatory interactions between muscle and the immune system during muscle regeneration. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2010 May; 298(5): p. R1173-87.

17. Costa RJ, Snipe RM, Camões-Costa V, Scheer V, Murray A. The impact of gastrointestinal symptoms and dermatological injuries on nutritional intake and hydration status during ultramarathon events. Sports Medicine - Open. 2016 Dec; 2(1): p. 16.
18. Peake JM, Neubauer O, Walsh NP, Simpson RJ. Recovery of the immune system after exercise. Journal of Applied Physiology. 2017 May; 122(5): p. 1077-87.
19. Impey SG, Hearris MA, Hammond KM, Bartlett JD, Louis J, Close GL, et al. Fuel for the work required: a theoretical framework for carbohydrate periodization and the glycogen threshold hypothesis. Sports Medicine. 2018 May; 48(5): p. 1031-48.
20. Roy BD. Milk: the new sports drink? A Review. Journal of the International Society of Sports Nutrition. 2008 Dec; 5(1): p. 15.
21. Burke LM, Peeling P. Methodologies for investigating performance changes with supplement use. International Journal of Sports Nutrition and Exercise Metabolism. 2018 Mar; 28(2): p. 159-69.
22. Mitchell JH, Haskell W, Snell P, Van Camp SP. Classification of sports. Journal of the American College of Cardiology. 2005 Apr; 45(8): p. 1364-7.
23. Wilmore JH, Costill DL, Kenney WL. Physiology of Sport and Exercise. In Human Kinetics. IL: Champaign; 1994.
24. Burke LM, Mujika I. Nutrition for Recovery in Aquatic Sports. International Journal of Sports Nutrition and Exercise Metabolism. 2014 Aug; 24(4): p. 425-36.
25. Costa RJ, Walters R, Bilzon JL, Walsh NP. Effects of immediate postexercise carbohydrate ingestion with and without protein on neutrophil degranulation. International Journal of Sport Nutrition and Exercise Metabolism. 2011 Jun; 21(3): p. 205-13.
26. Holloszy JO. Biochemical adaptations in muscle effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. Journal of Biological Chemistry. 1967 May; 242(9): p. 2278-82.
27. Bergström J, Hermansen L, Hultman E, Saltin B. Diet, muscle glycogen and physical performance. Acta Physiologica Scandinavica. 1967 Oct; 71(2): p. 140-50.
28. Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E, et al. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. American Journal of Physiology-Endocrinology And Metabolism. 1993 Sep; 265(3): p. E380-91.
29. Jentjens R, Jeukendrup AE. Determinants of post-exercise glycogen synthesis during short-term recovery. Sports Medicine. 2003 Feb; 33(2): p. 117-44.
30. Maehlum S, Hermansen L. Muscle glycogen concentration during recovery after prolonged severe exercise in fasting subjects. Scandinavian Journal of Clinical and Laboratory Investigation. 1978 Oct; 38(6): p. 557-60.
31. McCoy MI, Proietto JO, Hargreaves MA. Skeletal muscle GLUT-4 and postexercise muscle glycogen storage in humans. Journal of Applied Physiology. 1996 Feb; 80(2): p. 411-5.
32. Danforth WH. Glycogen synthetase activity in skeletal muscle interconversion of two forms and control of glycogen synthesis. Journal of Biological Chemistry. 1965 Feb; 240(2): p. 588-93.
33. Zachwieja JJ, Costill DL, Pascoe DD, Robergs RA, Fink WJ. Influence of muscle glycogen depletion on the rate of resynthesis. Medicine and Science in Sports and Exercise. 1991 Jan; 23(1): p. 44-8.
34. Bergström J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. Scandanavian Journal of Clinical and Laboratory Investigation. 1975 Jan; 35(7): p. 609-16.
35. Roden M, Shulman GI. Applications of NMR spectroscopy to study muscle glycogen metabolism in man. Annual Review of Medicine. 1999 Feb; 50(1): p. 277-90.

36. Greene J, Louis J, Korostynska O, Mason A. State-of-the-Art Methods for Skeletal Muscle Glycogen Analysis in Athletes-The Need for Novel Non-Invasive Techniques. Biosensors. 2017 Feb; 23(1).
37. Phillips SM, Hartman JW, Wilkinson SB. Dietary protein to support anabolism with resistance exercise in young men. Journal of the American College of Nutrition. 2005 Apr; 24(2): p. 134S-9S.
38. Biolo G, Maggi SP, Williams BD, Tipton KD, Wolfe RR. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. American Journal of Physiology-Endocrinology And Metabolism. 1995 Mar; 268(3): p. E514-20.
39. Phillips SM, Tipton KD, Aarsland AS, Wolf SE, Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. American Journal of Physiology-Endocrinology and Metabolism. 1997 Jul; 273(1): p. E99-107.
40. Carraro FA, Stuart CA, Hartl WH, Rosenblatt JU, Wolfe RR. Effect of exercise and recovery on muscle protein synthesis in human subjects. American Journal of Physiology-Endocrinology And Metabolism. 1990 Oct; 259(4): p. E470-6.
41. Sheffield-Moore M, Yeckel CW, Volpi E, Wolf SE, Morio B, Chinkes DL, et al. Postexercise protein metabolism in older and younger men following moderate-intensity aerobic exercise. American Journal of Physiology-Endocrinology and Metabolism. 2004 Sep; 287(3): p. E513-22.
42. Camera DM, Edge J, Short MJ, Hawley JA, Coffey VG. Early time course of Akt phosphorylation after endurance and resistance exercise. Medicine & Science in Sports and Exercise. 2010 Oct; 42(10): p. 1843-52.
43. Hornberger TA, Esser KA. Mechanotransduction and the regulation of protein synthesis in skeletal muscle. Proceedings of the Nutrition Society. 2004 May; 63(2): p. A37.
44. Coffey VG, Hawley JA. The molecular bases of training adaptation. Sports Medicine. 2007 Sep; 37(9): p. 737-63.
45. Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes and ageing. Nature Reviews Molecular Cell Biology. 2011 Jan; 12(1): p. 21-35.
46. Coffey VG, Zhong Z, Shield A, Canny BJ, Chibalin AV, Zierath JR, et al. Early signaling responses to divergent exercise stimuli in skeletal muscle from well-trained humans. The FASEB Journal. 2006 Jan; 20(1): p. 190-2.
47. Mascher H, Andersson H, Nilsson PA, Ekblom B, Blomstrand E. Changes in signalling pathways regulating protein synthesis in human muscle in the recovery period after endurance exercise. Acta Physiologica. 2007 Sep; 191(1): p. 67-75.
48. Wilkinson SB, Phillips SM, Atherton PJ, Patel R, Yarasheski KE, Tarnopolsky MA, et al. Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. The Journal of Physiology. 2008 Aug; 586(15): p. 3701-17.
49. Rennie MJ, Smith KE, Watt PW. Measurement of human tissue protein synthesis: an optimal approach. American Journal of Physiology-Endocrinology And Metabolism. 1994 Mar; 266(3): p. E298-307.
50. Wilkinson DJ, Brook MS, Smith K, Atherton PJ. Stable isotope tracers and exercise physiology: past, present and future. J Physiol. 2017 May; 595(9).
51. McCartney D, Desbrow B, Irwin C. The effect of fluid intake following dehydration on subsequent athletic and cognitive performance: A systematic review and meta-analysis. Sports Medicine Open. 2017 Dec; 3(1): p. 13.
52. Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, Stachenfeld NS. American College of Sports Medicine position stand. Exercise and fluid replacement. Medicine and Science in Sports and Exercise. 2007 Feb; 39(2): p. 377-90.

53. Sawka MN, Coyle EF. Influence of body water and blood volume on thermoregulation and exercise performance in the heat. Exercise and Sports Science Review. 199 Jan; 14(1): p. E112.
54. Cheuvront SN, Carter R, Montain SJ, Sawka MN. Daily body mass variability and stability in active men undergoing exercise-heat stress. International Journal of Sports Nutrition and Exercise Metabolism. 2004 Oct; 14(5): p. 532-40.
55. Holmes N, Miller V, Bates G, Zheo Y. The effect of exercise intensity on sweat rate and sweat sodium loss in well trained athletes. Journal of Science and Medicine and Sport. 2011 Dec; 14(1): p. E112.
56. Baker LB. Sweating rate and sweat sodium concentration in athletes: a review of methodology and intra/interindividual variability. Sports Medicine. 2017 Mar; 47(1): p. 111-28.
57. Armstrong LE, Pumerantz AC, Fiala KA, Roti MW, Kavouras SA, Casa DJ, et al. Human hydration indices: acute and longitudinal reference values. International Journal of Sport Nutrition and Exercise Metabolism. 2010 Apr; 20(2): p. 145-53.
58. Armstrong LE. Assessing hydration status: the elusive gold standard. Journal of the American College of Nutrition. 2007 Oct; 26(Supp5): p. 575S-84S.
59. Popowski LA, Oppliger RA, Lambert GP, Johnson RF, Johnson AK, Gisolfi CV. Blood and urinary measures of hydration status during progressive acute dehydration. Medicine & Science in Sports and Exercise. 2001 May; 33(5): p. 747-53.
60. Walsh NP. Recommendations to maintain immune health in athletes. European Journal of Sport Science. 2018 Jul; 18(6): p. 820-31.
61. Walsh NP, Gleeson M, Pyne DB, Nieman DC, Dhabhar FS, Shephard RJ, et al. Position statement part two: maintaining immune health. 2011.
62. Peters ED. Ultramarathon running and upper respiratory tract infections-an epidemiological survey. South African Medical Journal. 1983 Oct; 64(16): p. 582-4.
63. Nieman DC, Johanssen LM, Lee JW, Arabatzis K. Infectious episodes in runners before and after the Los Angeles Marathon. Journal of Sports Medicine and Physical Fitness. 1990 Sep; 30(3): p. 316-28.
64. Costa RJ, Camões-Costa V, Snipe RM, Dixon D, Russo I, Huschtscha Z. The Impact of a Dairy Milk Recovery Beverage on Bacterially Stimulated Neutrophil Function and Gastrointestinal Tolerance in Response to Hypohydration Inducing Exercise Stress. International Journal of Sport Nutrition and Exercise Metabolism. 2020 May; 30(4): p. 237-48.
65. Gleeson M. Immunological aspects of sport nutrition. Immunology and Cell Biology. 2016 Feb; 94(2): p. 117-23.
66. Rehrer NJ, Smets A, Reynaert H, Goes E, De Meilleir K. Effect of exercise on portal vein blood flow in man. Medicine and Science in Sports and Exercise. 2001 Sep; 33(9): p. 1533-7.
67. Rowell LB, Blackmon JR, Bruce RA. Indocyanine green clearance and estimated hepatic blood flow during mild to maximal exercise in upright man. The Journal of Clinical Investigation. 1964 Aug; 43(8): p. 1677-90.
68. Lang JA, Gisolfi CV, Lambert GP. Effect of exercise intensity on active and passive glucose absorption. International Journal of Sport Nutrition and Exercise Metabolism. 2006 Oct; 16(5): p. 485-93.
69. Snipe RM, Khoo A, Kitic CM, Gibson PR, Costa RJ. Carbohydrate and protein intake during exertional heat stress ameliorates intestinal epithelial injury and small intestine permeability. Applied Physiology, Nutrition, and Metabolism. 2017; 42(12): p. 1283-92.
70. Van Wijck K, Pennings B, Van Bijnen AA, Senden JM, Buurman WA, Dejong CH, et al. Dietary protein digestion and absorption are impaired during acute post-exercise recovery in young men. American Journal of Physiology-Heart and Circulatory Physiology. 2012 Dec; 26.

71. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Annals of Internal Medicine. 2009 Aug; 151(4): p. 264-9.
72. Higgins JP, Green S. Cochrane handbook for systematic reviews of interventions. In.: John Wiley & Sons; 2011.
73. Battram DS, Shearer J, Robinson D, Graham TE. Caffeine ingestion does not impede the resynthesis of proglycogen and macroglycogen after prolonged exercise and carbohydrate supplementation in humans. Journal of Applied Physiology. 2004 Mar; 96(3): p. 943-50.
74. Betts JA, Williams C, Boobis L, Tsintzas K. Increased carbohydrate oxidation after ingesting carbohydrate with added protein. Medicine and Science in Sports and Exercise. 2008 May; 40(5): p. 491-6.
75. Blom PC, Høstmark AT, Vaage O, Kardel KR, Maehlum SV. Effect of different post-exercise sugar diets on the rate of muscle glycogen synthesis. Medicine and Science in Sports and Exercise. 1987 Oct; 19(5): p. 491-6.
76. Carrithers JA, Williamson DL, Gallagher PM, Godard MP, Schulze KE, Trappe SW. Effects of postexercise carbohydrate-protein feedings on muscle glycogen restoration. Journal of Applied Physiology. 2000 Jun; 88(6): p. 1976-82.
77. Cogan KE, Evans M, Iuliano E, Melvin A, Susta D, Neff K, et al. Co-ingestion of protein or a protein hydrolysate with carbohydrate enhances anabolic signaling, but not glycogen resynthesis, following recovery from prolonged aerobic exercise in trained cyclists. European Journal of Applied Physiology. 2018 Feb; 118(2): p. 349-59.
78. Cramer MJ, Dumke CL, Hailes WS, Cuddy JS, Ruby BC. Postexercise glycogen recovery and exercise performance is not significantly different between fast food and sport supplements. International Journal of Sport Nutrition and Exercise Metabolism. 2015 Oct; 25(5): p. 448-55.
79. Detko E, O'Hara JP, Thelwall PE, Smith FE, Jakovljevic DG, King RF, et al. Liver and muscle glycogen repletion using 13 C magnetic resonance spectroscopy following ingestion of maltodextrin, galactose, protein and amino acids. British Journal of Nutrition. 2013 Sep; 110(5): p. 848-55.
80. Fuchs CJ, Gonzalez JT, Beelen M, Cermak NM, Smith FE, Thelwall PE, et al. Sucrose ingestion after exhaustive exercise accelerates liver, but not muscle glycogen repletion compared with glucose ingestion in trained athletes. Journal of Applied Physiology. 2016 Jun; 120(11): p. 1328-34.
81. Ivy JL, Katz AL, Cutler CL, Sherman WM, Coyle EF. Muscle glycogen synthesis after exercise: effect of time of carbohydrate ingestion. Journal of Applied Physiology. 1988 Apr; 64(4): p. 1480-5.
82. Ivy JL, Lee MC, Brozinick JJ, Reed MJ. Muscle glycogen storage after different amounts of carbohydrate ingestion. Journal of Applied Physiology. 1988 Nov; 65(5): p. 2018-23.
83. Jentjens RL, Van Loon LJ, Mann CH, Wagenmakers AJ, Jeukendrup AE. Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis. Journal of Applied Physiology. 2001 Aug; 91(2): p. 839-46.
84. Naperalsky M, Ruby B, Slivka D. Environmental temperature and glycogen resynthesis. International Journal of Sports Medicine. 2010 Aug; 31(8): p. 561-6.
85. Pederson DJ, Lessard SJ, Coffey VG, Churchley EG, Wootton AM, Ng T, et al. High rates of muscle glycogen resynthesis after exhaustive exercise when carbohydrate is coingested with caffeine. Journal of Applied Physiology. 2008 Jul; 105(1): p. 7-13.
86. PiehlAulin K, Söderlund K, Hultman E. Muscle glycogen resynthesis rate in humans after supplementation of drinks containing carbohydrates with low and high molecular masses. European Journal of Applied Physiology. 2000 Jan; 81(4): p. 346-51.

87. Reed MJ, Brozinick JJ, Lee MC, Ivy JL. Muscle glycogen storage postexercise: effect of mode of carbohydrate administration. Journal of Applied Physiology. 1989 Feb; 66(2): p. 720-6.
88. Ruby BC, Gaskill SE, Slivka D, Harger SG. The addition of fenugreek extract (Trigonella foenum-graecum) to glucose feeding increases muscle glycogen resynthesis after exercise. Amino Acids. 2005 Feb; 28(1): p. 71-6.
89. Tsintzas K, Williams C, Boobis L, Symington S, Moorehouse J, Garcia-Roves P, et al. Effect of carbohydrate feeding during recovery from prolonged running on muscle glycogen metabolism during subsequent exercise. International Journal of Sports Medicine. 2003 Aug; 24(6): p. 452-8.
90. Van Hall G, Saris WH, Van de Schoor PA, Wagenmakers AJ. The effect of free glutamine and peptide ingestion on the rate of muscle glycogen resynthesis in man. International Journal of Sports Medicine. 2000 Jan; 21(1): p. 25-30.
91. Van Hall G, Shirreffs SM, Calbet JA. Muscle glycogen resynthesis during recovery from cycle exercise: no effect of additional protein ingestion. Journal of Applied Physiology. 2000 May; 88(5): p. 1631-6.
92. Van Loon LJ, Saris WH, Kruijshoop M, Wagenmakers AJ. Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. The American Journal of Clinical Nutrition. 2000 Jul; 72(1): p. 106-11.
93. Wallis GA, Hulston CJ, Mann CH, Roper HP, Tipton KD, Jeukendrup AE. Postexercise muscle glycogen synthesis with combined glucose and fructose ingestion. Medicine and Science in Sports and Exercise. 2008 Oct; 40(10): p. 1789-94.
94. Wilkinson SB, Kim PL, Armstrong D, Phillips SM. Addition of glutamine to essential amino acids and carbohydrate does not enhance anabolism in young human males following exercise. Applied Physiology Nutrition and Metabolism. 2006 Oct; 31(5): p. 518-29.
95. Williams MB, Raven PB, Fogt DL, Ivy JL. Effects of recovery beverages on glycogen restoration and endurance exercise performance. The Journal of Strength and Conditioning Research. 2003 Feb; 17(1): p. 12-9.
96. Yaspelkis BB. The effect of a carbohydrate-arginine supplement on postexercise carbohydrate metabolism. International Journal of Sports Nutrition and Exercise Metabolism. 1999 Sep; 9(3): p. 241-50.
97. Zawadzki KM, Yaspelkis BB, Ivy JL. Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. Journal of Applied Physiology. 1992 May; 72(5): p. 1854-9.
98. Bowtell JL, Gelly K, Jackman ML, Patel A, Simeoni M, Rennie MJ. Effect of different carbohydrate drinks on whole body carbohydrate storage after exhaustive exercise. Journal of Applied Physiology. 2000 May; 88(5): p. 1529-36.
99. Bowtell JL, Gelly K, Jackman ML, Patel A, Simeoni M, Rennie MJ. Effect of oral glutamine on whole body carbohydrate storage during recovery from exhaustive exercise. Journal of Applied Physiology. 1999 Jun; 86(6): p. 1770-7.
100. Casey A, Mann R, Banister K, Fox J, Morris PG, Macdonald IA, et al. Effect of carbohydrate ingestion on glycogen resynthesis in human liver and skeletal muscle, measured by 13C MRS. American Journal of Physiology-Endocrinology and Metabolism. 2000 Jan; 278(1): p. E65-75.
101. Cheng IS, Huang SW, Lu HC, Wu CL, Chu YC, Lee SD, et al. Oral hydroxycitrate supplementation enhances glycogen synthesis in exercised human skeletal muscle. British Journal of Nutrition. 2012 Apr; 107(7): p. 1048-55.
102. Tsai TW, Chang CC, Liao SF, Liao YH, Hou CW, Tsao JP, et al. Effect of green tea extract supplementation on glycogen replenishment in exercised human skeletal muscle. British Journal of Nutrition. 2017 May; 117(10): p. 1343-50.

103. Tsao JP, Liao SF, Korivi M, Hou CW, Kuo CH, Wang HF, et al. Oral conjugated linoleic acid supplementation enhanced glycogen resynthesis in exercised human skeletal muscle. Journal of Sports Sciences. 2015 May; 33(9): p. 915-23.
104. Carper MJ, Richmond SR, Whitman SA, Acree LS, Godard MP. Muscle Glycogen Restoration in Females and Males Following Moderate Intensity Cycling Exercise in Differing Ambient Temperatures. Journal of Exercise Physiology Online. 2013 Aug; 16(4).
105. Ferguson-Stegall L, McCleave EL, Ding Z, Doerner III PG, Wang B, Liao YH, et al. Postexercise carbohydrate–protein supplementation improves subsequent exercise performance and intracellular signaling for protein synthesis. The Journal of Strength and Conditioning Research. 2011 May; 25(5): p. 1210-24.
106. Flynn S, Rosales A, Hailes W, Ruby B. Males and females exhibit similar muscle glycogen recovery with varied recovery food sources. European Journal of Applied Physiology. 2020 Mar; 25: p. 1-2.
107. Kammer L, Ding Z, Wang B, Hara D, Liao YH, Ivy JL. Cereal and nonfat milk support muscle recovery following exercise. Journal of the International Society of Sports Nutrition. 2009 Dec; 6(1): p. 11.
108. Tarnopolsky MA, Bosman M, Macdonald JR, Vandeputte D, Martin J, Roy BD. Postexercise protein-carbohydrate and carbohydrate supplements increase muscle glycogen in men and women. Journal of Applied Physiology. 1997 Dec; 83(6): p. 1877-83.
109. Wang B, Ding Z, Wang W, Hwang J, Liao YH, Ivy JL. The effect of an amino acid beverage on glucose response and glycogen replenishment after strenuous exercise. The European Journal of Applied Physiology. 2015 Jun; 115(6): p. 1283-94.
110. Breen L, Philip A, Witard OC, Jackman SR, Selby A, Smith K, et al. The influence of carbohydrate–protein co-ingestion following endurance exercise on myofibrillar and mitochondrial protein synthesis. The Journal of Physiology. 2011 Aug; 589(16): p. 4011-25.
111. Camera DM, Hawley JA, Coffey VG. Resistance exercise with low glycogen increases p53 phosphorylation and PGC-1α mRNA in skeletal muscle. European Journal of Applied Physiology. 2015 Jun; 115(6): p. 1185-94.
112. Churchward-Venne TA, Pinckaers PJ, Smeets JS, Peeters WM, Zorenc AH, Schierbeek H, et al. Myofibrillar and mitochondrial protein synthesis rates do not differ in young men following the ingestion of carbohydrate with whey, soy, or leucine-enriched soy protein after concurrent resistance-and endurance-type exercise. The Journal of Nutrition. 2019 Feb; 149(2): p. 210-20.
113. Rowlands DS, Nelson AR, Phillips SM, Faulkner JA, Clarke J, Burd NA, et al. Protein–leucine fed dose effects on muscle protein synthesis after endurance exercise. Medicine and Science in Sport and Exercise. 2015 Mar; 47(3): p. 547-55.
114. Harber MP, Konopka AR, Jemiolo B, Trappe SW, Trappe TA, Reidy PT. Muscle protein synthesis and gene expression during recovery from aerobic exercise in the fasted and fed states. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2010 Nov; 299(5): p. R1254-62.
115. Howarth KR, Moreau NA, Phillips SM, Gibala MJ. Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. Journal of Applied Physiology. 2009 Apr; 106(4): p. 1394-402.
116. Lunn WR, Pasiakos SM, Colletto MR, Karfonta KE, Carbone JW, Anderson JM, et al. Chocolate milk and endurance exercise recovery: protein balance, glycogen, and performance. Medicine and Science in Sports and Exercise. 2012 Apr; 44(4): p. 682-91.
117. Wong SH, Sun FH, Huang WY, Chen YJ. Effects of beverages with variable nutrients on rehydration and cognitive function. International Journal of Sports Medicine. 2014 Dec; 35(14): p. 1208-15.

118. Shirreffs SM, Taylor AJ, Leiper JB, Maughan RJ. Post-exercise rehydration in man: effects of volume consumed and drink sodium content. Medicine and Science in Sports and Exercise. 1996 Oct; 28(10): p. 1260.
119. Kovacs EM, Schmahl RM, Senden JM, Brouns F. Effect of high and low rates of fluid intake on post-exercise rehydration. International Journal of Sports Nutrition and Exercise Metabolism. 2002 Mar; 12(1): p. 14-23.
120. Amano T, Sugiyama Y, Okumura J, Fujii N, Kenny GP, Nishiyasu T, et al. Effects of isomaltulose ingestion on postexercise hydration state and heat loss responses in young men. Experimental Physiology. 2019 Oct; 104(10): p. 1494-504.
121. Jiminez CH, Koulmann NA, Mischler IS, Allevard AM, Launay JC, Savourey GU, et al. Plasma compartment filling after exercise or heat exposure. Medicine and Science in Sport and Exercise. 2002 Oct; 34(10): p. 209-15.
122. Maughan RJ, Owen JH, Shirreffs SM, Leiper JB. Post-exercise rehydration in man: effects of electrolyte addition to ingested fluids. European Journal of Applied Physiology and Occupational Physiology. 1994 May; 69(3): p. 209-15.
123. Evans GH, Shirreffs SM, Maughan RJ. Postexercise rehydration in man: the effects of osmolality and carbohydrate content of ingested drinks. Nutrition. 2009 Sep; 25(9): p. 905-13.
124. Evans GH, Mattin L, Ireland I, Harrison W, Yau AM, McIver V, et al. Bolus ingestion of whey protein immediately post-exercise does not influence rehydration compared to energy-matched carbohydrate ingestion. Nutrients. 2018 Jun; 10(6): p. 769.
125. Hobson R, James L. The addition of whey protein to a carbohydrate–electrolyte drink does not influence post-exercise rehydration. Journal of Sports Sciences. 2014 May; 33(1): p. 77-84.
126. James LJ, Mattin L, Aldiss P, Adebishi R, Hobson RM. Effect of whey protein isolate on rehydration after exercise. Amino Acids. 2014 May; 46(5): p. 1217-24.
127. Li L, Sun FH, Huang WY, Wong SH. Effects of whey protein in carbohydrate-electrolyte drinks on post-exercise rehydration. European Journal of Sport Science. 2018 May; 18(5): p. 685-94.
128. Maughan RJ, Leiper JB. Sodium intake and post-exercise rehydration in man. European Journal of Applied Physiology and Occupational Physiology. 1995 Sep; 71(4): p. 311-9.
129. Maughan RJ, McArthur M, Shirreffs SM. Influence of menstrual status on fluid replacement after exercise induced dehydration in healthy young women. British Journal of Sports Medicine. 1996 Mar; 30(1): p. 41-7.
130. Seery S, Jakeman P. A metered intake of milk following exercise and thermal dehydration restores whole-body net fluid balance better than a carbohydrate–electrolyte solution or water in healthy young men. British Journal of Nutrition. 2016 Sep; 116(6): p. 1013-21.
131. Shirreffs SM, Maughan RJ. Volume repletion after exercise-induced volume depletion in humans: replacement of water and sodium losses. American Journal of Physiology-Renal Physiology. 1998 May; 274(5): p. F868-75.
132. Shirreffs SM, Watson P, Maughan RJ. Milk as an effective post-exercise rehydration drink. British Journal of Nutrition. 2007 Jul; 98(1): p. 173-80.
133. Watson P, Love TD, Maughan RJ, Shirreffs SM. A comparison of the effects of milk and a carbohydrate-electrolyte drink on the restoration of fluid balance and exercise capacity in a hot, humid environment. European Journal of Applied Physiology. 2008 Nov; 104(4): p. 633-42.
134. Wong SH, Chen Y. Effect of a carbohydrate-electrolyte beverage, lemon tea, or water on rehydration during short-term recovery from exercise. International Journal of Sport Nutrition and Exercise Metabolism. 2011 Aug; 21(4): p. 300-10.

135. Wong SH, Williams C, Adams N. Effects of ingesting a large volume of carbohydrate-electrolyte solution on rehydration during recovery and subsequent exercise capacity. International Journal of Sports Nutrition and Exercise Metabolism. 2000 Dec; 10(4): p. 375-93.
136. McCartney D, Irwin C, Cox GR, Desbrow B. Fluid, energy, and nutrient recovery via ad libitum intake of different commercial beverages and food in female athletes. Applied Physiology Nutrition and Metabolism. 2019; 44(1): p. 37-46.
137. Thomas DR, Cote TR, Lawhorne L, Levenson SA, Rubenstein LZ, Smith DA, et al. Understanding clinical dehydration and its treatment. Journal of the American Medical Directors Association. 2008 Jun; 9(5): p. 292-301.
138. Costa RJ, Oliver SJ, Laing SJ, Walters R, Bilzon JL, Walsh NP. Influence of timing of postexercise carbohydrate-protein ingestion on selected immune indices. International Journal of Sport Nutrition and Exercise Metabolism. 2009 Aug; 19(4): p. 366-84.
139. Costa RJ, Fortes MB, Richardson K, Bilzon JL, Walsh NP. The effects of postexercise feeding on saliva antimicrobial proteins. International Journal of Sport Nutrition and Exercise Metabolism. 2012 Jun; 22(3): p. 184-91.
140. Burke LM, Hawley JA, Wong SH, Jeukendrup AE. Carbohydrates for Training and Competition. Journal of Sports Science. 2011 Jan; 29(Supp1): p. S17-27.
141. Beelen M, Burke LM, Gibala MJ, Van Loon LJ. Nutritional strategies to promote postexercise recovery. International Journal of Sport Nutrition and Exercise Metabolism. 2010 Dec; 20(6): p. 515-32.
142. Costill DL, Bowers R, Branam G, Sparks K. Muscle glycogen utilization during prolonged exercise on successive days. Journal of Applied Physiology. 1971 Dec; 31(6): p. 834-8.
143. Hickner RC, Fisher JS, Hansen PA, Racette SB, Mier CM, Turner MJ, et al. Muscle glycogen accumulation after endurance exercise in trained and untrained individuals. Journal of Applied Physiology. 1997 Sep; 83(3): p. 897-903.
144. Areta JL, Hopkins WG. Skeletal muscle glycogen content at rest and during endurance exercise in humans: A meta-analysis. Sports Medicine. 2018 Sep; 48(9): p. 2091-102.
145. Børsheim E, Cree MG, Tipton KD, Elliott TA, Aarsland A, Wolfe RR. Effect of carbohydrate intake on net muscle protein synthesis during recovery from resistance exercise. Journal of Applied Physiology. 2004 Feb; 1.
146. Biolo G, Tipton KD, Klein S, Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. American Journal of Physiology-Endocrinology And Metabolism. 1997 Jun; 273(1): p. E122-9.
147. Marquet LA, Brisswalter J, Louis J, Tiollier E, Burke LM, Hawley JA, et al. Enhanced endurance performance by periodization of CHO intake:" Sleep Low" strategy. Medicine and Science in Sports and Exercise. 2016 Apr; 48(4): p. 663-72.
148. Marquet LA, Hausswirth C, Molle O, Hawley JA, Burke LM, Tiollier E, et al. Periodization of carbohydrate intake: Short-term effect on performance. Nutrients. 2016 Dec; 8(12): p. 755.
149. Burke LM, Hawley JA, Jeukendrup A, Morton JP, Stellingwerff T, Maughan RJ. Toward a common understanding of diet–exercise strategies to manipulate fuel availability for training and competition preparation in endurance sport. International Journal of Sport Nutrition and Exercise Metabolism. 2018 Sep; 28(5): p. 451-63.
150. Areta JL, Burke LM, Ross ML, Camera DM, West DW, Broad EM, et al. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. The Journal of Physiology. 2013 May; 591(9): p. 2319-31.
151. Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, et al. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. The American Journal of Clinical Nutrition. 2009 Jan; 89(1): p. 161-8.

152. Witard OC, Jackman SR, Breen L, Smith K, Selby A, Tipton KD. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. The American Journal of Clinical Nutrition. 2014 Jan; 99(1): p. 86-95.
153. Moore DR, Camera DM, Areta JL, Hawley JA. Beyond muscle hypertrophy: why dietary protein is important for endurance athletes. Applied Physiology, Nutrition, and Metabolism. 2014; 39(9): p. 987-97.
154. Mitchell CJ, Churchward-Venne TA, Parise G, Bellamy L, Baker SK, Smith K, et al. Acute post-exercise myofibrillar protein synthesis is not correlated with resistance training-induced muscle hypertrophy in young men. PLoS One. 2014 Feb; 9(2).
155. Noakes T. Fluid replacement during marathon running. Clinical Journal of Sport Medicine. 2003 Sep; 13(5): p. 309-18.
156. McCubbin AJ, Costa RJ. The impact of dietary sodium intake on sweat sodium concentration in response to endurance exercise: a systematic review. International Journal of Sports Science. 2018 Jul; 8(1): p. 25-37.
157. McCubbin AJ, Lopez MB, Cox GR, Odgers JN, Costa RJ. Impact of 3-day high and low dietary sodium intake on sodium status in response to exertional-heat stress: A double-blind randomized control trial. European Journal of Applied Physiology. 2019 Sep; 119(9): p. 2105-18.
158. Walrand S, Guillet C, Boirie Y, Vasson MP. In vivo evidences that insulin regulates human polymorphonuclear neutrophil functions. Journal of Leukocyte Biology. 2004 Dec; 76(6): p. 1104-10.
159. Walrand S, Guillet C, Boirie Y, Vasson MP. Insulin differentially regulates monocyte and polymorphonuclear neutrophil functions in healthy young and elderly humans. The Journal of Clinical Endocrinology and Metabolism. 2006 Jul; 91(7): p. 2738-48.
160. Burke LM, Van Loon LJ, Hawley JA. Postexercise muscle glycogen resynthesis in humans. Journal of Applied Physiology. 2017 May; 122(5): p. 1055-67.
161. Phillips SM, Van Loon LJ. Dietary protein for athletes: from requirements to optimum adaptation. Journal of Sports Sciences. 2011 Jan; 29(Sup1): p. S29-38.
162. Gaskell SK, Snipe RM, Costa RJ. Test–retest reliability of a modified visual analog scale assessment tool for determining incidence and severity of gastrointestinal symptoms in response to exercise stress. International Journal of Sport Nutrition and Exercise Metabolism. 2019 Jul; 29(4): p. 411-9.
163. Costa RJ, Miall A, Khoo A, Rauch C, Snipe RM, Camões-Costa V, et al. Gut-training: The impact of two weeks repetitive gut-challenge during exercise on gastrointestinal status, glucose availability, fuel kinetics, and running performance. Applied Physiology, Nutrition, and Metabolism. 2017; 42(5): p. 547-57.
164. Miall A, Khoo A, Rauch C, Snipe RM, Camões-Costa V, Gibson PR, et al. Two weeks of repetitive gut-challenge reduce exercise-associated gastrointestinal symptoms and malabsorption. Scandinavian Journal of Medicine and Science in Sports. 2018 Feb; 28(2): p. 630-40.
165. Maughan RJ, Watson P, Cordery PA, Walsh NP, Oliver SJ, Dolci A, et al. A randomized trial to assess the potential of different beverages to affect hydration status: development of a beverage hydration index. The American Journal of Clinical Nutrition. 2016 Mar; 103(3): p. 717-23.
166. Pritchett K, Pritchett R. Chocolate milk: a post-exercise recovery beverage for endurance sports. Acute Topics in Sport Nutrition. 2012; 59: p. 127-34.
167. Saunders MJ. Carbohydrate-protein intake and recovery from endurance exercise: Is chocolate milk the answer? Current Sports Medicine Reports. 2011 Jul; 10(4): p. 203-10.

168. Moore DR. Nutrition to support recovery from endurance exercise: optimal carbohydrate and protein replacement. Current Sports Medicine Reports. 2015 Jul; 14(4): p. 294-300.
169. Alcantara JM, Sanchez-Delgado G, Martinez-Tellez B, Labayen I, Ruiz JR. Impact of cow's milk intake on exercise performance and recovery of muscle function: a systematic review. Journal of the International Society of Sports Nutrition. 2019 Dec; 16(1): p. 22.
170. Amiri M, Ghiasvand R, Kaviani M, Forbes SC, Salehi-Abargouei A. Chocolate milk for recovery from exercise: a systematic review and meta-analysis of controlled clinical trials. European Journal of Clinical Nutrition. 2019 Jun; 73(6): p. 835-49.
171. James LJ, Stevenson EJ, Rumbold PL, Hulston CJ. Cow's milk as a post-exercise recovery drink: implications for performance and health. European Journal of Sport Science. 2019 Jan; 19(1): p. 40-8.
172. Pegoretti C, Antunes AE, de Barros Manchado-Gobatto F, Capitani CD. Milk: an alternative beverage for hydration? Food and Nutrition Sciences. 2015 Apr; 6(6): p. 547.
173. Spaccarotella KJ, Andzel WD. Building a beverage for recovery from endurance activity: a review. The Journal of Strength and Conditioning Research. 2011 Nov; 25(11): p. 3198-204.
174. Baguley BJ, Zilujko J, Leveritt MD, Desbrow B, Irwin C. The effect of ad libitum consumption of a milk-based liquid meal supplement vs. a traditional sports drink on fluid balance after exercise. International Journal of Sport Nutrition and Exercise Metabolism. 2016 Aug; 26(4): p. 347-55.
175. Campagnolo N, Iudakhina E, Irwin C, Schubert M, Cox GR, Leveritt M, et al. Fluid, energy and nutrient recovery via ad libitum intake of different fluids and food. Physiology and Behavior. 2017 Mar; 15(171): p. 228-35.
176. Desbrow B, Jansen S, Barrett A, Leveritt MD, Irwin C. Comparing the rehydration potential of different milk-based drinks to a carbohydrate–electrolyte beverage. Applied Physiology, Nutrition, and Metabolism. 2014; 39(12): p. 1366-72.
177. Brown MA, Green BP, James LJ, Stevenson EJ, Rumbold PL. The effect of a dairy-based recovery beverage on post-exercise appetite and energy intake in active females. Nutrients. 2016 Jun; 8(6): p. 355.
178. Thomas K, Morris P, Stevenson EJ. Improved endurance capacity following chocolate milk consumption compared with 2 commercially available sport drinks. Applied Physiology, Nutrition, and Metabolism. 2009 Feb; 34(1): p. 78-82.
179. Rumbold P, Shaw E, James L, Stevenson EJ. Milk consumption following exercise reduces subsequent energy intake in female recreational exercisers. Nutrients. 2015 Jan; 7(1): p. 293-305.
180. Upshaw AU, Wong TS, Bandegan A, Lemon PW. Cycling time trial performance 4 hours after glycogen-lowering exercise is similarly enhanced by recovery nondairy chocolate beverages versus chocolate milk. International Journal of Sport Nutrition and Exercise Metabolism. 2016 Feb; 26(1): p. 65-70.
181. Karp JR, Johnston JD, Tecklenburg S, Mickleborough TD, Fly AD, Stager JM. Chocolate milk as a post-exercise recovery aid. International Journal of Sport Nutrition and Exercise Metabolism. 2006 Feb; 16(1): p. 78-91.
182. Potter JA, Fuller B. The effectiveness of chocolate milk as a post-climbing recovery aid. Journal of Sports Medicine and Physical Fitness. 2015 Dec; 55(12): p. 1438-44.
183. Pritchett K, Bishop P, Pritchett R, Green M, Katica C. Acute effects of chocolate milk and a commercial recovery beverage on postexercise recovery indices and endurance cycling performance. Applied Physiology, Nutrition, and Metabolism. 2009 Dec; 34(6): p. 1017-22.
184. Sudsa-ard K, Kijbooncho K, Chavasit V, Chaunchaiyakul R, Nio AQ, Lee JK. Lactose-free milk prolonged endurance capacity in lactose intolerant Asian males. Journal of the International Society of Sports Nutrition. 2014 Dec; 11(1): p. 1-6.

185. Phillips SM. Dietary protein for athletes: from requirements to metabolic advantage. Applied Physiology, Nutrition, and Metabolism. 2006 Dec; 31(6): p. 647-54.
186. Burd NA, Tang JE, Moore DR, Phillips SM. Exercise training and protein metabolism: influences of contraction, protein intake, and sex-based differences. Journal of Applied Physiology. 2009 May; 106(5): p. 1692-701.
187. Volpi E, Ferrando AA, Yeckel CW, Tipton KD, Wolfe RR. Exogenous amino acids stimulate net muscle protein synthesis in the elderly. The Journal of Clinical Investigation. 1998 May; 101(9): p. 2000-7.
188. Pennings B, Boirie Y, Senden JM, Gijsen AP, Kuipers H, Van Loon LJ. Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. The American Journal of Clinical Nutrition. 2011 May; 93(5): p. 997-1005.
189. Dickinson JM, Fry CS, Drummong MJ, Gundermann DM, Walker DK, Glynn EL, et al. Mammalian target of rapamycin complex 1 activation is required for the stimulation of human skeletal muscle protein synthesis by essential amino acids. The Journal of Nutrition. 2011 May; 141(5): p. 856-62.
190. Ahlborg B, Bergström J, Brohult J, Ekelund LG, Hultman E, Maschio G. Human muscle glycogen content and capacity for prolonged exercise after different diets. Forsvarsmedicin. 1967; 3(Supp1): p. 85-9.
191. Cartee GD, Young DA, Sleeper MD, Zierath JU, Wallberg-Henriksson H, Hollozy JO. Prolonged increase in insulin-stimulated glucose transport in muscle after exercise. American Journal of Physiology-Endocrinology And Metabolism. 1989 Apr; 256(4): p. E494-9.
192. Ivy JL, Goforth Jr HW, Damon BM, McCauley TR, Parsons EC, Price TB. Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. Journal of Applied Physiology. 2002 Oct.
193. Roy BD, Tarnopolsky MA. Influence of differing macronutrient intakes on muscle glycogen resynthesis after resistance exercise. Journal of Applied Physiology. 1998 Mar; 84(3): p. 890-6.
194. Hunt JN, Stubbs DF. The volume and energy content of meals as determinants of gastric emptying. The Journal of Physiology. 1975 Feb; 245(1): p. 209-25.
195. Owen JA, Fortes MB, Rahman SU, Jibani M, Walsh NP, Oliver SJ. Hydration marker diagnostic accuracy to identify mild intracellular and extracellular dehydration. International Journal of Sport Nutrition and Exercise Metabolism. 2019 Nov; 29(6): p. 604-11.
196. Alcock R, McCubbin A, Camões-Costa V, Costa RJ. Case study: Nutritional support for self-sufficient multi-stage ultra-marathon: Rationed versus full energy provisions. Wilderness and Environmental Medicine. 2018; 29(4): p. 508-20.
197. Costa RJ, Swancott AJ, Gill S, Hankey J, Scheer V, Murray A, et al. Compromised energy and macronutrient intake of ultra-endurance runners during a multi-stage ultra-marathon conducted in a hot ambient environment. International Journal of Sports Science. 2013; 3(2): p. 51-62.
198. Peake J, Della Gatta PM, Suzuki K, Nieman D. Cytokine expression and secretion by skeletal muscle cells: regulatory mechanisms and exercise effects. Exercise Immunology Review. 2015; 21: p. 8-25.
199. Camões-Costa V, Snipe RM, Dixon D, Costa RJ. Dairy milk beverage anhances neutrophil function after immuno-depressive exercise in response to a bacterial challenge. In Sports Dietitians Australia Conference; 2017.
200. Costa RJ, Gaskell SK, McCubbin AJ, Snipe RM. Exertional-heat stress-associated gastrointestinal perturbations during Olympic sports: Management strategies for athletes preparing and competing in the 2020 Tokyo Olympic Games. Temperature. 2020 Jan; 7(1): p. 58-88.

201. Huschtscha Z, Russo I, Snipe RM, Dixon D, Camões-Costa V, Costa RJ. Exercise-induced dehydration causes malabsorption of carbohydrate rich pre-exercise meal. In International Journal of Sports Nutrition and Exercise Metabolism; 2017.
202. Burke LM. Fueling strategies to optimize performance: training high or training low? Scandinavian Journal of Medicine and Science in Sport. 2010 Oct; 20: p. 48-58.
203. Hawley JA, Burke LM. Carbohydrate availability and training adaptation: effects on cell metabolism. Exercise and Sport Sciences Reviews. 2010 Oct; 38(4): p. 152-60.
204. Costa RJ, Teixeira A, Rama L, Swancott AJ, Hardy LD, Lee B, et al. Water and sodium intake habits and status of ultra-endurance runners during a multi-stage ultra-marathon conducted in a hot ambient environment: an observational field based study. Nutrition Journal. 2013 Dec; 12(1): p. 1-6.
205. Gill SK, Teixeira A, Rama L, Rosado F, Hankey J, Scheer V, et al. Circulatory endotoxin concentration and cytokine profile in response to exertional-heat stress during a multi-stage ultra-marathon competition. Exercise Immunology Review. 2015 Jan; 21.
206. Russo I, Camões-Costa V, Gaskell SK, Porter J, Burke LM, Costa RJ. Systematic literature review: The effect of dairy milk on markers of recovery optimisation in response to endurance exercise. International Journal of Sports Science. 2019 Aug; 9(4): p. 69-85.
207. Suzuki K. Involvement of neutrophils in exercise-induced muscle damage and its modulation. General Internal Medicine and Clinical Innovations. 2018; 3: p. 1-8.
208. Bennett CJ, Henry R, Snipe RM, Costa RJ. Is the gut microbiota bacterial abundance and composition associated with intestinal epithelial injury, systemic inflammatory profile, and gastrointestinal symptoms in response to exertional-heat stress? Journal of Science and Medicine in Sport. 2020 Jun; 23(12): p. 1141-53.
209. Suzuki K, Tominaga T, Ruhee RT, Ma S. Characterization and modulation of systemic inflammatory response to exhaustive exercise in relation to oxidative stress. Antioxidants. 2020 May; 9(5): p. 401.
210. Snipe RM, Khoo A, Kitic CM, Gibson PR, Costa RJ. The impact of mild heat stress during prolonged running on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profiles. International Journal of Sports Medicine. 2018 Apr; 39(4): p. 255-63.
211. Wan HY, Stickford JL, Dawkins EJ, Lindeman AK, Stager JM. Acute modulation in dietary behaviour following glycogen depletion and postexercise supplementation in trained cyclists. Applied Physiology Nutrition and Metabolism. 2018; 43(12): p. 1326-1333.
212. Gaskell SK, Taylor B, Muir J, Costa RJ. Impact of 24-h high and low fermentable oligo-, di-, monosaccharide, and polyol diets on markers of exercise-induced gastrointestinal syndrome in response to exertional heat stress. Applied Physiology Nutrition and Metabolism. 2020 Jun; 45(6): p. 569-80.
213. Borg GA. Psychophysical bases of perceived exertion. Med & Sci in Sports & Exerc. 1982; 14(5).
214. Hollies NR, Goldman RF. Psychological scaling in comfort assessment. Clothing comfort: Interaction of thermal, ventilation, construction, and assessment factors. 1977.
215. Duncan MJ, Smith M, Cook K, James RS. The acute effect of a caffeine-containing energy drink on mood state, readiness to invest effort, and resistance exercise to failure. The Journal of Strength & Conditioning Research. 2012 Aug; 26(10): p. 771-6.
216. Snipe RM, Costa RJ. Does the temperature of water ingested during exertional-heat stress influence gastrointestinal injury, symptoms, and systemic inflammatory profile? Journal of Science and Medicine in Sport. 2018 Aug; 21(8): p. 771-6.
217. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydrations. Journal of Applied Physiology. 1974; 37(2): p. 247-8.

218. Bergmeyer HU. Methods of enzymatic analysis. Verlag Chemie. 1974.
219. Harris RC, Hultman E, Nordesjö LO. Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. Scandinavian Journal of Clinical and Laboratory Investigation. 1974 Jan; 33(2): p. 109-20.
220. Camera DM, Smiles WJ, Hawley JA. Exercise-induced skeletal muscle signaling pathways and human athletic performance. Free Radical Biology and Medicine. 2016 Sep; 1(98): p. 131-43.
221. Oliver SJ, Laing SJ, Wilson S, Bilzon JL, Walsh NP. Endurance running performance after 48 h of restricted fluid and/or energy intake. Medicine and Science in Sport and Exercise. 2007 Feb; 39(2): p. 316.
222. Oliver SJ, Costa RJ, Laing SJ, Bilzon JL, Walsh NP. One night of sleep deprivation decreases treadmill endurance performance. European Journal of Applied Physiology. 2009 Sep; 107(2): p. 155-61.
223. Bate JP, Irving PM, Barrett JS, Gibson PR. Benefits of breath hydrogen testing after lactulose administration in analysing carbohydrate malabsorption. European Journal of Gastroenterology and Hepatology. 2010 Mar; 22(3): p. 318-26.
224. Barrett JS, Irving PM, Shepherd SJ, Muir JG, Gibson PR. Comparison of the prevalence of fructose and lactose malabsorption across chronic intestinal disorders. Alimentary Pharmacology and Therapeutics. 2009 Jul; 30(2): p. 165-74.
225. Rumessen JJ, Gudmand-Høyer E. Absorption capacity of fructose in healthy adults. Comparison with sucrose and its constituent monosaccharides. Gut. 1986 Oct; 27(10): p. 1161-8.
226. Mazzulla M, Parel JT, Beals JW, Van Vliet S, Abou Swan S, West DW, et al. Endurance exercise attenuates postprandial whole-body leucine balance in trained men. Medicine and Science in Sports and Exercise. 2017 Dec; 49(12): p. 2585-2592.
227. Leiper JB. Fate of ingested fluids: factors affecting gastric emptying and intestinal absorption of beverages in humans. Nutrition Reviews. 2015 Sep; 73(Supp2): p. 57-72.
228. Gill SK, Allerton DM, Ansley-Robson P, Hemmings K, Cox M, Costa RJ. Does short-term high dose probiotic supplementation containing lactobacillus casei attenuate exertional-heat stress induced endotoxaemia and cytokinaemia? International Journal of Sport Nutrition and Exercise Metabolism. 2016 Jun; 26(3): p. 268-75.
229. Gill S, Hankey J, Weight A, Marczak S, Hemming K, Allerton D, et al. The impact of a 24-h ultra-marathon on circulatory endotoxin and cytokine profile. International Journal of Sports Medicine. 2015; 36(8): p. 688-95.
230. Gaskell SK, Rauch CE, Parr A, Costa RJ. Diurnal versus Nocturnal Exercise-Impact on the Gastrointestinal Tract. Medicine and Science in Sports and Exercise. 2020 Oct; In Press.
231. Cowan DF, Leeming ER, Sinclair A, Dordevic AL, Truby H, Gibson SJ. Effect of whole foods and dietary patterns on markers of subclinical inflammation in weight-stable overweight and obese adults: a systematic review. Nutrition Reviews. 2020 Jan; 78(1): p. 19-38.
232. Asp S, Daugaard JR, Richter EA. Eccentric exercise decreases glucose transporter GLUT4 protein in human skeletal muscle. The Journal of Physiology. 1995 Feb; 482(3): p. 705-12.
233. Huschtscha Z, Porter J, Costa RJ. The impact of dairy protein intake on muscle mass, muscle strength, and physical performance in middle-aged to older adults with or without existing sarcopenia. Advances in Nutrition. 2020; 11(1): p. 175-6.
234. Russo I, Della Gatta PA, Garnham A, Porter J, Burke LM, Costa RJ. Does the nutritional composition of dairy milk based recovery beverages influence post-exercise gastrointestinal and immune status, and subsequent markers of recovery optimisation in response to high intensity interval exercise? Frontiers in Nutrition. 2020; 7(343): p. In Press.

235. Costa RJ, Crockford MJ, Moore JP, Walsh NP. Heat acclimation responses of an ultra-endurance running group preparing for hot desert based competition. European Journal of Sports Science. 2014 Jan; 14(Supp1): p. S131-41.
236. Knapick JJ, Steelman RA, Hoedebecke SS, Austin KG, Farina EK, Lieberman HR. Prevalence of dietary supplement use by athletes: systematic review and meta-analysis. Sports Medicine. 2016 Jan; 46(1): p. 103-23.
237. Burke LM, Castell LM, Casa DJ, Close GL, Costa RJ, Desbrow B, et al. International Association of Athletics Federations consensus statement 2019: Nutrition for athletics. International Journal of Sport Nutrition and Exercise Metabolism. 2019 Mar; 29(2): p. 73-84.
238. Van Leishout GA, Lambers TT, Bragt MC, Hettinga KA. How processing may affect milk protein digestion and overall physiological outcomes: A systematic review. Critical Reviews in Food Science and Nutrition. 2019 Aug.
239. Costa RJ, Cartner L, Oliver SJ, Laing SJ, Walters R, Bilzon JL, et al. No effect of a 30-h period of sleep deprivation on leukocyte trafficking, neutrophil degranulation and saliva IgA responses to exercise. European Journal of Applied Physiology. 2008 Feb; 105(3): p. 499-504.
240. Costa RJ, Smith AH, Oliver SJ, Walters R, Maassen N, Bilzon JL, et al. The effects of two nights of sleep deprivation with or without energy restriction on immune indices at rest and in response to cold exposure. European Journal of Applied Physiology. 2010 Jun; 109(3): p. 417-28.
241. Jaconi ME, Lew DP, Carpentier JL, Magnusson KE, Sjögren M, Stendahl O. Cytosolic free calcium elevation mediates the phagosome-lysosome fusion during phagocytosis in human neutrophils. Journal of Cell Biology. 1990 May; 110(5): p. 1555-64.
242. Davison G, Kehaya C, Diment BC, Walsh NP. Carbohydrate supplementation does not blunt the prolonged exercise-induced reduction of in vivo immunity. European Journal of Nutrition. 2016 Jun; 55(4): p. 1583-93.
243. Caradonna L, Amati L, Magrone T, Pellegrino NM, Jirillo E, Caccavo D. Invited review: Enteric bacteria, lipopolysaccharides and related cytokines in inflammatory bowel disease: biological and clinical significance. Journal of Endotoxin Research. 2000 Jun; 6(3): p. 205-14.
244. Gnauck A, Lentle RG, Kruger MC. The characteristics and function of bacterial lipopolysaccharides and their endotoxic potential in humans. International Reviews of Immunology. 2016 May; 35(3): p. 189-218.
245. Churchward-Venne TA, Snijders T, Linkens AM, Hamer HM, Van Kranenburg J, Van Loon LJ. Ingestion of casein in a milk matrix modulates dietary protein digestion and absorption kinetics but does not modulate postprandial muscle protein synthesis in older men. The Journal of Nutrition. 2015 Jul; 145(7): p. 1438-45.
246. Gorissen SH, Burd NA, Hamer HM, Gijsen AP, Groen BB, Van Loon LJ. Carbohydrate coingestion delays dietary protein digestion and absorption but does not modulate postprandial muscle protein accretion. The Journal of Clinical Endocrinology and Metabolism. 2014 Jun; 99(6): p. 2250-8.
247. Murray R. The effects of consuming carbohydrate-electrolyte beverages on gastric emptying and fluid absorption during and following exercise. Sports Medicine. 1987 Sep; 4(5): p. 322-51.
248. Maughan RJ, Watson P, Cordery PA, Walsh NP, Oliver SJ, Dolci A, et al. Sucrose and Sodium but not Caffeine Content Influence the Retention of Beverages in Humans Under Euhydrated Conditions. Int J Sport Nutr Exerc Metab. 2019 Jan; 29(1).
249. Philip A, Hargreaves M, Baar K. More than a store: regulatory roles for glycogen in skeletal muscle adaptation to exercise. American Journal of Physiology-Endocrinology And Metabolism. 2012 Jun; 302(11): p. E1343-51.

250. Ørtenblad N, Nielsen J, Saltin B, Holmberg HC. Role of glycogen availability in sarcoplasmic reticulum Ca2+ kinetics in human skeletal muscle. The Journal of Physiology. 2011 Feb; 589(3): p. 711-25.
251. Shiose K, Yamada Y, Motonaga K, Sagayama H, Higaki Y, Tanaka H, et al. Segmental extracellular and intracellular water distribution and muscle glycogen after 72-h carbohydrate loading using spectroscopic techniques. J Appl Physiol. 2016 Jul; 12(1).
252. Howarth KR, Phillips SM, MacDonald MJ, Richards D, Moreau NA, Gibala MJ. Effect of glycogen availability on human skeletal muscle protein turnover during exercise and recovery. Journal of Applied Physiology. 2010 Aug; 109(2): p. 431-8.
253. Scheer V, Basset P, Giovanelli N, Vernillo G, Millet GP, Costa RJ. Defining off-road running: A Position statement from the Ultra Sports Science Foundation. International Journal of Sports Medicine. 2020 May; 41(5): p. 275-284.
254. Impey SG, Hammond KM, Shepherd SO, Sharples AP, Stewart C, Limb M, et al. Fuel for the work required: a practical approach to amalgamating train-low paradigms for endurance athletes. Physiological Reports. 2016 May; 4(10).
255. Yeo WK, Paton CD, Garnham AP, Burke LM, Carey AL, Hawley JA. Skeletal muscle adaptation and performance responses to once a day versus twice every second day endurance training regimens. Journal of Applied Physiology. 2008 Nov; 105(5): p. 1462-70.
256. Widrick JJ, Costill DL, Fink WJ, Hickey MS, McConell GK, Tanaka H. Carbohydrate feedings and exercise performance: effect of initial muscle glycogen concentration. Journal of Applied Physiology. 1993 Jun; 74(6): p. 2998-3005.
257. Glynn EL, Fry CS, Drummond MJ, Dreyer HC, Dhanani S, Volpi E, et al. Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2010 Aug; 299(2): p. R533-40.
258. Doyle JA, Sherman WM, Strauss RL. Effects of eccentric and concentric exercise on muscle glycogen replenishment. Journal of Applied Physiology. 1993 Apr; 74(4): p. 1848-55.
259. O'Reiley KP, Warhol MJ, Fielding RA, Frontera WR, Meredith CN, Evans WJ. Eccentric exercise-induced muscle damage impairs muscle glycogen repletion. Journal of Applied Physiology. 1987 Jul; 63(1): p. 252-6.
260. Zehnder M, Muelli M, Buchli R, Kuehne G, Boutellier U. Further glycogen decrease during early recovery after eccentric exercise despite a high carbohydrate intake. European Journal of Nutrition. 2004 Jun; 43(3): p. 148-59.
261. Kirwan JP, Hickner RC, Yarasheski KE, Withop BV, Hollozy JO. Eccentric exercise induces transient insulin resistance in healthy individuals. Journal of Applied Physiology. 1992 Jun; 72(6): p. 2197-202.
262. King DS, Feltmeyer TL, Baldus PJ, Sharp RL, nespor JA. Effects of eccentric exercise on insulin secretion and action in humans. Journal of Applied Physiology. 1993 Nov; 75(5): p. 2151-6.
263. Kristiansen S, Asp SV, Richter EA. Decreased muscle GLUT-4 and contraction-induced glucose transport after eccentric contractions. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 1996 Aug; 271(2): p. R477-82.
264. Del Aguila LF, Krishnan RK, Ulbrecht JS, Farrell PA, Correll PH, Lang CH, et al. Muscle damage impairs insulin stimulation of IRS-1, PI 3-kinase, and Akt-kinase in human skeletal muscle. American Journal of Physiology-Endocrinology And Metabolism. 2000 Jul; 279(1): p. E206-12.
265. Anderson OE, Nielsen OB, Overgaard K. Early effects of eccentric contractions on muscle glucose uptake. Journal of Applied Physiology. 2019 Feb; 126(2): p. 376-85.
266. Kogan KE, Evans M, Iuliano E, Melvin A, Susta D, Neff K, et al. Co-ingestion of protein or a protein hydrolysate with carbohydrate enhances anabolic signaling, but not glycogen

resynthesis, following recovery from prolonged aerobic exercise in trained cyclists. European Journal of Applied Physiology. 2018 Feb; 118(2): p. 349-59.

267. Witard OC, Turner JE, Jackman SR, Kies AK, Jeukendrup AE, Bosch JA, et al. High dietary protein restores overreaching induced impairments in leukocyte trafficking and reduces the incidence of upper respiratory tract infection in elite cyclists. Brain Behav Immun. 2013 Jul; 39.

Appendix 1

The Influence Of Biological Sex And Fitness Status On Markers Of Recovery Optimisation In Response To Prolonged High Intensity Interval Exercise.

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Abstract This study aimed to characterise recovery outcomes and repeated performance following 2h high-intensity interval training (HIIT) exercise, followed by consumption of 1.2g/kg body mass (BM) and 0.4g/kgBM of carbohydrate and protein, respectively, between biological sex and fitness status categories. Venous blood samples, muscle biopsies, BM, body water, and breath samples were collected, and gastrointestinal symptoms (GIS) were measured, pre-exercise, and throughout 4h recovery. The following morning, participants returned to assess performance outcomes. Significantly greater body water losses were experienced by high (BM loss: 2.3%) vs. moderate fitness athletes (1.8%; P=0.009), but there were no differences between sexes (1.9%). Intestinal injury, carbohydrate malabsorption, and GIS occurred amongst all groups, with no differences. Phosphorylation of mTOR (P<0.001) and Akt (P=0.031), but not rpS6, increased from 0-2 h recovery in both fitness groups. Greater overall phosphorylation of GSK-3β was observed amongst high fitness (P=0.033). There were no group differences for glucose, insulin, cortisol, leukocyte or cytokine markers. A decline in neutrophil functional responses (36%) occurred for all groups. The following day, rates of carbohydrate oxidation were greater amongst males at all intensities. Rates of fat oxidation rates were greater at 50% and 60% $\dot{V}O_{2max}$, and carbohydrate oxidation were greater at 70% and 80% $\dot{V}O_{2max}$ amongst high compared to moderate fitness athletes. Absolute performance was greater amongst high vs. moderate fitness; however, there were no differences in relative performance between groups. Recovery optimisation markers following a 2h HIIT exercise protocol and consumption of carbohydrate and protein at 1.2g/kgBM and 0.4g/kgBM, respectively, are similarly achieved by male and female athletes of moderate and high fitness status. Registration: This sub-group analysis was part of a larger study that was prospectively registered with ANZCTR (reference number 375090).

Keywords muscle glycogen, protein synthesis, hydration, immune, gastrointestinal, performance.

1. Introduction

The implementation of recovery nutrition strategies to support training adaptations and performance outcomes is common practice amongst athletes at all levels of competition [1]. Generally, these strategies focus on replacement of substrate and body water losses, and repair of damaged tissues (e.g., skeletal muscle). Indeed, over several decades, an extensive body of literature has developed to determine the ideal intake of carbohydrate, protein, and water to support muscle and liver glycogen replenishment, skeletal muscle protein synthesis, and rehydration, respectively [2,3]. To date, however, few studies have considered nutritional support for restoring immunocompetency in response to immunodepressive exercise, and (or) the impact of exercise-induced gastrointestinal syndrome on nutrient assimilation in the post-exercise recovery period [4-7]. Moreover, these guidelines acknowledge that fitness status and biological sex may influence individual nutrient requirements, but currently lack data to provide definitive and (or) quantitative recommendations.

It is well established that the extent of physiological (i.e., thermoregulatory and immune stress) and biochemical (i.e., substrate depletion, local and systemic inflammatory responses, and accumulation of metabolic by-products) disturbances induced by the specific exercise stress are major determinants of recovery nutrition requirements [3]. Prolonged strenuous exercise is known to reduce muscle glycogen and body water content, and induce skeletal muscle damage. For example, steady state treadmill running (i.e., 90-

120 min at 70-75% $\dot{V}O_{2max}$) in thermoneutral conditions has been reported to deplete muscle glycogen content below 250 mmol/kg dry weight (dw) and induce body mass (BM) loss >2.5% [8-12]. Likewise, skeletal muscle damage, as indicated by biochemical (i.e., increased creatine kinase and (or) myoglobin) and functional (i.e., reduced isometric maximal voluntary contraction, increased muscle soreness) markers, is induced by prolonged intermittent running (i.e., 90 min Loughborough Intermittent Shuttle Test) and eccentric and (or) plyometric type contractions (e.g., 5 x 20 plyometric drop jumps) exercise protocols [13,14]. However, characterisation of these exercise-induced perturbations is bias towards young well-trained male athletic populations, with a scarcity in female athlete population recovery research.

Prolonged strenuous exercise is proposed to induce an acute immune alteration, characteristics of: 1) an 'open window' that may lead to increased risk of microorganism-borne infectious episode as a result of pathogenic exposure, and 2) an acute systemic inflammatory response [15,16]. Steady state running (i.e., 70-75% $\dot{V}O_{2max}$) for 2 h in thermoneutral conditions has consistently been shown to decrease innate immune system responses, including depressed in-vitro bacterially challenged (E.coli lipopolysaccharide) neutrophil elastase release by >30%, despite the post-exercise neutrophilia, and also increasing the systemic inflammatory cytokine profile (SIR profile) [4,5,17-19]. Moreover, skeletal muscle (i.e., ultrastructural myofibrillar disruption, swelling, efflux of myocellular proteins) and gastrointestinal tissue damage (i.e., ischemic epithelial cell death, disruption to function and regulation of tight-junction proteins) occur following prolonged and muscle-damaging exercise, and launch local and systemic inflammatory responses, that further disrupts regulation of gastrointestinal and sarcolemma barriers, respectively [6,20]. Impaired functionality of epithelial tissue and sarcolemma may interfere with assimilation of recovery nutrition, from the lumen into circulation, and form circulation into intracellular compartment, respectively. A functional and coordinated immune response is required for removal of damaged skeletal muscle tissue for subsequent growth and adaptation, and to repair gastrointestinal-associated lymphoid tissue structure and function. There is mounting evidence to describe the interrelated nature of the targeted systems and (or) organs of recovery nutrition (i.e., skeletal muscle glycogen repletion and tissue repair, hydration status, immune and gastrointestinal function), and the importance of timely and adequate nutritional interventions. The concept of 'exercise recovery optimisation' integrates recovery strategies that maximise desired outcomes while minimising those that cause detrimental outcomes within the complex and interrelated responses to exercise [21].

Studies to date investigating post-exercise nutritional strategies to support immune and gastrointestinal function have predominantly employed well-trained (i.e., $\dot{V}O_{2max}$ >60 ml/kgBM/min) male athletes [4,5,17,22], while the effects of fitness status and biological sex on the responsiveness to recovery optimisation nutrition interventions have not been systematically and comprehensively researched. Although it is well established that differences in fluid dynamics, substrate oxidation, and relative performance intensities exist between male and female athlete populations (i.e., biological sex differences), and well-trained (elite or high competitive) and moderately-trained athletes (recreationally active), the implications of these differences on post-exercise recovery nutrient bioavailability and assimilation, and subsequent nutritional requirements, between these subgroups remain poorly characterised [23-30]. With this in mind, the current study aimed to characterise differences in recovery outcomes following 2 h high-intensity interval training (HIIT) exercise protocol and consumption of a recovery beverage, and subsequent performance the following day, between 1) male and female athletes, and 2) moderate and high fitness athletes. The influence of biological sex and fitness status on assimilation and integration of recovery nutrition was assessed using global markers of recovery optimisation including exercise-induced gastrointestinal syndrome, immune function response previously confirmed to depress after exercise and respond to recovery nutrition (e.g., in-vitro bacterially-stimulated neutrophil elastase release), muscle glycogen resynthesis, protein synthesis, rehydration, and performance outcomes. It was hypothesised male athletes and high fitness status athletes would experience greater exercise-induced perturbations in response to 2 h HIIT exercise. It was further hypothesised that following the exercise stress, greater exercise induced perturbations amongst male and high fitness athletes would result in suboptimal delivery, absorption and assimilation of recovery nutrition leading to impaired recovery outcomes and diminished subsequent performance.

2. Methods

2.1. Participants

Thirty-five (n= 27 male, n= 8 female) endurance trained athletes volunteered to participate in the study. Participants were grouped according to their $\dot{V}O_{2max}$ (i.e., <55 ml/kg BM/min or >60 ml/kg BM/min) for fitness status, and in accordance with their biological sex. Baseline characteristics are summarised in Table 1. All participants gave written informed consent. This sub-group analysis was part of a larger study that was prospectively registered with ANZCTR (reference number 375090), received approval from the local ethics committee (MUHREC: 12799) and conformed to the Helsinki Declaration for Human Research Ethics. Inclusion and exclusion criteria has been previously reported in Russo et al [31].

2.2. Preliminary Measures

Baseline measurements were recorded one to three weeks prior to the first experimental trial. Height (stadiometer, Holtain Limited, United Kingdom) and BM (Seca 515 MBCA, Seca Group, Germany) were recorded. $\dot{V}O_{2max}$ (Vmax Encore Metabolic Cart, Carefusion, USA) was estimated using a continuous incremental exercise test to volitional exhaustion on a motorised treadmill (MyRun Technogym; Technogym, Italy) as previously reported [4]. To determine running speeds for the exercise trials, the speed at approximately 50%, 60%, 70%, and 80% $\dot{V}O_{2max}$ and 1% gradient was determined and verified from the $\dot{V}O_2$ work rate relationship.

Table 1. Baseline characteristics of participants by biological sex and fitness status.

	Biological sex		**Fitness status**	
	Male	**Female**	**$\dot{V}O_{2max}$ ≤55ml/kg BM/min**	**$\dot{V}O_{2max}$ ≥60 ml/kg BM/min**
N	27	8	21	10
Age (years)	30 (26 to 33)	35 (29 to 41)	33 (30 to 37)	26$^{\#}$ (22 to 31)
Height (cm)	178 (175 to 181)	167$^{\#\#}$ (163 to 171)	174 (170 to 177)	178 (171 to 184)
Body mass (kg)	73.8 (70.3 to 77.3)	62.1$^{\#\#}$ (55.2 to 69.1)	70.8 (65.7 to 75.9)	69.9 (64.2 to 75.7)
BMI	23.3 (22.6 to 24.0)	22.1 (20.2 to 24.0)	23.1 (22.1 to 24.1)	22.7 (21.5 to 23.9)
Body fat (%)	14.7 (12.9 to 16.4)	21.8$^{\#\#}$ (17.4 to 26.1)	18.3 (15.6 to 21.0)	12.6$^{\#\#}$ (10.7 to 14.6)
Skeletal muscle mass (kg)	30.3 (28.8 to 31.8)	22.1$^{\#\#}$ (19.5 to 24.7)	27.2 (24.8 to 29.6)	29.5 (26.6 to 32.5)
$\dot{V}O_{2max}$ (ml/kg BM/min)	55.8 (53.9 to 57.7)	51.3 (47.7 to 55.3)	51.1 (49.8 to 52.3)	61.8$^{\#\#}$ (60.4 to 63.2)
$\dot{V}O_{2max}$ (ml/kg FFM/min)	65.4 (63.7 to 67.1)	65.7 (61.2 to 70.3)	62.5 (60.5 to 64.6)	70.8$^{\#\#}$ (68.4 to 73.2)

Mean (95% CI): $^{\#\#}P< 0.01$ and $^{\#}P< 0.05$ *vs.* comparator.

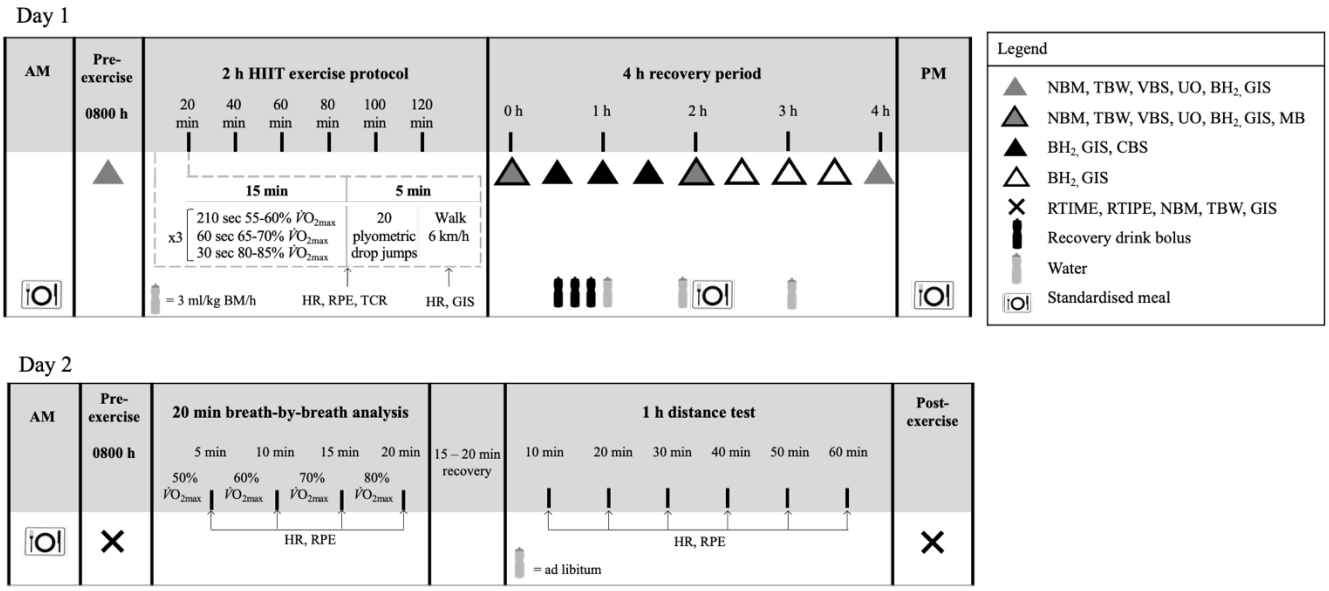
2.3. Experimental Protocol

Participants were required to consume a standardised low FODMAP (<2 g/meal) diet during the 24 h prior to, and throughout the experimental trial. Daily intake met current nutrition guidelines for endurance athletes (overall mean (SD):, energy 10.3 (2.5) MJ/day, protein 99 (29) g/day, fat 58 (29) g/day, carbohydrate 358 (84) g/day, fibre 44 (11) g/day, and water 2717 (1187) ml/day) [3,32]. Participants were asked to refrain from consuming alcohol, and performing strenuous exercise during the 48 h before the experimental trial, and from consuming caffeinated beverages during the 24 h before the experimental trial. Compliance to these instructions was checked via the completion of a 24 h pre-trial food and exercise diary.

Participants reported to the laboratory at 0800h after consuming the standardised low FODMAP mixed carbohydrate breakfast (energy 2.8 (0.9) MJ, protein 25 (10) g, fat 20 (6) g, carbohydrate 92 (32) g, fibre 12 (4) g, and 357 (243) ml water). Trials for female athletes were scheduled during the follicular phase of their menstrual cycle (n= 7) or when taking the active medication of the oral contraceptive pill (n= 1). Resting estrogen levels (DKO003/RUO; DiaMetra, Italy) were measured for verification (7.0 (3.4) pg/ml). Before commencing exercise, participants were asked to void, and pre-exercise nude BM and total body water (TBW) (Seca 515 MBCA, Seca Group, Germany) were recorded. Participants inserted a thermocouple 12 cm beyond the external anal sphincter to record pre- and post-exercise rectal temperature (Precision Temperature 4600 Thermometer, Alpha Technics, USA). Participants provided a breath sample into a 250 ml breath collection bag (Wagner Analysen Technick, Germany), and completed an exercise-specific modified visual analogue scale GIS assessment tool [33]. Blood was collected by venepuncture from an antecubital vein into three separate vacutainers (6 ml 1.5 IU/ml lithium heparin, 4 ml 1.6 mg/ml K_3EDTA, and 5 ml SST; BD, UK).

The exercise protocol consisted of a 2 h (initiated at 0900h) HIIT session in 23.4 (0.7) °C ambient temperature and 42 (8) % relative humidity on a motorised treadmill, as described in Figure 1. Immediately post-exercise, nude BM and rectal temperature were recorded. The recovery period commenced 30 min after the end of the exercise protocol to prepare for muscle biopsy sampling. Participants rested in a supine position in a sterile phlebotomy room for venous blood sampling followed by the first muscle biopsy thereafter. TBW was measured immediately after muscle biopsy sampling. After sample collection and measurement recording, participants were provided with a commercially available chocolate flavoured dairy milk beverage. The

Figure 1. Schematic illustration of the experimental design.



NBM: nude body mass, TBW: total body water, VBS: venous blood sampling, UO: urine output and osmolality, BH_2: breath hydrogen, GIS: gastrointestinal symptoms, Tre: rectal temperature, HR: heart rate, TCR: thermal comfort rating, RPE: rating of perceived exertion, CBS: capillary blood sampling, RTIME: readiness to invest mental effort, RTIPE: readiness to invest physical effort, RER: respiratory exchange ratio, BM: body mass.

beverage was served in opaque bottles at ~7°C beverage temperature [34], in 3 equal boluses every 10 min, from 0.5 h of recovery. The volume of the beverage was calculated to provide 1.2 g/kg BM of carbohydrate and 0.4 g/kg BM of protein (energy 2.5 (0.3) MJ, protein 28 (4) g, fat 16 (2) g, and carbohydrate 84 (12) g). Additional water calculated to provide a total fluid intake of 35 ml/kg BM was provided at hourly intervals. Participants were instructed to drink as much as tolerable. Total fluid intake was recorded hourly. The percentage of fluid retained was calculated from the difference between ingested fluid and urine output, as a fraction of total fluid intake [35]. An additional muscle biopsy samples was taken at 2 h into the recovery period. Blood samples, nude BM and TBW were collected again at 2 h and 4 h of recovery. Breath samples were collected and GIS recorded every 30 min throughout the recovery period. Total urine output was collected throughout the total

recovery period. Weight of urine output was recorded at 2 h and 4 h of recovery. After sample collection and measurement recording at 2 h of recovery, participants received a standardised recovery meal (energy 2.8 (0.6) MJ, protein 31 (7) g, fat 4 (1) g, carbohydrate 130 (29) g, fibre 9 (2) g, and water 409 (90) ml), and were instructed to consume as much as tolerable. The total weight of the meal consumed was recorded. In addition, participants were provided with a standardised low FODMAP evening meal to consume after leaving the laboratory (energy 3.0 (1.4) MJ, protein 29 (14) g, fat 17 (16) g, carbohydrate 98 (50) g, fibre18 (7) g, and water 897 (712) ml).

The following morning, participants returned to the laboratory (0800h) to assess psychophysiological parameters and exercise performance. Due to unforeseen circumstances, 5 participants did not return for the second day of testing (n= 3 male, n= 2 female), therefore these participants are limited to day 1 data metrics. A standardised low FODMAP mixed carbohydrate breakfast (energy 2.7 (1.0) MJ, protein 24 (11) g, fat 19 (6) g, carbohydrate 89 (37) g, fibre 11 (4) g, and water 389 (232) ml) was consumed at 0700h. Nude BM, TBW and GIS were recorded on arrival and again after the performance test. Before and after the performance test, participants completed measures of readiness to invest mental and physical effort, on a rating scale from 0-10 [36]. Participants completed a 20 min running exercise bout to measure oxygen uptake and oxidation rates at four submaximal exercise intensities for 5 min each, before undertaking a 1 h performance test in 23.0 (1.3) °C ambient temperature and 46 (9) % relative humidity. Participants were instructed to run the maximal distance they are capable of running in 1 h, with the incline set at 1%.

2.4. Muscle Biopsy Procedure

Nineteen male and three female participants consented to providing muscle samples. Muscle biopsies were performed using a modified 5 mm Bergstrom biopsy needle. Samples were obtained from the vastus lateralis of the ipsilateral leg for the first trial, and contralateral leg for the second. The skin of the lateral aspect of the mid-thigh was washed well (10% Povidone – Iodine solution) then 2-3 ml of local anaesthetic (lidocaine 1%) was infiltrated subcutaneously over vastus lateralis to anaesthetise the skin and superficial fascia. After the anaesthetic had taken effect, two 5mm stab incisions ~15 mm apart were made through skin and fascia, with one incision made for each muscle biopsy sample. Samples were then extracted, immediately submerged in liquid nitrogen and stored at -80°C prior to further analysis.

2.5. Sample Analysis

Blood glucose concentration, hemoglobin, haematocrit, total and differential leukocyte counts, were determined as previously described [17,32]. Coefficient of variation (CV) for blood glucose concentration, hemoglobin, haematocrit, and leukocyte counts were 5.5%, 2.3%, 0.6% and 11.8%, respectively. Hemoglobin and hematocrit values were used to estimate changes in plasma volume (PV) relative to baseline, and used to correct plasma variables. To determine the blood glucose response to the recovery beverage, immediately before and every 30 min thereafter for 2 h, blood glucose concentration was measured from capillary blood samples (CV 3.7%). To determine in-vitro bacterially-stimulated elastase release, 1 ml of whole blood was pipetted into a microcentrifuge tube containing 50 µg of 1 mg/ml bacterial stimulant (lipopolysaccharide from E.coli, Sigma, Poole, UK) within 5 min of collection and gently vortex-mixed. Samples were incubated in a water bath (Labline, Thermo Fisher Scientific Australia, Scoresby, Victoria, Australia) at 37°C for 1 h, and further mixed by gentle inversion at 30 min. Bacterially challenged samples were then centrifuged at 4000 rpm (1500 g) for 10 min, and supernatant was aspirated into 1.5 ml micro-storage tubes and stored at -80ºC for further analysis. PMN elastase (BMS269; Affymetrix EBioscience, Vienna, Austria) was determined by ELISA. The remaining whole blood processing and analysis (i.e., insulin, cortisol, aldosterone, I-FABP, and sCD14 ELISA, and multiplex system for systemic inflammatory profile) were performed, as previously reported [17,22]. Plasma osmolality (P_{Osmol}) was determined by freezepoint osmometry (Osmomat 030, Gonotec, Germany) (CV 0.6%). The CVs for ELISAs were ≤10.0% and for cytokine profile multiplex was 17.5%. Breath samples (20 ml) were analysed (CV 2.0%) for hydrogen (H_2) content using a gas-sensitive analyser (Breathtracker Digital Microlyzer, Quintron, USA). Plasma sodium, potassium and calcium concentrations were determined using ion selective electrodes (Cobas c 501, Roche Diagnostics, Switzerland) and analysed by local pathology services (Cabrini Pathology, Australia).

2.6. Western Blot Analysis

Approximately 30 mg of skeletal muscle was solubilized in radioimmunoprecipitation buffer (Millipore, Bayswater, Victoria, Australia) with 1 µl/ml protease inhibitor cocktail (Sigma- Aldrich, Castle Hill, New South Wales, Australia) and 10 µl/ml Halt Phosphatase Inhibitor Single-Use Cocktail (Thermo Scientific, Australia, North Ryde, New South Wales, Australia). The concentration of protein per sample was determined by the bicinchoninic acid assay (BCA Protein Assay Kit#23225, Thermo Scientific). 20 µg of skeletal muscle protein lysate was loaded onto into either Bio-Rad precast Criterion TGX Stain-Free 4-12% gels (Bio-Rad, Gladesville, New South Wales, Australia). SDS-PAGE

was conducted following manufacturer's instructions. Protein was then transferred to PVDF membranes and blocked for 1 h in 5% bovine serum albumin (BSA) solution in Tris-buffered saline-Tween, (pH 7.6, 20 mmol/L Tris and 150 mmol/L NaCl, 0.1% Tween) (TBST) at room temperature. Membranes were then incubated in primary antibodies diluted in 5% BSA/TBST overnight at 4 °C. Following washing in TBST, membranes were incubated for 1 h with fluorescent secondary antibodies (phospho-mammalian target of rapamycin (mTOR)^{Ser2448}, phospho-protein kinase B (Akt)^{Ser473}, phospho-ribosomal protein S6 (rpS6)$^{Ser235/236}$, and phosphor-glycogen synthase kinase 3β (GSK-3β)^{Ser9}) (Anti-Rabbit IgG (H+L) DylightTM 800 Conjugate; Anti-mouse IgG (H+L) DylightTM 680 Conjugate) (Cell Signalling Technologies®, Danvers, Massachusetts, USA) diluted 1:10,000 in TBST. Following 2 further washes in TBST and 1 wash in phosphate buffered saline (PBS) membranes were scanned using the LiCOR® Odyssey CLx® Imaging System (Millennium Science, Mulgrave, Victoria, Australia). All targets were normalized to total protein using either the Bio-Rad stain-free system.

2.7. Muscle Glycogen Analysis

One fraction of muscle sample (approx. 20-25 mg (ww)) was freeze-dried, after which collagen, blood and other non-muscle material were removed from the muscle fibres. Samples were then pulverized and powdered. Samples were extracted with 0.5 M perchloric acid ($HClO_4$) containing 1 nmol EDTA and neutralised using 2.2 M $KHCO_3$. Adenosine triphosphate, phosphocreatine, and creatine was determined from the supernatant by enzymatic spectrophotometric assays [37,38]. Muscle glycogen content was determined from 2 aliquots of freeze-dried muscle (2–3 mg) as previously reported [37]

2.8. Statistical Analysis

Confirmation of adequate statistical power was determined from the applied statistical test, mean, standard deviation, and effect size of original research data extracted from a systematic literature review process on markers of recovery optimisation a priori [21]. Using a standard alpha (0.05) and beta value (0.80), the current participant sample size, within an independent cohort design, is estimated to provide adequate statistical power (power* 0.80-0.99) for detecting significant group differences (G*Power 3.1, Kiel, Germany). Data in the text and tables are presented as mean (SD) for experimental descriptive method, and mean and 95% confidence interval (CI) for primary variable, as indicated. For clarity, data in figures are presented as mean and standard error of the mean (SEM), and/or mean and individual responses, as indicated. Systemic inflammatory cytokine responses are presented as raw values and systemic inflammatory response profile (SIR-profile), as previously reported [39]. Only participants with full data sets within each specific variable were included in the data analysis. Due to low rates of consent to muscle biopsy procedures amongst female participants, intramuscular markers are limited to analysis by fitness status only. All data were checked for normal distribution (Shapiro-Wilks test of normality) by calculating skewness and kurtosis coefficients. Variables with singular data points were examined using independent sample t-tests, or non-parametric Mann-Whitney U test, when appropriate. Variables with multiple data points were examined using a two-way repeated-measures ANOVA, with fitness status and biological sex as between-subject factors. Assumptions of homogeneity and sphericity were checked, and when appropriate adjustments to the degrees of freedom were made using the Greenhouse- Geisser correction method. Main effects were analysed by Tukey's post hoc HSD. Statistics were analysed using SPSS statistical software (V.26.0, IBM Corp, Armonk, NY) with significance accepted at $P \leq 0.05$.

3. Results

3.1. Participant Characteristics

$\dot{V}O_{2max}$ relative to BM and fat free mass (FFM) did not differ between male and female athletes (Table 1). TBW was significantly greater (Table 2), and body fat percentage lower (Table 1) amongst male and high fitness athletes, compared to female and moderate fitness athletes, respectively. As intended, there was a significant difference in $\dot{V}O_{2max}$ between high and moderate fitness athletes (Table 1). High fitness athletes were younger than their counterparts (P= 0.034).

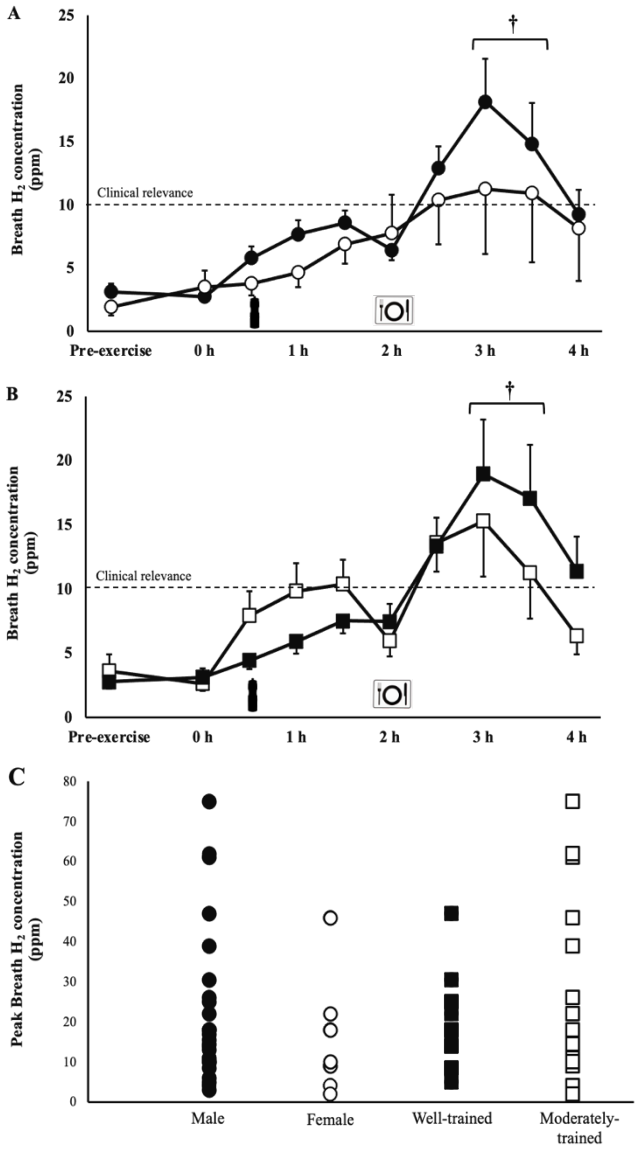
3.2. Exertional Strain

During exercise, a main effect of time (MEOTime) was observed for peak (overall mean and 95% CI otherwise specified: 158 (156 to 160) bpm; P< 0.001) and recovered HR (122 (120 to 124) bpm; P< 0.001), RPE (13 (12 to 13); P< 0.001) and TCR (9 (8 to 9); P< 0.001); whereby HR, RPE and TCR increased as exercise progressed for all groups. A trend towards a fitness*time interaction (P= 0.053) was observed for recovered HR, such that values were lower amongst high fitness athletes as the exercise progressed. Rectal temperature increased pre- (36.6 (36.3 to 36.9) °C) to post-exercise (37.8 (37.5 to 38.1) °C) for all groups (P< 0.001). Plasma cortisol concentration decreased significantly from 0 h to 2 h and 4 h recovery (P< 0.01), with no group differences (Table 2).

3.3. Gastrointestinal Integrity and Symptoms

There were no differences in exercise-associated increases in plasma I-FABP concentration between groups (Table 2). There were no significant group differences in severity of GIS during exercise and recovery period (Table 3). There was a MEOTime for breath H_2 ($P< 0.001$), with all groups reaching a peak of clinical significance (male: 22 (15 to 29) ppm, and female: 15 (5 to 25) ppm), high fitness: 21 (13 to 28) ppm, moderate fitness: 22 (13 to 32) ppm at 3.5 h post exercise (Figure 2). No significant main effects or interaction were observed for plasma sCD14 concentration (Table 2).

Figure 2. Breath hydrogen response between A) male athletes ● vs. female athletes ○, B) high fitness athletes ■ vs. moderate fitness athletes □, and C) and individual peak breath hydrogen responses after 2 h HIIT exercise in temperate ambient conditions and consumption of a dairy milk beverage.

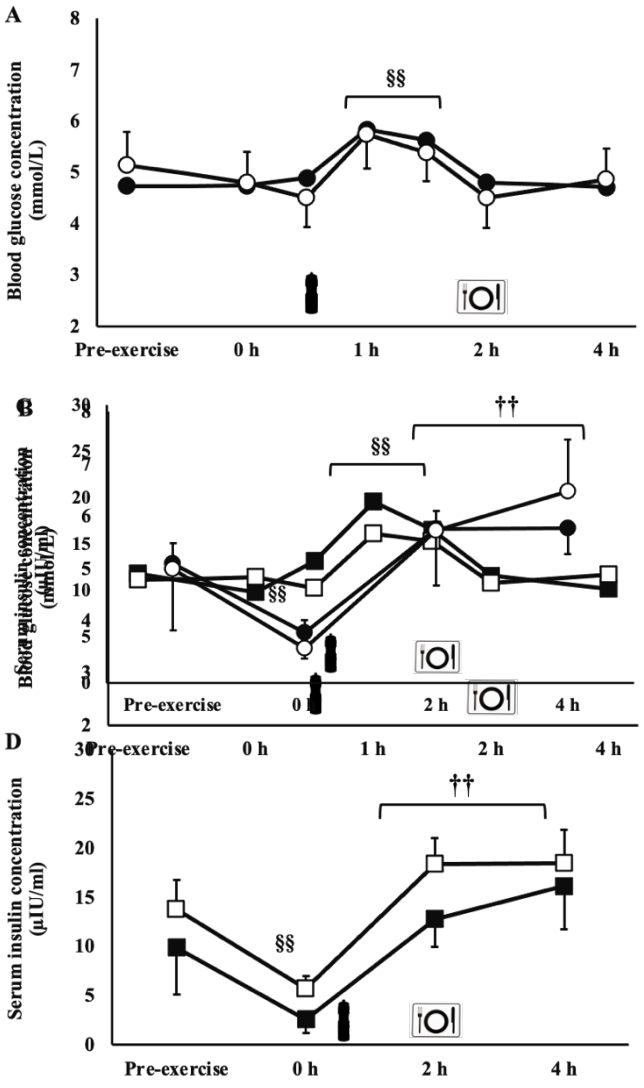


Figures 2A and 2B; mean ±SEM responses (n= 35): MEOTime † $P< 0.05$ vs. 0 h recovery.

3.4. Blood Glucose Availability and Serum Insulin Response

A MEOTime occurred for blood glucose and serum insulin, imposed by feeding during the recovery period (Figure 3). Blood glucose concentration was significantly greater 1 h and 1.5 h into recovery, compared to pre-exercise values ($P< 0.01$). Serum insulin was significantly lower at 0 h recovery compared to all other time points ($P< 0.01$). No group differences were observed.

Figure 3. Blood glucose (A and B) and serum insulin (C and D) concentrations after 2 h HIIT exercise protocol in temperate ambient conditions and consumption of a dairy milk beverage (male athletes ● vs. female athletes ○, and high fitness athletes ■ vs. moderate fitness athletes □).



Mean ±SEM responses (n= 35). MEOTime §§ $P< 0.01$ vs. pre-exercise, MEOTime †† $P< 0.01$ vs. 0 h recovery.

3.5. Immune Responses

An exercise-induced leukocytosis (10.2 (9.3 to 11.1) $x10^9$/L; P< 0.001), neutrophilia (6.9 (6.2 to 7.7) $x10^9$/L; P< 0.001), monocytosis (0.6 (0.6 to 0.7) $x10^9$/L; P= 0.004), and increased neutrophil:lymphocyte ratio (2.9 (2.6 to 3.3); P< 0.001) were observed in the recovery period in all groups. No main effects or interaction were observed for unstimulated plasma elastase concentration (178 (116 to 239) ng/ml). A MEOTime was observed for total bacterially-stimulated plasma elastase concentration such that values increased from pre-exercise (3.3 (2.0 to 4.7) µg/ml) to 2 h recovery (5.3 (3.7 to 6.8) µg/ml) (P< 0.01). Neutrophil function decreased during the recovery period (36%); however no main effects or interaction were observed for bacterially-stimulated elastase release per neutrophil (544 (428 to 660) fg/cell). A biological sex*time effect was observed for IL-10 concentrations, whereby values were significantly greater amongst females pre-exercise and 0 h recovery, and 2 h recovery. A MEOTime occurred for plasma IL-1ra concentrations, whereby IL-1ra values increased at 0 h recovery and remained elevated throughout the remainder of the recovery period (P< 0.05). No main effects or interaction were observed for plasma IL-1β, TNF-α, IL-6, and IL-8 concentrations (Table 2). No difference in exercise-induced SIR-profile (male: 32 (21 to 43) arb.unit, and female: 45 (11 to 78) arb.unit, high fitness: 37 (17 to 57) arb.unit, moderate fitness: 40 (13 to 66) arb.unit) and recovery beverage post-prandial SIR-profile (male: 8 (-1 to 17) and female: 11 (-28 to 50) arb.unit, high fitness: -1 (-13 to 10) arb.unit, moderate fitness: 13 (-11 to 37)) was observed between groups.

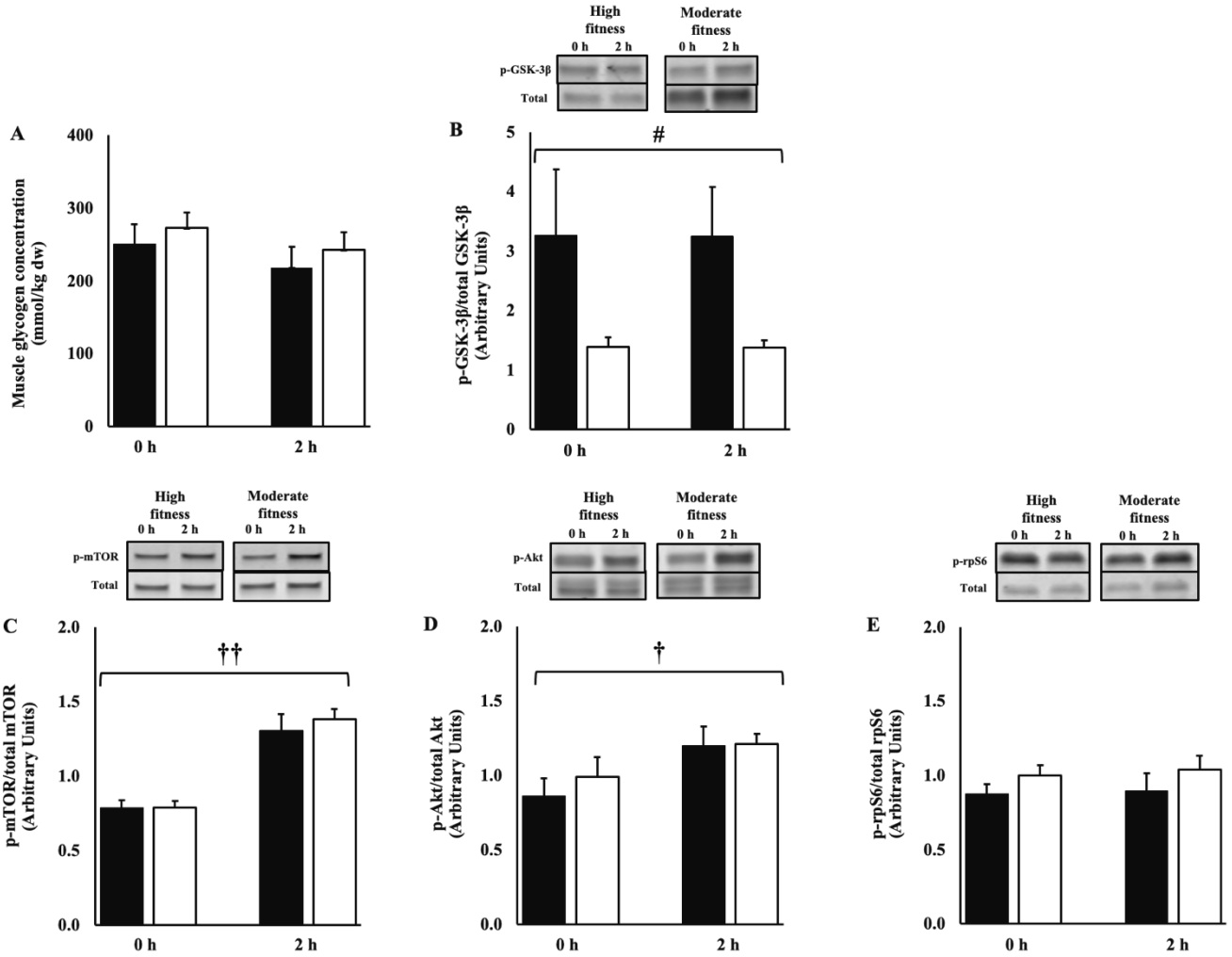
3.6. Intramuscular Markers

Post-exercise muscle glycogen content was 264 (232 to 297) mmol/kg dw and did not differ by fitness status (Figure 4). The early rate of muscle glycogen formation did not differ between trials (-15.3 (-27.3 to -3.3) mmol/kg dw/h). A group effect was observed for the ratio of phosphorylated GSK-3β to total GSK-3β such that values were greater amongst high fitness athletes (P= 0.033); however there was no effect of time for either group. A MEOTime occurred for the ratio of phosphorylated mTOR (P< 0.001) and Akt (P= 0.031) to total protein, such that values increased following consumption of the recovery beverage. No main effects or interaction was observed for phosphorylation of rpS6.

3.7. Hydration and Plasma Electrolyte Status

Baseline P_{Osmol} was significantly higher amongst male compared to female athletes (P= 0.006), and remained higher throughout the experimental trial (P= 0.004); however, values remained within range of euhydration for all groups throughout the experimental trial (Table 2). An effect of biological sex was observed for plasma sodium concentration, as values were lower amongst female athletes (P= 0.004). Body water losses were greater amongst high compared to moderate fitness athletes (P= 0.009), and a significant correlation was observed between $\dot{V}O_{2max}$ and BM loss (rs= 0.412, n= 35, P= 0.014). Total fluid intake during the recovery period did not differ between groups (male: 24 (22 to 26) ml/kgBM, female: 22 (18 to 26) ml/kgBM, high: 24 (22 to 27) ml/kgBM, moderate: 23 (21 to 25) ml/kgBM), and fluid retention at the end of the recovery period did not differ between groups (79 (76 to 83) %). A MEOTime occurred for TBW (P< 0.001) including extracellular (P< 0.001) water, with values returning to near baseline values 2 h into the recovery period. Plasma volume increased significantly at 2 and 4 h, compared to 0 h recovery for all groups (P< 0.01); however values were more negative amongst female compared to male athletes (P= 0.037). Plasma calcium and aldosterone concentrations decreased from pre- to post-exercise, before returning to resting values (P< 0.01).

Figure 4. Muscle glycogen content (A), phosphorylated GSK3-β to total GSK3-β (B), ratio of phosphorylated mTOR to total mTOR (C), phosphorylated Akt to total Akt (D), and phosphorylated rpS6 to total rpS6 (E) after 2 h HIIT exercise in temperate ambient conditions and consumption of a dairy milk beverage for high fitness ■ vs. moderate fitness athletes □.



Mean ±SEM responses (n= 22): MEOTime †† P< 0.01 and † P< 0.05 vs. 0 h recovery, Group effect # P< 0.05.

3.8. Psychophysiological Parameters & Performance Outcomes

There was a group effect for mental readiness to invest effort (P= 0.021), whereby male athletes reported greater readiness to perform (7 (6 to 8)) compared to female athletes 5 (3 to 6)). TBW was significantly greater amongst male (61 (60 to 62) %) and high fitness athletes (62 (61 to 64) %), compared to female (56 (53 to 59) %) and moderate fitness athletes (59 (57 to 61) %), respectively (P< 0.01). During the breath-by-breath test, rates of carbohydrate oxidation were greater at all intensities amongst male compared to female athletes (P= 0.008; Table 4). $\dot{V}O_2$ values were greater amongst high compared to moderate fitness athletes (P< 0.001). Rates of fat oxidation rates were significantly greater at 50% (P= 0.044) and 60% $\dot{V}O_{2max}$ (P= 0.024) and carbohydrate oxidation were significantly greater at 70% (P= 0.030) and 80% $\dot{V}O_{2max}$ (P= 0.031) amongst high fitness athletes compared to moderate fitness athletes. Mean HR (169 (167 to 171), RPE (15 (15 to 16)) and water intake (514 (297 to 731) ml) did not differ across the distance test. Relative (75 (73 to 78) % $\dot{V}O_{2max}$) and absolute (11.4 (10.8 to 11.9) km) performance was not different between male and female athletes. Total distance covered over 1 h was significantly greater amongst high fitness (12.6 (11.8 to 13.5) km) compared to moderate fitness athletes (10.6 (9.9 to 11.3) km; P= 0.001), however relative intensity did not differ (75 (72 to 78) % $\dot{V}O_{2max}$.

4. Discussion

The current study aimed to characterise differences in recovery outcomes following 2 h high-intensity interval training (HIIT) exercise protocol and consumption of a recovery beverage, and subsequent performance the following day, between 1) male and female athletes, and 2) moderate and high fitness athletes. Contrary to our hypothesis, exercise-induced increases in plasma I-FABP, carbohydrate malabsorption, GIS and decline in neutrophil function did not differ between biological sexes. Total body water, plasma sodium and plasma osmolality were greater, and plasma volume change was lesser amongst male compared to female athletes. Carbohydrate oxidation rates were greater amongst male athletes at all intensities the following morning, but no differences in fat oxidation rates were observed. In accordance with our hypothesis, the 2 h HIIT exercise stress model used in the current study induced greater BM loss amongst high level fitness athletes compared to moderate fitness athletes. Plasma I-FABP concentration, carbohydrate malabsorption and associated GIS did not differ between fitness groups. Decline in neutrophil function occurred amongst both fitness status groups, with no differences. Fitness status did not influence post-exercise glycogen concentration or glycogen resynthesis in the acute timeframe, but a greater phosphorylation of GSK-3β was observed amongst high compared with moderate fitness athletes. Increased phosphorylation of mTOR and Akt protein signalling was observed in response to consumption of the recovery beverage amongst both fitness groups. Total body water was greater amongst high fitness athletes at all time points, but no other differences in fluid-electrolyte status were observed. The following day, rates of carbohydrate oxidation were greater at 50% and 60% $\dot{V}O_{2max}$, and fat oxidation were greater at 70% and 80% $\dot{V}O_{2max}$ amongst high compared to moderate fitness athletes. Absolute performance was greater amongst high fitness athletes, however there were no differences in relative performance between groups. Findings from the current study suggest that recovery optimisation following a 2 h HIIT exercise protocol and consumption of 1.2 g/kg BM carbohydrate and 0.4 g/kg BM protein, using a dairy milk recovery beverage, is similarly achieved by male and female athletes of moderate and high fitness status.

4.1. Gastrointestinal Response to Recovery Nutrition

Exercise-induced gastrointestinal syndrome is a recently recognised term used to describe the gastrointestinal disturbances caused by exercise stress, including intestinal epithelial injury, impair gastrointestinal function, and associated GIS, mediated by neuroendocrine and circulatory pathways [6]. Exercise-induced gastrointestinal syndrome may impair nutrient availability via impaired tolerance and (or) nutrient losses (i.e., vomiting, malabsorption, or diarrhoea), thereby impacting overall recovery optimisation outcomes [6,31,40-43]. In response to the 2 h HIIT exercise, there were no differences between biological sexes in plasma I-FABP or plasma cortisol concentrations. Subsequently, there were no differences in carbohydrate malabsorption and (or) GIS incidence or severity between sexes. In line with the current findings, previous literature has shown comparable responses in intestinal injury and symptomology amongst male and female athletes, following prolonged strenuous exercise (i.e., 2 h running at 60% and 70% $\dot{V}O_{2max}$) and adhering to the same dietary control [17,19].

To our knowledge, this is the first study to explore the effect of training status on EIGS with post-exercise nutrition. Contrary to our hypothesis, the present findings suggest that circulatory- and (or) neuroendocrine-mediated gastrointestinal responses are unaffected by fitness status, as evidenced by no differences in intestinal injury (i.e., I-FABP), or stress (i.e., cortisol) and blood glucose responses, respectively. Consequently, it appears that assimilation of recovery nutrition does not differ by fitness status in response to a 2 h HIIT at the same relative intensity. Indeed, breath H_2 concentration reached clinical significance (e.g., >10 ppm breath H_2) amongst all groups (i.e., male, female, high fitness and moderate fitness athletes) following the exercise stress and consumption of the dairy milk beverage. These values are consistent with those seen amongst healthy individuals after consuming a solution containing 50 g lactose at rest [44], and are possibly related to the dosage and timing of nutrient intake and gastrointestinal presence. Similarly, the present findings show wide individual variation with regards to epithelial injury (Table 2) and carbohydrate malabsorption (Figure 2) that does not appear to be attributed to biological sex, nor fitness status, within the current cohort. Possible underlying physiological mechanisms include a) intestinal enterocyte carbohydrate transporter saturation capacity; and (or) b) exercise associated impairment of carbohydrate transporter translocation and activity effectiveness at the enterocyte brush border, secondary to increased enterocyte damage (i.e., circulatory gastrointestinal pathway of exercise-induced gastrointestinal syndrome) and (or) sympathetic activation (i.e., neuroendocrine gastrointestinal pathway of exercise-induced gastrointestinal syndrome). Transporter capacity and (or) individual capacity to curb circulatory and neuroendocrine strain on gastrointestinal integrity and function may be influenced by gut training [6], usual fibre and FODMAP intake [32], microbiota composition [39], or stress responses [45].

4.2. Immune Responses to Recovery Nutrition

Exercise-associated immune perturbations, characterised by, circulatory leukocytosis and neutrophilia, with an adjunct reduction in neutrophil function, and inflammatory cytokine responses occurs during the acute post-exercise period, proportional to the intensity and duration of the exercise stress [15,16]. In the current exercise protocol, these immune perturbations were observed amongst all groups, with no group differences observed for all immune markers measures. An interesting observation was that, in all groups, the recovery beverage ingested after exercise failed to prevent the decline in in-vitro bacterially-stimulated neutrophil elastase release (i.e., indicative of neutrophil degranulation function); an immune marker repeatedly shown to be sensitive to recovery nutrition responses compared with other immune response markers [4,5,7,15-17,46], and an immune functional response essential for coping with exercise-associated luminal originated bacterial pathogenic endotoxins (e.g., lipopolysaccharide) [6,47,48]. This effect was likely attributed to the delay (i.e., 60 min) in provision of the recovery beverage [4], considering immediate feeding has consistently been shown to prevent this immune functional decline [4,5,17]. We have recently provided evidence to show that following 2 h running at 70% $\dot{V}O_{2max}$ in thermoneutral conditions, immediate consumption of a dairy milk beverage (i.e., 1.2 g carbohydrate and 0.4 g protein/kg BM) increased bacterially-stimulated elastase release 3 h post-exercise [22]. It is hypothesised that the hyperinsulinemic and hypercalcemic effect on increased neutrophil chemotaxis, phagocytosis, and bactericidal capacity, and the effectiveness of neutrophil phagocytosis and degranulation processes, respectively, are possible contributing mechanisms [48-51]; whereas, delaying feeding may suppress the full activation of these mechanisms. Accordingly, no groups differences in serum insulin or plasma calcium concentrations were observed in response to consumption of the recovery beverage.

To date, studies investigating nutritional interventions to support immune function following prolonged, strenuous exercise have been conducted exclusively amongst well-trained, male athletes [4,5,17,46]. In response to an acute exercise stress, research suggests that circulating leukocyte populations and cytokines amongst females during the follicular phase of the menstrual cycle, are comparable to their male counterparts [53]. In the present study, authors observed minor differences in cytokine profiles between sexes characterised by a greater pro-inflammatory (i.e., IL-1β) response amongst male athletes, while a greater anti-inflammatory (i.e., IL-10) response was observed amongst female athletes. In the current body of literature, sex-based differences in cytokine responses to prolonged exercise are mixed, likely due to lack of controlling for menstrual cycle and (or) oral contraceptive use; however, in accordance with the present findings, it is generally accepted that responses are not markedly different between trained male and female athletes and of clinical irrelevance [53].

Resting and exercise-induced leukocyte and inflammatory markers are reportedly unaffected by training status [54-56]. Consistent with this consensus, there were no differences in resting or exercise-induced leukocytosis, neutrophil activity or inflammatory profiles between high and moderate fitness athletes. Findings from the current study builds on previous evidence to show that innate immune markers are not affected by training or biological sex, following an acute bout of HIIT exercise and 1 h delayed nutritional intervention.

4.3. Muscle Glycogen and Recovery Nutrition

Restoration of skeletal muscle glycogen stores is a primary focus of recovery nutrition following prolonged, glycogen depleting exercise [57]. In the current study, following 2 h HIIT, muscle glycogen stores were reduced to <300 mmol/kg dw. High fitness athletes showed greater phosphorylation of GSK-3β, suggestive of greater cellular activity towards skeletal muscle glycogen disposal. These results align with previous studies that have shown enhanced muscle glycogen restoration following continuous concentric exercise (i.e., 2 h cycling at 75% $\dot{V}O_{2max}$) amongst well-trained endurance athletes [58]. Indeed, consumption of 1.4 g carbohydrate/kg BM/h resulted in ~2-fold greater muscle glycogen concentrations amongst well-trained (i.e., >59 ml/kg/min) compared to sedentary (i.e., $\dot{V}O_{2max}$ <40 ml/kg/min) adults, and that these rates correlated to sarcolemma glucose-transporter 4 (GLUT-4) concentration [58]. Authors also observed a more pronounced blood glucose and insulin response amongst sedentary adults. In the current study, despite a training effect of phosphorylation of GSK-3β, there were no group differences in the magnitude of change of GSK-3β phosphorylation, and all groups failed to achieve muscle glycogen disposal towards repletion of muscle glycogen stores within 90 min of consumption of 1.2 g carbohydrate/kg BM. Previous research has shown compromised muscle glycogen resynthesis >24 h after eccentric exercise, likely associated with reduced translocation of the GLUT-4 transporter and (or) insulin sensitivity of the damaged muscle sarcolemma [59-61]. Indices of muscle damage (i.e., creatine kinase or myoglobin) were not collected in the current study, as they are known to peak >24 h after muscle damaging exercise [62]. Potential limitations within the current protocol include the inability to quantify the extent of muscle damage between groups and metabolic activity towards muscle glycogen resynthesis beyond the 4 h acute recovery period. Moreover, due to low rates of consent to muscle biopsies, it was not possible to compare male and female muscle glycogen responses. Differences in GLUT-4 concentration, exercise-induced muscle damage and potential recovery nutrition

requirements to mediate damaging effects amongst different athletic populations warrants further investigation.

4.4. Muscle Protein and Recovery Nutrition

It is well established that the male phenotype is characterised by greater skeletal muscle mass and a greater relative contribution of protein, in particular leucine, to substrate oxidation [63,64]. In light of these phenotypic and metabolic differences, has been suggested that female daily protein requirements relative to BM are 15-20% less than their male counterparts [65]. Current recovery nutrition guidelines, however, do not differentiate male and female protein requirements [3]. As previously noted, analysis of differences in intramuscular signalling proteins between males and females was not possible. Quantification of fractional synthetics rates is necessary to establish sex-based differences in muscle protein synthesis.

Phosphorylation of mTOR and Akt has consistently been observed following endurance exercise in endurance trained and untrained adults [66-68]. Indeed, in the current study, the exercise stress and subsequent intake of 0.4 g protein/kg BM increased phosphorylation of mTOR and Akt in both high and moderate fitness athletes. The current body of literature investigating protein requirements to support skeletal muscle protein synthesis following endurance exercise has employed athletes with $\dot{V}O_{2max}$ ranging from 45-66 ml/kg/min [68]. Although research is limited, the current findings support the notion that there do not appear to be any training-associated differences in the acute anabolic responses to a standardised relative intensity endurance exercise stress and (or) protein feeding.

4.5. Hydration and Recovery Nutrition

Total body water and fluid dynamics are known to differ between sexes and fitness groups, owing to differences in body composition (i.e., fat free mass vs. fat mass) [69]. TBW and ECW differed between all groups throughout the experimental trial, corresponding to differences in skeletal muscle mass and fat mass (Table 1, 2). Although resting P_{Osmol} was significantly different between male and female athletes, all participants commenced exercise within range of euhydration [17]. Likewise, all groups achieved euhydration at the end of the recovery period as indicated by body water, BM, P_V, and P_{Osmol}, with no group differences in fluid retention. It has previously been reported that women typically experience lower sweat rates compared to men [25,26]. In the current study, however, no differences in BM loss were observed. Greater decreases in P_V and sodium concentrations were observed amongst female athletes, however there were no groups differences in aldosterone concentrations. No other differences in hydration status after exercise or throughout the recovery period were observed between sexes. In the current study, all women completed trials during the follicular phase of their menstrual cycle, or when taking the active oral contraceptive pill. Maughan, McArthur & Shirreffs, have previously reported that menstrual phase did not influence fluid retention, net fluid balance, urine or plasma electrolyte losses following 1.8% exercise-induced BM loss, and provided 150% fluid replacement [70]. Consistent with current exercise and fluid replacement guidelines, sex-based differences in fluid-electrolyte balance during exercise are inconsequential, and as such there do not appear any differences in rehydration requirements [2].

Endurance-training is known to refine thermoregulatory responses, characterised by a more rapid onset of sweating and (or) greater overall sweat rates [23,24]. Accordingly, a significantly greater change in BM occurred amongst high fitness athletes in the current study. However, P_V, P_{Osmol}, TBW, electrolyte status, absolute and relative fluid intake, and fluid retention did not differ between groups. These findings support the notion that water losses <2.5% BM loss are mild in nature and do not significantly perturb fluid-electrolyte status. As such, aggressive hydration strategies are unnecessary, as adequate water intake can be achieved by drinking to thirst and consuming sodium-containing meals [71].

4.6. Performance Outcomes

It has previously been reported that female athletes are more reliant on fats as an energy substrate [28,29], and reach their fat max at a greater relative intensity compared to age and fitness matched males [30]. Due to greater energy requirements, absolute carbohydrate oxidation rates were significantly greater amongst males in the current study; however, relative contributions of carbohydrates and fats did not differ between sexes at any intensity (Table 4). It is generally accepted that at the elite level, female performance in endurance events is 7-12% slower than their male counterparts [72,73]. In the current study, however, absolute and relative intensities maintained during the performance trial were the same between males and females. Indeed, sex-based differences in the progression of recovery from muscle damaging exercise appear to be minor or inconsequential. Collectively, these findings suggest that the recovery nutrition provided (i.e., recovery beverage and meals), supported recovery optimisation similarly between male and female athletes.

Endurance trained athletes display enhanced respiratory capacity of the muscle and increase propensity for fat oxidation during submaximal exercise [74-76]. In the current study, high fitness athletes demonstrated greater rates of fat

oxidation rates at 70% $\dot{V}O_{2max}$. In addition, although greater distances were covered by high fitness athletes, there were no differences between groups in the relative intensity during the performance test. This conflicts with previous literature that has shown trained athletes reach their lactate threshold at a higher percentage of their $\dot{V}O_{2max}$, and maintain a higher percentage of their $\dot{V}O_{2max}$ during endurance events [27]. It is possible that differences in fitness status were insufficient to detect significant differences.

4.7. Strengths and Limitations

This is the first study to comprehensively compare markers of recovery optimisation between male and female, and moderate and high fitness athletes. Moreover, this is the first study to comprehensively examine the effect of post-exercise nutrition on multiple markers of innate immune function amongst female athletes. Although preceding studies have been indispensable in formulating current recovery nutrition guidelines, such studies have examined recovery outcomes in isolation amongst limited participant cohorts. The present findings highlight the inter-related nature of recovery optimisation outcomes, as well as the wide individual variation in exercise induced perturbations and recovery outcomes, particularly those associated with EIGS and nutrient assimilation. This reinforces the importance of recovery nutrition requirements being provided on an individual basis. A noted limitation within the current study is the imbalance between male and female participant numbers. The authors experienced difficulty recruiting female athletes to take part in the current study to completion, presumably due to the high burden and invasive nature of the experimental protocol. Despite lower female participant numbers, power calculations indicated n= 8 was sufficient to detect differences of a magnitude of practical and clinical relevance for the investigated markers.

5. Conclusion

The current 2 h HIIT exercise protocol resulted in comparable exercise-induced increases in plasma I-FABP, carbohydrate malabsorption, GIS and decline in neutrophil function amongst male and female athletes of moderate at high fitness. Total body water, plasma sodium and plasma osmolality were greater, and plasma volume change was lesser amongst male compared to female athletes. The following morning, carbohydrate oxidation rates were greater amongst male athletes at all intensities, but no differences in fat oxidation rates were observed. In response to the 2 h HIIT, body water losses were significantly greater amongst high fitness athletes compared to moderate fitness athletes, but there were no differences in post-exercise muscle glycogen. The recovery beverage containing 0.4 g protein/kg BM resulted in increased phosphorylation of mTOR and Akt protein signalling amongst high and moderate fitness groups. Greater phosphorylation of GSK-3β was observed amongst high fitness athletes, but there were no group differences in early-phase muscle glycogen resynthesis. The following day, rates of carbohydrate oxidation were greater at 50% and 60% $\dot{V}O_{2max}$, and fat oxidation were greater at 70% and 80% $\dot{V}O_{2max}$ amongst high compared to moderate fitness athletes. Findings from the current study suggest that recovery optimisation following a 2 h HIIT exercise protocol and consumption of 1.2 g/kg BM carbohydrate and 0.4 g/kg BM protein, using a dairy milk recovery beverage, is similarly achieved by male and female athletes of moderate and high fitness status. Considering the professional practice implications of the present findings, individual assessment of body composition, fluid dynamics, and gastrointestinal response to exercise and subsequent absorptive capacity, should be primary considerations for post-exercise nutritional recommendations, before biological sex and fitness status.

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DISCLOSURE

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Table 2. Change in hydration and recovery biomarkers in response to 2 h HIIT exercise (between 60 and 80% $\dot{V}O_{2max}$) in temperate ambient conditions and after consumption of dairy milk recovery beverage by (A) male and female and (B) moderate and high fitness athletes.

A	Male				Female			
	Pre-exercise	**0 h**	**2 h**	**4 h**	**Pre-exercise**	**0 h**	**2 h**	**4 h**
Total body water (%)	61.9 (60.4 to 62.7)	62.5[§§] (61.4 to 63.6)	61.3 (60.3 to 62.4)	61.4 (60.3 to 62.5)	56.5[aa] (54.1 to 59.0)	56.8[§§##] (54.1 to 59.3)	56.1[##] (53.7 to 58.5)	56.3[##] (53.7 to 58.8)
L	44.4 (42.5 to 46.3)	44.2[§§] (42.4 to 46.1)	44.2[§§] (42.3 to 46.1)	44.7 (42.7 to 46.6)	35.3[##] (32.3 to 38.3)	34.8[§§##] (31.5 to 38.1)	34.9[§§##] (31.8 to 38.1)	35.3[##] (32.1 to 38.5)
Extracellular water (%)	24.2 (23.6 to 24.8)	24.1[§§] (23.6 to 24.6)	23.9 (23.4 to 24.4)	23.9 (23.3 to 24.4)	24.6 (23.7 to 25.4)	24.4[§§] (23.2 to 25.5)	24.1 (23.1 to 25.1)	24.1 (23.1 to 25.1)
L	17.6 (16.8 to 18.4)	17.2[§§] (16.4 to 18.0)	17.3[§§] (16.5 to 18.1)	17.5[§§] (16.6 to 18.3)	15.5[##] (14.2 to 16.9)	15.2[§§##] (13.8 to 16.6)	15.2[§§##] (13.8 to 16.6)	15.3[§§##] (14.0 to 16.7)
P_{Osmol} (mOsmol/kg)	295 (292 to 298)	297 (294 to 300)	295 (293 to 298)	294 (291 to 297)	286[##] (280 to 292)	286[##] (281 to 291)	288[##] (283 to 292)	289[##] (283 to 296)
Δ plasma volume (%)	---	-2.0 (-4.2 to 0.2)	1.6[††] (-1.0 to 4.2)	1.6[††] (-0.2 to 3.5)	---	-5.6 (-9.5 to -1.8)	-3.8[††] (-8.0 to 0.4)	-2.5[††] (-6.8 to 1.7)
Aldosterone (nmol/L)	138 (103 to 173)	374[§§] (294 to 454)	131 (105 to 157)	110 (87 to 133)	115 (70 to 160)	329[§§] (159 to 498)	140 (84 to 197)	106 (68 to 144)
Serum sodium (nmol/L)	141 (140 to 142)	141 (138 to 145)	142 (139 to 146)	142 (139 to 144)	139[##] (137 to 140)	132[##] (126 to 138)	134[##] (126 to 141)	134[##] (128 to 141)

Serum potassium (nmol/L)	4.5 (4.3 to 4.6)	4.6 (4.4 to 4.7)	4.4 (4.2 to 4.5)	4.5 (4.3 to 4.6)	4.7 (4.2 to 5.2)	4.3 (3.8 to 4.8)	4.4 (3.7 to 5.0)	4.6 (4.1 to 5.2)
Serum calcium (nmol/L)	2.4 (2.3 to 2.4)	2.3[§§] (2.2 to 2.3)	2.4 (2.3 to 2.4)	2.4 (2.3 to 2.4)	2.3 (2.3 to 2.4)	2.2[§§] (2.1 to 2.2)	2.3 (2.1 to 2.4)	2.3 (2.2 to 2.4)
Cortisol (nmol/L)	550 (448 to 651)	576 (451 to 701)	390[††] (323 to 457)	285[††] (224 to 346)	766 (469 to 1064)	764 (505 to 1023)	619[††] (182 to 1057)	457[††] (132 to 782)
I-FABP (pg/ml)	587 (412 to 763)	1616[§§] (1073 to 2158)	----	----	673 (335 to 1010)	1086[§§] (656 to 1516)	----	----
sCD14 (µg/ml)	2.3 (2.1 to 2.5)	2.3 (2.1 to 2.5)	----	----	2.2 (1.6 to 2.8)	1.9 (1.2 to 2.6)	----	----
IL-1β (pg/ml)	2.3 (0.8 to 3.7)	2.5 (1.0 to 4.0)	2.7 (1.2 to 4.1)	2.7 (1.2 to 4.2)	3.7 (0.4 to 7.0)	3.1 (0.6 to 5.7)	3.5 (0.7 to 6.3)	4.2 (<0.1 to 8.5)
TNF-α (pg/ml)	9.8 (7.7 to 11.8)	10.2 (8.0 to 12.3)	10.2 (8.1 to 12.3)	11.8 (8.2 to 15.5)	15.3 (6.5 to 24.1)	13.4 (5.7 to 21.2)	14.8 (6.1 to 23.6)	14.7 (2.9 to 26.6)
IL-6 (pg/ml)	35.7 (4.5 to 66.9)	37.9 (4.1 to 71.7)	36.4 (5.2 to 67.6)	35.2 (3.3 to 67.1)	23.9 (<0.1[*] to 65.4)	23.7 (<0.1[*] to 64.4)	29.1 (<0.1[*] to 81.4)	23.1 (<0.1[*] to 62.7)
IL-8 (pg/ml)	16.7 (5.0 to 28.5)	17.4 (5.3 to 29.4)	16.8 (5.0 to 28.6)	17.0 (5.1 to 28.9)	13.6 (<0.1[*] to 28.5)	12.5 (<0.1[*] to 26.6)	13.7 (<0.1[*] to 32.1)	12.1 (<0.1[*] to 26.3)
IL-10 (pg/ml)	14.2 (6.7 to 21.6)	21.6[§§] (14.6 to 28.6)	14.3 (8.2 to 20.4)	16.0 (8.6 to 23.5)	23.7[##] (9.7 to 37.7)	38.3[§§] (13.1 to 63.6)	19.8[#] (6.8 to 32.9)	15.4 (5.9 to 25.0)

IL-1rα (pg/ml)	27.7 (17.0 to 38.5)	35.0§ (22.8 to 47.3)	36.4§ (24.9 to 47.9)	34.8§ (24.7 to 44.9)	34.6 (20.3 to 48.8)	40.0§ (21.6 to 58.4)	55.7§ (26.5 to 84.8)	37.2§ (21.6 to 52.9)

B	$\dot{V}O_{2max}$ ≤55 ml/kg BM/min				$\dot{V}O_{2max}$ ≥60 ml/kg BM/min			
	Pre-exercise	**0 h**	**2 h**	**4 h**	**Pre-exercise**	**0 h**	**2 h**	**4 h**
Total body water (%)	58.9 (57.2 to 60.6)	59.9§§ (58.1 to 61.7)	58.7 (57.1 to 60.3)	58.8 (57.1 to 60.5)	63.3## (62.0 to 64.5)	63.5§§## (61.9 to 65.2)	62.9## (61.5 to 64.4)	62.9## (61.6 to 64.2)
L	40.8 (38.0 to 43.5)	40.7§§ (38.0 to 43.4)	40.6§§ (37.8 to 43.4)	41.0 (38.2 to 43.8)	43.7 (39.9 to 47.5)	43.2§§ (39.0 to 47.4)	43.4§§ (39.4 to 47.3)	43.8 (39.8 to 47.9)
Extracellular water (%)	23.9 (23.2 to 24.6)	23.9§§ (23.2 to 24.5)	23.6 (22.9 to 24.2)	23.6 (22.9 to 24.3)	25.1a (24.7 to 25.5)	24.9§§# (24.7 to 25.4)	24.7# (24.2 to 25.3)	24.7# (24.3 to 25.1)
L	16.5 (15.5 to 17.5)	16.2§§ (15.2 to 17.2)	16.3§§ (15.3 to 17.3)	16.5§§ (15.4 to 17.5)	18.0 (16.6 to 19.3)	17.5§§ (16.1 to 19.0)	17.6§§ (16.2 to 19.0)	17.8§§ (16.3 to 19.2)
P_{Osmol} (mOsmol/kg)	293 (290 to 297)	294 (290 to 298)	294 (290 to 297)	294 (290 to 298)	294 (288 to 300)	298 (291 to 304)	295 (292 to 298)	294 (289 to 298)
Δ plasma volume (%)	---	-3.0 (-5.6 to 0.5)	-0.3†† (-3.7 to 3.2)	-0.4†† (-3.0 to 2.2)	---	-3.4 (-7.1 to 0.3)	1.8†† (-1.5 to 5.2)	1.8†† (-0.8 to 4.4)
Aldosterone (nmol/L)	158 (116 to 199)	394§§ (286 to 502)	150 (144 to 186)	117 (87 to 147)	111 (67 to 155)	363§§ (264 to 461)	115 (89 to 140)	98 (71 to 125)
Serum sodium (nmol/L)	140 (139 to 141)	139 (134 to 144)	140 (134 to 145)	139 (135 to 143)	140 (139 to 141)	136 (131 to 141)	142 (137 to 147)	141 (137 to 146)

Serum potassium (nmol/L)	4.4 (4.2 to 4.7)	4.5 (4.3 to 4.7)	4.3 (4.1 to 4.5)	4.5 (4.3 to 4.7)	4.7 (4.3 to 5.0)	4.5 (4.2 to 4.9)	4.5 (4.1 to 5.0)	4.5 (4.1 to 5.0)
Serum calcium (nmol/L)	2.3 (2.3 to 2.4)	2.2§§ (2.2 to 2.3)	2.3 (2.3 to 2.4)	2.3 (2.3 to 2.4)	2.4 (2.3 to 2.4)	2.2§§ (2.1 to 2.3)	2.4 (2.3 to 2.5)	2.4 (2.3 to 2.4)
Cortisol (nmol/L)	634 (485 to 783)	702 (542 to 861)	492†† (326 to 659)	355†† (228 to 482)	533 (361 to 705)	513 (334 to 692)	339†† (227 to 452)	274†† (152 to 395)
I-FABP (pg/ml)	600 (444 to 755)	1671§§ (1022 to 2320)	----	----	681 (257 to 1105)	1365§§ (634 to 2095)	----	----
sCD14 (µg/ml)	2.2 (1.9 to 2.6)	2.1 (1.8 to 2.5)	----	----	2.3 (2.2 to 2.5)	2.2 (2.0 to 2.5)	----	----
IL-1β (pg/ml)	2.1 (0.8 to 3.4)	1.9 (0.9 to 2.9)	2.2 (1.1 to 3.4)	2.4 (1.1 to 3.7)	3.7 (0.1 to 7.2)	3.8 (0.3 to 7.3)	4.0 (0.3 to 7.7)	4.7 (0.7 to 8.8)
TNF-α (pg/ml)	9.7 (6.9 to 12.5)	9.4 (7.0 to 11.8)	10.1 (7.8 to 12.4)	11.1 (6.2 to 15.9)	12.8 (6.5 to 19.2)	12.8 (7.1 to 18.5)	12.8 (5.8 to 19.8)	15.7 (7.5 to 23.9)
IL-6 (pg/ml)	32.2 (<0.1* to 67.8)	32.9 (<0.1* to 71.8)	33.8 (<0.1* to 70.2)	33.6 (<0.1* to 72.5)	27.8 (<0.1* to 69.2)	29.5 (<0.1* to 70.2)	28.1 (<0.1* to 69.4)	27.5 (<0.1* to 66.6)
IL-8 (pg/ml)	14.0 (2.1 to 25.8)	13.3 (1.4 to 25.2)	14.1 (1.4 to 26.8)	14.4 (1.0 to 27.9)	16.5 (<0.1* to 35.3)	17.8 (<0.1* to 36.0)	16.2 (<0.1* to 34.4)	17.3 (0.3 to 34.4)
IL-10 (pg/ml)	13.6 (7.2 to 20.0)	20.1§§ (12.4 to 27.8)	12.3 (6.9 to 17.8)	13.0 (6.1 to 19.8)	21.5 (3.8 to 39.2)	32.5§§ (12.7 to 52.2)	20.6 (6.6 to 34.6)	22.2 (7.0 to 37.5)

IL-1rα (pg/ml)	25.4 (16.6 to 34.2)	31.1[§] (20.7 to 41.4)	38.9[§] (24.5 to 53.2)	31.0[§] (20.9 to 41.1)	34.0 (11.7 to 56.3)	43.9[§] (18.1 to 69.7)	43.4[§] (20.4 to 66.5)	43.5[§] (25.0 to 62.0)

Mean (95% CI): MEOTime †† $P< 0.01$ and † $P< 0.05$ vs. post-exercise, MEOTime §§ $P< 0.01$ and § $P< 0.05$ vs. pre-exercise, [##] $P< 0.01$ and [#] $P< 0.05$ vs. comparator, [*] under detectable lowest standard.

Table 3. Incidence of gastrointestinal symptoms and severity of gut discomfort, total, upper-, and lower-gastrointestinal symptoms in response to 2 h HIIT exercise (between 60% and 80% $\dot{V}O_{2max}$) in temperate ambient conditions and after consumption of dairy milk recovery beverage by (A) male and female and (B) moderate and high fitness athletes.

A	**Male**					**Female**				
	Exercise		**Recovery**			**Exercise**		**Recovery**		
	Incidence	**Severity**	**Incidence**	**Severity**		**Incidence**	**Severity**	**Incidence**	**Severity**	
	% (severe)		**% (severe)**	**Acute (0-2 h)**	**Total (0-4 h)**	**% (severe)**		**% (severe)**	**Acute (0-2 h)**	**Total (0-4 h)**
Gut discomfort	NA	6 (1-27)	NA	4 (1-19)	10 (2-41)	NA	4 (6-10)	NA	5 (2-24)	15 (3-51)
Total GIS[a]	70 (56)	8 (1-52)	78 (52)	4 (1-21)	11 (2-59)	50 (50)	5 (6-11)	74 (63)	5 (2-24)	16 (3-51)
Upper GIS[b]	41 (22)	2 (1-19)	41 (22)	2 (1-19)	5 (1-37)	50 (38)	4 (4-11)	50 (50)	5 (14-24)	13 (5-51)
Belching	33 (19)	2 (1-9)	7 (0)	0 (1-1)	0 (1-1)	38 (25)	2 (2-10)	25 (0)	0 (1-1)	0 (1-2)
Heartburn	15 (7)	1 (2-10)	4 (0)	0	0 (3-3)	13 (0)	0 (2)	13 (0)	0	1 (4-4)
Bloating	4 (0)	0 (2)	33 (19)	2 (2-19)	5 (2-36)	13 (0)	1 (4)	38 (38)	5 (13-24)	12 (5-51)
Stomach pain	4 (0)	0 (1)	4 (4)	0	0 (5-5)	13 (13)	1 (7)	0	0	0
Urge to regurgitate	4 (0)	0 (1)	0	0	0	0	0	0	0	0
Regurgitation	0	0	0	0	0	0	0	0	0	0
Lower GIS[b]	37 (15)	1 (1-10)	44 (30)	1 (1-10)	4 (2-23)	25 (0)	0 (1-2)	50 (25)	0	3 (2-16)
Flatulence	19 (0)	0 (1-3)	15 (7)	0 (2-3)	1 (3-6)	13 (0)	0 (1)	25 (13)	0	1 (3-5)

Lower bloating	19 (0)	0 (1-3)	26 (7)	0	1 (1-6)	13 (0)	0 (2)	38 (25)	0	2 (2-11)
Urge to defecate	19 (0)	0 (1-3)	26 (7)	1 (1-8)	2 (2-20)	0	0	0	0	0
Intestinal pain	7 (0)	0 (3-4)	0	0	0	0	0	0	0	0
Abnormal defecation[c]	0	0	11 (11)	0 (10-10)	0 (10)	0	0	0	0	0
Others										
Nausea	15 (7)	1 (1-11)	7 (4)	0 (5-5)	0 (4-5)	0	0	0	0	0
Dizziness	48 (30)	3 (2-13)	33 (7)	1 (1-7)	1 (1-9)	13 (0)	0 (3)	13 (0)	0 (2)	0 (2)
Stitch[d]	7 (4)	1 (1-19)	0	0	0	0	0	0	0	0
Appetite	78 (67)	11 (1-33)	96 (89)	19 (2-44)	24 (2-68)	100 (100)	18 (6-39)	100 (100)	19 (8-28)	24 (14-32)
Thirst	96 (93)	20 (1-46)	96 (96)	15 (2-27)	20 (6-40)	100 (100)	30 (22-44)	100 (100)	18 (11-30)	26 (15-42)

B	$\dot{V}O_{2max}$ ≤55 ml/kg BM/min					$\dot{V}O_{2max}$ ≤60 ml/kg BM/min				
	Exercise		Recovery			Exercise		Recovery		
	Incidence	Severity	Incidence	Severity		Incidence	Severity	Incidence	Severity	
	% (severe)		% (severe)	Acute (0-2 h)	Total (0-4 h)	% (severe)		% (severe)	Acute (0-2 h)	Total (0-4 h)
Gut discomfort	NA	7 (4-14)	NA	2 (1-11)	5 (3-15)	NA	5 (1-27)	NA	5 (2-24)	14 (2-51)
Total GIS[a]	80 (60)	8 (4-27)	70 (40)	2 (1-11)	6 (3-17)	67 (57)	8 (1-52)	76 (62)	5 (2-24)	16 (2-59)
Upper GIS[b]	50 (20)	2 (2-7)	30 (10)	0 (1-1)	2 (1-14)	43 (29)	3 (1-19)	48 (38)	4 (2-24)	10 (2-51)
Belching	30 (20)	2 (2-7)	10 (0)	0 (1-1)	0 (1-1)	38 (19)	2 (1-10)	14 (0)	0 (1)	0 (1-2)
Heartburn	10 (0)	0 (2)	0	0	0	19 (10	1 (2-10)	5 (0)	0	0 (4-4)
Bloating	10 (0)	0 (4)	20 (10)	0	2 (2-14)	5 (0)	0 (2)	43 (33)	4 (2-24)	10 (2-51)
Stomach pain	10 (0)	0 (1)	0	0	0	5 (5)	0 (7)	5 (5)	0	0 (5)
Urge to regurgitate	0	0	0	0	0	0	0	0	0	0
Regurgitation	0	0	0	0	0	0	0	0	0	0
Lower GIS[b]	50 (20)	2 (1-10)	50 (30)	2 (1-10)	4 (3-14)	33 (10)	1 (1-6)	43 (29)	0 (2-3)	4 (2-23)
Flatulence	20 (0)	0 (1-3)	20 (0)	0	1 (3-3)	19 (0)	0 (1-3)	19 (14)	0 (2-3)	1 (3-6)

Lower bloating	20 (0)	1 (2-3)	20 (10)	0	1 (2-6)	19 (0)	0 (1-3)	38 (14)	0	2 (1-11)
Urge to defecate	30 (0)	0 (1-3)	30 (10)	1 (1-4)	2 (2-5)	10 (0)	0 (1-3)	10 (0)	0	0 (3-4)
Intestinal pain	10 (0)	0 (4)	0	0	0	5 (0)	0 (3)	0	0	0
Abnormal defecation[c]	0	0	10 (10)	1 (10-10)	1 (10-10)	0	0	14 (14)	0	1 (10-10)
Others										
Nausea	20 (0)	0 (1)	0	0	0	10 (10)	1 (5-11)	10 (5)	0 (5-5)	0 (4-5)
Dizziness	50 (30)	4 (2-13)	30 (0)	1 (1-3)	1 (1-3)	38 (19)	2 (2-8)	29 (5)	1 (1-7)	1 (1-9)
Stitch[d]	10 (0)	0 (1)	0	0	0	5 (5)	1 (19)	0	0	0
Appetite	70 (70)	9 (6-29)	100 (100)	23 (12-35)	28 (18-43)	95 (81)	15 (1-39)	100 (95)	18 (2-44)	24 (2-68)
Thirst	90 (90)	20 (11-44)	100 (100)	14 (4-25)	19 (6-35)	100 (100)	25 (13-46)	100 (100)	18 (7-30)	24 (8-42)

Values are presented as means and range of participants reporting GIS incidence (*n*= 35). GIS incidence during exercise and recovery are presented as percentage of total participants reporting GIS ≥1 on the mVAS. GIS severity during exercise and recovery are presented as mean summative accumulation of mVAS rating scale of measured time periods and individual range of participant reporting GIS incidence [33]. [a]Summative accumulation of upper-, lower-, and other gastrointestinal symptoms, [b]summative accumulation of upper- or lower- gastrointestinal symptoms, [c]abnormal defecation including loose watery stools, diarrhoea and blood in stools, and [d]acute transient abdominal pain. NA: not applicable. Wilcoxon signed-rank tests showed no differences between A) moderate and high fitness status athletes, and B) male and female athletes for GIS. Hedge's *g* measurement of effect size for GIS and feeding tolerance severity between A) moderate and high fitness status athletes, and B) male and female athletes was determined as >0.50 and >0.80 for medium and large effects, respectively; however, no medium or large effects size value were detected between groups.

REFERENCES

[1] Garthe I, Maughan RJ. Athletes and supplements: prevalence and perspectives. Int J Sport Nut Exerc Metab. 2018;28(2):126-38.

[2] Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, Stachenfeld NS. American College of Sports Medicine position stand. Exercise and fluid replacement. Med Sci Sports Exerc. 2007;39(2):377-90.

[3] Thomas DT, Erdman KA, Burke LM. Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: nutrition and athletic performance. J Acad Nutr Diet. 2016;116(3):501-28.

[4] Costa RJS, Oliver SJ, Laing SJ, Walters R, Bilzon JL, Walsh NP. Influence of timing of postexercise carbohydrate-protein ingestion on selected immune indices. Int J Sport Nut Exerc Metab. 2009;19(4):366-84.

[5] Costa RJS, Walters R, Bilzon JL, Walsh NP. Effects of immediate postexercise carbohydrate ingestion with and without protein on neutrophil degranulation. Int J Sport Nut Exerc Metab. 2011;21(3):205-13.

[6] Costa RJS, Snipe RMJ, Kitic CM, Gibson PR. Systematic review: exercise- induced gastrointestinal syndrome—implications for health and intestinal disease. Aliment Pharmacol Ther. 2017;46(3):246-65.

[7] Peake JM, Neubauer O, Walsh NP, Simpson RJ. Recovery of the immune system after exercise. J Appl Physiol. 2017;122(5):1077-87.

[8] Alghannam AF, Jedrzejewski D, Bilzon J, Thompson D, Tsintzas K, Betts JA. Influence of post-exercise carbohydrate-protein ingestion on muscle glycogen metabolism in recovery and subsequent running exercise. Int J Sport Nut Exerc Metab. 2016;26(6):572-80.

[9] Betts J, Williams C, Duffy K, Gunner F. The influence of carbohydrate and protein ingestion during recovery from prolonged exercise on subsequent endurance performance. J Sport Sci. 2007;25(13):1449-60.

[10] Tsintzas K, Williams C, Boobis L, Symington S, Moorehouse J, Garcia-Roves P, Nicholas C. Effect of carbohydrate feeding during recovery from prolonged running on muscle glycogen metabolism during subsequent exercise. Int J Sport Med. 2003;24(06):452-8.

[11] Wong SH, Williams C, Adams N. Effects of ingesting a large volume of carbohydrate-electrolyte solution on rehydration during recovery and subsequent exercise capacity. Int J Sport Nut Exerc Metab. 2000;10(4):375-93.

[12] Wong SH, Williams C. Influence of different amounts of carbohydrate on endurance running capacity following short term recovery. Int J Sport Med. 2000;21(06):444-52.

[13] Magalhães J, Rebelo A, Oliveira E, Silva JR, Marques F, Ascensão A. Impact of Loughborough Intermittent Shuttle Test versus soccer match on physiological, biochemical and neuromuscular parameters. Eur J Appl Physiol. 2010;108(1):39.

[14] Miyama MO, Nosaka KA. Influence of surface on muscle damage and soreness induced by

consecutive drop jumps. J Strength Cond Res. 2004;18(2):206-11.

[15] Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop N, Fleshner M, Green C, Pedersen BK, Hoffman-Goete L, Rogers CJ. Position statement part one: immune function and exercise. 2011.

[16] Walsh NP, Gleeson M, Pyne DB, Nieman DC, Dhabhar FS, Shephard RJ, Oliver SJ, Bermon S, Kajeniene A. Position statement part two: maintaining immune health. 2011.

[17] Costa RJS, Camões-Costa V, Snipe RM, Dixon D, Russo I, Huschtscha Z. The Impact of a dairy milk recovery beverage on bacterially stimulated neutrophil function and gastrointestinal tolerance in response to hypohydration inducing exercise stress. Int J Sport Nut Exerc Metab. 2020;30(4):237-48.

[18] Snipe RMJ, Khoo A, Kitic CM, Gibson PR, Costa RJS. The impact of exertional-heat stress on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profile. Eur J Appl Physiol. 2018;118(2):389-400.

[19] Snipe RMJ, Costa RJS. Does biological sex impact intestinal epithelial injury, small intestine permeability, gastrointestinal symptoms and systemic cytokine profile in response to exertional-heat stress?. J Sport Sci. 2018;36(24):2827-35.

[20] Peake J, Della Gatta P, Suzuki K, Nieman D. Cytokine expression and secretion by skeletal muscle cells: regulatory mechanisms and exercise effects. Exerc Immunol Rev. 2015;21:8-25.

[21] Russo I, Camões-Costa V, Gaskell SK, Porter J, Burke LM, Costa RJS. Systematic literature review: The effect of dairy milk on markers of recovery optimisation in response to endurance exercise. Int J Sport Sci. 2019;9(4):69-85.

[22] Costa RJS, Camões-Costa V, Snipe RM, Dixon D, Russo I, Huschtscha Z. Impact of exercise-induced hypohydration on gastrointestinal integrity, function, symptoms, and systemic endotoxin and inflammatory profile. J Appl Physiol. 2019;126(5):1281-91.

[23] Inoue Y, Havenith G, Kenney WL, Loomis JL, Buskirk ER. Exercise- and methylcholine-induced sweating responses in older and younger men: effect of heat acclimation and aerobic fitness. Int J Biometeorol. 1999;42:210–6.

[24] Armstrong LE, Maresh CM. Effects of training, environment, and host factors on the sweating response to exercise. Int J Sports Med. 1998;19:S103–5.

[25] Sawka MN, Coyle EF. Influence of body water and blood volume on thermoregulation and exercise performance in the heat. Exerc Sport Sci Rev. 1999;27:167-218.

[26] Shapiro Y, Pandolf KB, Avellini BA, Pimental NA, Goldman RF. Physiological responses of men and women to humid and dry heat. J Appl Physiol. 1980;49(1):1-8.

[27] Demello JJ, Cureton KJ, Boineau RE, Singh MM. Ratings of perceived exertion at the lactate threshold in trained and untrained men and women. Med Sci Sports Exerc. 1987;19(4):354-62.

[28] Carter SL, Rennie C, Tarnopolsky MA. Substrate utilization during endurance exercise in men and women after endurance training. Am J Physiol Endocrinol Metab. 2001;280(6):E898-E907.

[29] Tarnopolsky LJ, MacDougall JD, Atkinson SA, Tarnopolsky MA, Sutton JR. Gender differences in substrate for endurance exercise. J Appl Physiol. 1990;68(1):302-8.

[30] Venables MC, Achten J, Jeukendrup AE. Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. J Appl Physiol. 2005;98(1):160-7.

[31] Russo I, Della Gatta PA, Garnham A, Porter J, Burke LM, Costa RJS. Does the nutritional composition of dairy milk based recovery beverages influence post-exercise gastrointestinal and immune status, and subsequent markers of recovery optimisation in response to high intensity interval exercise? Frontiers Nutr. [In Press]

[32] Gaskell SK, Taylor B, Muir J, Costa RJS. Impact of 24-h high and low fermentable oligo-, di-, monosaccharide, and polyol diets on markers of exercise-induced gastrointestinal syndrome in response to exertional heat stress. Appl Physiol Nutr Metab. 2020;45(6): 569-580.

[33] Gaskell SK, Snipe RMJ, Costa RJS. Test–Retest Reliability of a Modified Visual Analog Scale Assessment Tool for Determining Incidence and Severity of Gastrointestinal Symptoms in Response to Exercise Stress. Int J Sport Nut Exerc Metab. 2019;29(4):411-9.

[34] Snipe RMJ, Costa RJS. Does the temperature of water ingested during exertional-heat stress influence gastrointestinal injury, symptoms, and systemic inflammatory profile? J Sci Med Sport. 2018;21(8):771-6.

[35] Maughan RJ, Leiper JB. Sodium intake and post-exercise rehydration in man. Eur J Appl Physiol Occup Physiol. 1995;71(4):311-9.

[36] Duncan MJ, Smith M, Cook K, James RS. The acute effect of a caffeine-containing energy drink on mood state, readiness to invest effort, and resistance exercise to failure. J Strength Cond Res. 2012;26(10):2858-65.

[37] Bergmeyer HU. Methods of enzymatic analysis. Verlag Chemie; 1974.

[38] Harris RC, Hultman E, Nordesjö LO. Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. Scan J Clin Lab Invest. 1974;33(2):109-20.

[39] Bennett CJ, Henry R, Snipe RM, Costa RJ. Is the gut microbiota bacterial abundance and composition associated with intestinal epithelial injury, systemic inflammatory profile, and gastrointestinal symptoms in response to exertional-heat stress?. J Sci Med Sport. 2020 [In press].

[40] Costa RJS, Snipe RMJ, Camões-Costa V, Scheer V, Murray A. The impact of gastrointestinal symptoms and dermatological injuries on nutritional intake and hydration status during ultramarathon events. Sport Med Open. 2016;2(1):16.

[41] Costa RJ, Miall A, Khoo A, Rauch C, Snipe R, Camões-Costa V, Gibson P. Gut-training: The impact of two weeks repetitive gut-challenge during exercise on gastrointestinal status, glucose availability, fuel kinetics, and running performance. Appl Physiol Nutr Metab. 2017;42(5):547-57.

[42] Lang JA, Gisolfi CV, Lambert GP. Effect of exercise intensity on active and passive glucose absorption. Int J Sport Nut Exerc Metab. 2006;16(5):485-93.

[43] Van Wijck K, Pennings B, van Bijnen AA, Senden JM, Buurman WA, Dejong CH, Van Loon LJ, Lenaerts K. Dietary protein digestion and absorption are impaired during acute post-exercise recovery in young men. Am J Physiol Heart Circ Physiol. 2012;304(5):R356-R361.

[44] Barrett JS, Irving PM, Shepherd SJ, Muir JG, Gibson PR. Comparison of the prevalence of fructose and lactose malabsorption across chronic intestinal disorders. Aliment Pharmacol Ther. 2009;30(2):165-74.

[45] Gaskell SK, Parr A, Rauch C, Costa RJS. Diurnal versus nocturnal exercise – impact on the gastrointestinal tract. Med Sci Sport Exerc. 2020; [In press].

[46] Costa RJS, Fortes MB, Richardson K, Bilzon JL, Walsh NP. The effects of postexercise feeding on saliva antimicrobial proteins. Int J Sport Nut Exerc Metab. 2012;22(3):184-91.

[47] Peake JM. Exercise-induced alterations in neutrophil degranulation and respiratory burst activity: possible mechanisms of action. Exerc Immunol Rev. 2002;8:49.

[48] Costa RJ, Gaskell SK, McCubbin AJ, Snipe RM. Exertional-heat stress-associated gastrointestinal perturbations during Olympic sports: Management strategies for athletes preparing and competing in the 2020 Tokyo Olympic Games. Temperature. 2020 Jan 2;7(1):58-88.

[49] Jaconi ME, Lew DP, Carpentier JL, Magnusson KE, Sjögren M, Stendahl O. Cytosolic free calcium elevation mediates the phagosome-lysosome fusion during phagocytosis in human neutrophils. J Cell Biol. 1990;110(5):1555-64.

[50] Tapper H, Furuya W, Grinstein S. Localized exocytosis of primary (lysosomal) granules during phagocytosis: role of Ca2+-dependent tyrosine phosphorylation and microtubules. J Immunol. 2002;168(10):5287-96.

[51] Walrand S, Guillet C, Boirie Y, Vasson MP. In vivo evidences that insulin regulates human polymorphonuclear neutrophil functions. J Leuk Biol. 2004;76(6):1104-10.

[52] Walrand S, Guillet C, Boirie Y, Vasson MP. Insulin differentially regulates monocyte and polymorphonuclear neutrophil functions in healthy young and elderly humans. J Clin Endocrinol Metab. 2006;91(7):2738-48.

[53] Gillum TL, Kuennen MR, Schneider S, Moseley P. A review of sex differences in immune function after aerobic exercise. Exerc Immunol Rev. 2011;17.

[54] Nieman DC, Johanssen LM, Lee JW, Arabatzis K. Infectious episodes in runners before and after the Los Angeles Marathon. J Sports Med Phys Fitness. 1990;30(3):316-28.

[55] Moyna NM, Acker GR, Fulton JR, Weber K, Goss FL, Robertson RJ, Tollerud DJ, Rabin BS. Lymphocyte function and cytokine production during incremental exercise in active and sedentary males and females. Int J Sports Med. 1996;17(08):585-91.

[56] Moyna NM, Acker GR, Weber KM, Fulton JR, Goss FL, Robertson RJ, Rabin BS. The effects of incremental submaximal exercise on circulating leukocytes in physically active and sedentary males and females. Eur J Appl Physiol Occup Physiol. 1996;74(3):211-8.

[57] Burke LM, Hawley JA, Wong SH, Jeukendrup AE. Carbohydrates for training and

competition. J Sport Sci. 2011;29(sup1):S17-27.

[58] Hickner RC, Fisher JS, Hansen PA, Racette SB, Mier CM, Turner MJ, Holloszy JO. Muscle glycogen accumulation after endurance exercise in trained and untrained individuals. J Appl Physiol. 1997;83(3):897-903.

[59] Asp S, Daugaard JR, Richter EA. Eccentric exercise decreases glucose transporter GLUT4 protein in human skeletal muscle. J Physiol. 1995;482(3):705-12.

[60] Doyle JA, Sherman WM, Strauss RL. Effects of eccentric and concentric exercise on muscle glycogen replenishment. J Appl Physiol. 1993;74(4):1848-55.

[61] O'Reilly KP, Warhol MJ, Fielding RA, Frontera WR, Meredith CN, Evans WJ. Eccentric exercise-induced muscle damage impairs muscle glycogen repletion. J Appl Physiol. 1987;63(1):252-6.

[62] Paulsen G, Crameri R, Benestad HB, Fjeld JG, Mørkrid L, Hallén J, Raastad T. Time course of leukocyte accumulation in human muscle after eccentric exercise. Med Sci Sport Exerc. 2010;42(1):75-85.

[63] Janssen I, Heymsfield SB, Wang Z, Ross R. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. J Appl Physiol. 2000;89(1):1-88.

[64] Phillips SM, Atkinson SA, Tarnopolsky MA, MacDougall JD. Gender differences in leucine kinetics and nitrogen balance in endurance athletes. J Appl Physiol. 1993;75(5):2134-41.

[65] Tarnopolsky M. Protein requirements for endurance athletes. Eur J Sport Sci. 2004;4(1):1-5.

[66] Coffey VG, Zhong Z, Shield A, Canny BJ, Chibalin AV, Zierath JR, Hawley JA. Early signaling responses to divergent exercise stimuli in skeletal muscle from well-trained humans. FASEB J. 2006;20(1):190-2.

[67] Wilkinson SB, Phillips SM, Atherton PJ, Patel R, Yarasheski KE, Tarnopolsky MA, Rennie MJ. Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. J Physiol. 2008;586(15):3701-17.

[68] Moore DR, Camera DM, Areta JL, Hawley JA. Beyond muscle hypertrophy: why dietary protein is important for endurance athletes. Appl Physiol Nutr Metab. 2014;39(9):987-97.

[69] Institute of Medicine (US). Panel on Dietary Reference Intakes for Electrolytes, Water. DRI, dietary reference intakes for water, potassium, sodium, chloride, and sulfate. National Academy Press; 2005.

[70] Maughan RJ, McArthur M, Shirreffs SM. Influence of menstrual status on fluid replacement after exercise induced dehydration in healthy young women. Br J Sport Med. 1996;30(1):41-7.

[71] Campagnolo N, Iudakhina E, Irwin C, Schubert M, Cox GR, Leveritt M, Desbrow B. Fluid, energy and nutrient recovery via ad libitum intake of different fluids and food. Physiol Behav. 2017;171:228-35.

[72] Hunter SK, Stevens AA, Magennis K, Skelton KW, Fauth M. Is there a sex difference in the age of elite marathon runners?. Med Sci Sport Exerc. 2011;43(4).

[73] Senefeld J, Joyner MJ, Stevens A, Hunter SK. Sex differences in elite swimming with

advanced age are less than marathon running. Scand J Med Sci Sport. 2016;26(1):17-28.

[74] Coggan AR, Raguso CA, Gastaldelli A, Sidossis LS, Yeckel CW. Fat metabolism during high-intensity exercise in endurance-trained and untrained men. Metabolism. 2000;49(1):122-8.

[75] Jeukendrup AE, Mensink M, Saris WH, Wagenmakers AJ. Exogenous glucose oxidation during exercise in endurance-trained and untrained subjects. J Appl Physiol. 1997;82(3):835-40.

[76] Klein S, Coyle EF, Wolfe RR. Fat metabolism during low-intensity exercise in endurancetrained and untrained men. Am J Physiol Endocrinol Metab. 1994;267(6):E934-

Appendix 2

MONASH University

Monash University Human Research Ethics Committee

Approval Certificate

This is to certify that the project below was considered by the Monash University Human Research Ethics Committee. The Committee was satisfied that the proposal meets the requirements of the *National Statement on Ethical Conduct in Human Research* and has granted approval.

Project Number: 12799
Project Title: Sports Recovery Milk
Chief Investigator: Dr Ricardo Da Costa
Approval Date: 09/04/2018
Expiry Date: 09/04/2023

Terms of approval - failure to comply with the terms below is in breach of your approval and the *Australian Code for the Responsible Conduct of Research*.

1. The Chief Investigator is responsible for ensuring that permission letters are obtained, if relevant, before any data collection can occur at the specified organisation.
2. Approval is only valid whilst you hold a position at Monash University.
3. It is responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval and to ensure the project is conducted as approved by MUHREC.
4. You should notify MUHREC immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.
5. The Explanatory Statement must be on Monash letterhead and the Monash University complaints clause must include your project number.
6. Amendments to approved projects including changes to personnel must not commence without written approval from MHUREC.
7. Annual Report - continued approval of this project is dependent on the submission of an Annual Report.
8. Final Report - should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected completion date.
9. Monitoring - project may be subject to an audit or any other form of monitoring by MUHREC at any time.
10. Retention and storage of data - The Chief Investigator is responsible for the storage and retention of the original data pertaining to the project for a minimum period of five years.

Thank you for your assistance.

Professor Nip Thomson

Chair, MUHREC

CC: Dr Ricardo Da Costa, Dr Judi Porter, Assoc Professor David Kannar, Miss Isabella Russo, Professor Louise Burke

List of approved documents:

Document Type	File Name	Date	Version
Supporting Documentation	venepuncture SWF	12/06/2013	2
Supporting Documentation	SWP capillary blood sampling SWP	05/02/2014	1
Supporting Documentation	SWP Plasma Volume Change SWP	05/02/2014	1
Supporting Documentation	treadmill SWF	15/02/2014	1
Explanatory Statement	Explanatory statement	08/03/2018	1
Consent Form	Consent form	08/03/2018	1
Questionnaires / Surveys	Gastrointestinal Symptoms Assessment Tool 2018	08/03/2018	1
Questionnaires / Surveys	Profile of Mood States POM best	08/03/2018	1
Supporting Documentation	Participant recruitment advertisement	08/03/2018	1
Supporting Documentation	Pre-exercise questionnaire	08/03/2018	1
Supporting Documentation	Saliva Collection Steps	08/03/2018	1
Supporting Documentation	SOP VO2max	08/03/2018	1
Explanatory Statement	Explanatory statement	16/03/2018	2
Questionnaires / Surveys	Borg Scale	16/03/2018	1
Consent Form	Consent form v2	05/04/2018	2

DATA SHARING STATEMENT

The role of differing post-exercise beverages on markers of physiological and psychophysiological recovery in elite and habitually-trained athletes.

Registration number:	ACTRN12618000865213
Date registered:	22/05/2018
Date this registration last updated:	27/08/2020
Type of registration:	Prospectively registered
Date this document generated:	14/12/2020

Will individual participant data be available?	IPD is not available
Reason	
What additional, related documents will be available?	No other documents available
How or where can supporting documents be obtained?	

Appendix 4

Monash University
Department of Nutrition & Dietetics

EXPLANATORY STATEMENT

Project Title: Sports Recovery drink

MUHREC Project Number: 12799

Dr Ricardo Costa
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You are invited to take part in this study. Please read this Explanatory Statement in full before deciding whether or not to participate in this research. If you would like further information regarding any aspect of this project, you are encouraged to contact the researchers via the phone numbers or email addresses listed above.

Background: The food and fluid provided during the post-exercise period plays an essential role in recovery and adaptation processes. There has been a considerable amount of research investigating the ideal quantity and quality of nutrients (e.g., carbohydrate, protein, electrolytes and water) for optimising the key recovery processes (i.e., repair, healing, growth, glycogen resynthesis and rehydration) individually. We will be conducting a study to determine the effects of different sports beverages on the body's whole recovery processes.

What is the purpose of this research study?

This study aims to develop an ideal clinically proven beverage nutritional composition for exercise recovery.

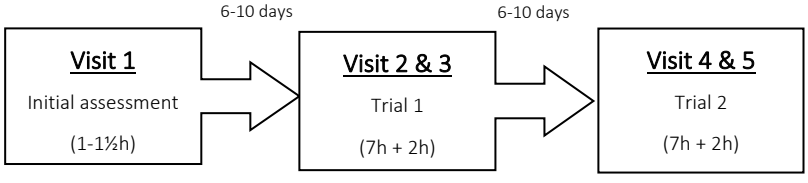
What does participation in this research project involve?

Prior to commencing the experimental trials you will be expected to:

- Complete an initial assessment (approximately 1-1½h in duration) that will include anthropometrical measures (height, weight, body composition assessment) and a maximal aerobic exercise test (~15-25 minutes) on an electric treadmill. The initial assessment and all experimental trials be conducted at the BASE Facility- Nutrition & Exercise Clinic.

Completing the experimental trials

- You will be required to take part in **two experimental trials** in a random order usually conducted two weeks apart.



All Trials: Include a self-paced warm-up (10min- not included in 2h), then 3 x 5min blocks (3min30sec jog at 55-60% VO_{2max}, 1min run at 70-75% VO_{2max}, and 30sec hard at 80-85% VO_{2max}) + 20 plyometric jumps (remaining time walking at 6km/h) in 20-25ºC and 30-40% relative humidity. Repeat until 2h. Intensity calculated from initial assessment VO_{2max} test. Water will be provided at 3ml/kg/h. The day after each trial, you will be required to return to the lab to perform a 1-hour distance trial. This involves a 1 hour run on a treadmill to the maximal distance you are capable of running. Before and after the distance test, we will measure your oxygen uptake and oxidation rates at four submaximal exercise intensities (50%, 60%, 70%, and 80% VO_{2max}) for 5 minutes each.

Whilst completing the experimental trials you will also be expected to:

- Consume the study food provided for 24 hours prior to each experimental trial, until the end of the 1-hour distance trial (~48 hours total). The study foods provided are low in fermentable carbohydrates (FODMAPs) and will be individually tailored to meet your daily energy requirements. As part of this meal plan a standardised breakfast will need to be consumed at home 2 hours before the exercise trials. All study food will be provided in advance and can be consumed at home or as part of your normal daily routine.
- Complete a basic food and exercise diary during the study.
- Provide a faecal sample before and urine sample before and during recovery from the exercise trials.
- To have blood samples taken from the antecubital vein by a trained researcher. These will occur immediately before and during recovery from the exercise trials (i.e., immediately after exercise cessation, 2 and 4 h after exercise cessation).
- To have a muscle biopsy taken from the quadriceps muscle by a trained Sports Physician.
- To have finger prick blood samples taken at 30 minute intervals during the initial 2 h of recovery (except for the occasions when venous samples are taken)
- Provide a breath sample before exercise and every 30 minutes during recovery.
- Have body mass measured before exercise and every hour during recovery.
- Have rectal body temperature measured before and after exercise.
- Wear a heart rate monitor during each trial, in which heart will be constantly monitored and recorded every 10 minutes. Answer questions relating to exertion, thermal comfort and gastrointestinal symptoms every 20 minutes during exercise protocol.
- After each experimental trial, you will be provided with a recovery meal and drink that meets the recovery nutrition and hydration guidelines and recommendation.
- Return to the lab the following day to perform a 1h distance test in 20-25ºC and 30-40% relative humidity. This will involve repeat measures of body mass, total body water and gastrointestinal symptom log.
- Complete a Likert scale questionnaire to collect information related to perception markers **(**mental and physical readiness to invest in physical performance and muscle soreness) before and after the 1 hour running test.

If you decide to take part in this study, there will be a number of constraints placed upon your normal everyday life and activities:

- You will be asked to consume the study food provided and follow a low FODMAP food guide for any additional food consumed during the 24h prior to each exercise trial, and continue until the end of the 1 hour distance trial (approx. 48 hours total)
- Refrain from strenuous exercise, alcohol and using non-steroidal anti-inflammatories for 48 hours prior to each exercise trial.
- You will be asked to refrain from consuming any dietary supplements (including probiotics) one month prior to and during the study.

Why were you chosen for this research?

Since you are a recreational, amateur and/or elite level endurance athlete, with experience in marathon, ultra-marathon and long-distance triathlon training and competition you have been invited to take part in this research investigation.

Consenting to participate in the project and withdrawing from the research

Once you have understood what the research study is about, if you would like to take part please sign the consent form at the end of this information sheet. You will be given a copy of this information sheet and consent form to keep.

Participation in this research study is completely voluntary. If you do not want to take part, you do not have to. You can withdraw from the study at any time without explanation and this will not affect your involvement with the University, Faculty, Department, or Nutrition & Exercise Clinic

Advantages of taking part

As part of the experimental design that will be conducted at the BASE Facility- Nutrition & Exercise Clinic, participants that complete the full experiment design (i.e., initial assessment, trial 1 and trial 2) will be provided with a report of the full fitness assessment and physiological responses on completion of the study. Such services comprise a substantial consultation value ($680). Individual results will be sent within four weeks after the completion of the experimental design.

By taking part in this study you will contribute towards the scientific knowledge within Sport & Exercise Nutrition. You will gain an insight into the scientific aspects of the study and you may find it interesting to know what we are trying to achieve or how various measurements are recorded.

By taking part in this study you will also gain an insight into how your body recovers from exercise and responds to nutrition interventions, which may provide valuable information that can be used within your normal sporting activities. Additionally, receive comprehensive feedback on the results and measurements made during the research, with full explanations; for your fitness level, gastrointestinal system status, and advice on strategies to avoid unwanted symptoms associated with strenuous exercise.

Participants that complete the full experimental design will receive a gift voucher to the value of $300.

Possible discomforts and risks of taking part

The discomforts and risks of taking part in this study, which you will probably be most concerned about are: blood sampling, muscle biopsy, physical exertion, gastrointestinal discomfort, monitoring body temperature and time commitment.

1. Anthropometrical measures

Height will be measured by a stadiometer and body mass by calibrated weighing scales. These measures are similar to those consistently measured for health monitoring in the GP setting, which pose no direct risk. Bioelectric Impedance Analysis, a technique that involves sending a small and safe electric current through your body that cannot shock you. The current travels at different speeds through body fat compared to the rest of the body, allowing us to estimate the amount of fat-free mass (the amount of your body weight that is not fat).

2. Blood sampling

Only qualified phlebotomy trained researchers with experience at performing this procedure will collect blood samples. To ensure you are completely comfortable with giving blood samples, we will familiarise you with the blood sample collection procedure during the initial assessment. The amount of blood collected during each trial (12ml) will not impact upon normal physiological functioning. Some participants may find this procedure may create a bruise around the puncture site. Fainting may also occur in 'at risk' participants. Familiarising you with the blood sample collection procedure during the initial assessment will determine if you are an 'at risk' participant for fainting during blood sampling.

3. Physical exertion

The physical components of this study include; the incremental aerobic fitness test, multiple 2 hour interval running components, plyometric exercise stress and a distance running test the next day. The incremental aerobic fitness test will only require you to run at your maximal capacity for approximately 1 minute. You most probably feel the same type of exhaustion and fatigue for longer periods during your normal training and competition habits. The 2 hours running exercise bouts at varying efforts may promote post-exercise fatigue. However, they are of a similar volume and intensity of effort to that experienced during your training and competitions. As such, the 2 hours running exercise components can also be seen as useful intensive and prolonged training sessions. Identical to exercise during training and competition, some participants may find the exercise element results in a variety of common symptoms: dizziness, headache, thirst, nausea, light-headedness, fainting, muscle and joint soreness.

4. Gastrointestinal discomfort

The experimental design may promote some gastrointestinal discomfort and may lower appetite. These are normal responses to exercise. The gastrointestinal symptoms that will be monitored during the study and may occur include: upper gastrointestinal symptoms (belching, heartburn, bloating, stomach cramps, and urge to vomit) lower gastrointestinal symptoms (flatulence, urge to defecate, intestinal cramp, loose stools, and diarrhoea) as well as appetite and thirst.

5. Faecal and urine samples

You will be required to provide a faecal sample at the beginning and urine samples throughout the experimental trials. You will be given absolute privacy when providing these samples. The researchers have been trained in the correct collection and disposal methods for these samples.

6. Body temperature

To monitor your body temperature for safety reasons, you will be asked to self-insert a thermal coupling 12cm in the rectal sphincter before and after exercise. The procedure of thermal coupling self-insertion is extremely easy, not harmful, and standard practice in thermoregulatory measurements.

As the study involves prolonged strenuous exercise, all safety measures will be discussed with you before any testing. You will also be asked to complete a heath/medical history questionnaire before any testing. If at any point during exercise you wish to stop, testing will immediately discontinue.

7. Time commitment

To complete all aspects of the study we will require you to visit the laboratory on:

- One occasion for the initial assessment (approx. 1-1½ hour).
- 2 x 2 occasions for the exercise trials (approx. 7 hours for trial + 2 hours for testing the next day)
  Total time required: 19 hours

In addition to the laboratory visits you will also be required to consume the study food provided for 24 hours prior to each trial, through until the end of the distance trial the following day, as part of your normal daily routine. For some study participants this may save time in food planning and/or preparation. The basic food and exercise diary should take no longer than 5 minutes to complete for each trial (e.g. an additional 15-20 minutes in total).

8. Muscle biopsy

Within each experimental trial two muscle samples (after exercise and 2 h after exercise; four in total) via the Bergstrom muscle biopsy technique, performed by a trained and qualified Sports Physician, will be required. This is a common technique in the field of exercise physiology and sports medicine enabling the identification of skeletal muscle function, structure, nutritional status and cell signalling before and after exercise stress. Firstly, the skin will be numbed with a topical anaesthetic cream. The Sports Physical will administer a local subcutaneous anaesthetic to numb the muscle area. **Those will anaesthetic allergies and sensitivity will be excluded from participating in the study**. Once the muscle area is prepared, a small insertion will be made ~14cm above the knee and 4-6cm laterally from the quadriceps midline. A Bergstrom muscle biopsy needle will then be used to collect a 50-70mg skeletal muscle sample. This procedure will result in a small puncture wound at the collection location, however the size of the puncture will not require any stitching and sufficient healing will occur prior to the distance test the following day. A Sports Physician will be on location to attend any issues arising from the muscle biopsy technique.

Confidentiality & data storage

To ensure protection of participants' right to privacy to the best of our ability, confidentiality will be maintained throughout data collection. All information collected during the study will be coded and treated confidentially. All

data is anonymous as soon as it is collected and will be stored electronically using participant codes so that individuals cannot be identified. Any data from your participation in the study will be used by the research team. It may also be disseminated externally at scientific conferences and publication in scientific journals to inform academic and professional practice; however your name or identity will remain anonymous. Data will be retained in the Department for at least five years and destroyed thereafter.

Complaints

Should you have any concerns or complaints about the conduct of the project, you are welcome to contact the Executive Officer, Monash University Human Research Ethics (MUHREC):

Executive Officer
Monash University Human Research Ethics Committee (MUHREC)
Room 111, Building 3e
Research Office
Monash University VIC 3800

Tel: +61 3 9905 2052 Email: muhrec@monash.edu Fax: +61 3 9905 3831

The funding for this project has been received from Lion Drinks and Dairy.

Thank you,

Dr Ricardo Costa

Appendix 5

CONSENT FORM

General consent form for exercise testing at the Be Active Eat Sleep (BASE) Facility, Monash University.
Exercise Physiology Lead: Dr Ricardo Costa

I have volunteered to take part in exercise testing at the BASE Facility at Monash University. I participate at my own free will and take full responsibility for my participation. I confirm I have read and/or have been informed, and understood all the relevant test procedures. The nature, demands, and risks have been explained to me. I have had the opportunity to consider the information, ask questions regarding the testing procedures, and have had these answered satisfactorily by the test operator. I also confirm that all the relevant health and safety aspects of the test have been explained to me. I hereby consent to participate in the exercise testing and acknowledge that I am free to withdraw from the test procedure at any time without giving a reason.

I consent to taking part in the following aspects of exercise physiology test procedures:	Yes	No
1. Continuous sub-maximal exercise test (treadmill).	☐	☐
2. Incremental exercise test to voluntary exhaustion (VO_{2max}) (treadmill).	☐	☐
3. Breath-by-breath IC analysis.	☐	☐
4. General anthropometry (i.e., weight, height, girth, lengths, widths).	☐	☐
5. Bioelectrical impedance analysis (body composition or hydration).	☐	☐
6. Capillary blood sampling for glucose or lactate.	☐	☐
7. Venous blood sampling for biomarker analysis.	☐	☐
8. Urine sampling.	☐	☐
9. Saliva sampling.	☐	☐
10. Breath analysis.	☐	☐
11. Gastrointestinal symptom visual analogue scale.	☐	☐
12. Gut challenge protocol (Costa et al 2017).	☐	☐
13. Rectal body temperature measurement	☐	☐
14. Dietary or nutritional intervention.	☐	☐
15. 1 hour distance test (treadmill)	☐	☐
16. Muscle biopsy	☐	☐
17. Other:	☐	☐
18: Other:	☐	☐
I consent for the data collected during my participant in the exercise testing at BASE Facility at Monash University be used for teaching, education, and/or research purposes.	☐	☐

Name of Participant _____

Participant Signature _____ Date _____