The Effect of Different Post-Exercise Beverages on Fluid Recovery, Nutrient Provision and Subsequent Athletic Performance

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Statement of Originality

This thesis describes original research conducted by Danielle Jade McCartney in the School of Allied Health Sciences at Griffith University. This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, this document contains no material previously published or written by another person except where due reference is made in the thesis itself.

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Abstract

Background: Athletes undertaking frequent training or those involved in sporting competitions with multiple heats or games may be required to complete two or more high-quality exercise sessions with limited recovery time between bouts (e.g. ≤4 h). Beverages may be an ideal way in which to consume the fluid and nutrients required to enhance short-term post-exercise recovery and subsequent athletic performance in these situations, owing to their appeal (i.e. capacity to "quench thirst") and gastrointestinal tolerability. However, studies investigating the ability of different beverages to influence these outcomes typically "prescribe" drinking (i.e. beverage volume and rate of ingestion) and deny participants access to food; an approach with limited ecological validity. The overall objective of this thesis was to develop a better understanding of how different beverages affect fluid restoration, nutrient provision and subsequent athletic performance when consumed between exercise sessions with limited (i.e. ≤4 h) recovery time; particularly, within the context of enhanced ecological validity.

Thesis Part I: Initially, the effect of fluid intake during or following dehydration on subsequent athletic performance was examined. The systematic review included 64 controlled trials that measured athletic (categorised as: *continuous*, *intermittent*, *resistance*, *sport-specific* and *balance* exercise) or cognitive performance ≤4 h after dehydration of participants with or (versus) without fluid consumption. Meta-analysis identified a significant positive effect of fluid consumption on continuous exercise performance; particularly, under heat-stress conditions. Research investigating the effect of fluid intake on other sports or types of exercise was relatively limited, and a narrative synthesis of the available evidence failed to indicate a clear improvement. Study 2 explored the effect of consuming fluid with carbohydrate (CHO) and protein during or following exercise on subsequent athletic performance. This systematic and meta-analytic review included 67 controlled trials that measured either endurance or anaerobic performance ≤4 h after an initial standardised exercise bout. Initially, the review investigated the effect of "adding" CHO to water, and subsequently, the "addition" of protein to a CHO-containing beverage. Fluid co-ingested with CHO significantly improved subsequent endurance and anaerobic performance compared to fluid intake alone. However, protein added to a CHO-containing beverage did not

influence subsequent endurance performance. Collectively, the results of Studies 1 & 2 suggest that individuals with limited recovery time between exercise sessions should prioritise CHO and fluid ingestion to enhance subsequent athletic (endurance) performance. Whether CHO-containing beverages improve short-term post-exercise recovery (i.e. the restoration of fluid and substrate losses) and subsequent athletic performance when consumed under ecologically valid conditions (e.g. *ad libitum* and with food) remained unclear.

Thesis Part II: Recent evidence suggests that different post-exercise beverages promote similar levels of fluid recovery but alter energy and nutrient provision in trained males when consumed *ad libitum* and with food. Study 3 investigated if similar effects are observed in trained females, who may exhibit contrasting dietary behaviours. On 4 separate occasions, 8 females lost ~2% of their body mass (BM) cycling before completing a 4 h recovery period with *ad libitum* access to water, a CHO-electrolyte sports beverage or one of two milk-based formulations and food. Results indicated that the different beverages were equally effective at replenishing fluid losses, but that caloric (CHO-containing) alternatives increased short-term (i.e. 4 h) and total daily energy and nutrient consumption. This could potentially affect short-term muscle glycogen resynthesis, and thus, subsequent athletic performance, as well as chronic exercise–nutrient interactions. Study 4 investigated the effect of consuming different post-exercise beverages *ad libitum* with food on short-term (i.e. ≤4 h) fluid recovery, nutrient provision and subsequent athletic performance. On 2 separate occasions, 16 endurance-trained cyclists (8 males) completed 1 h of fixed-intensity cycling followed by a 4 h recovery period with *ad libitum* access to water or a CHO-electrolyte sports beverage and food, and later, an endurance cycling performance test. Once again, results indicated that both beverages were effective at replenishing fluid losses and that the sports beverage increased short-term (i.e. 4 h) energy and CHO consumption. However, this additional nutrition did not translate to an improvement in cycling performance; possibly because individuals already consumed enough CHO from food alone to meet post-exercise refuelling recommendations when water was provided. Collectively, the results of Studies 3 & 4 suggest that a CHO-containing post-exercise beverage is more likely to influence nutrient provision than fluid restoration or subsequent athletic performance.

Conclusion: The research presented in this thesis indicates that individuals with limited recovery time between exercise sessions should consume CHO and fluid to enhance subsequent athletic performance. However, when consumed *ad libitum* and with food, CHO-containing beverages do not appear to be any more effective than water at promoting fluid restoration; may not enhance subsequent athletic (endurance) performance; and may increase total daily energy consumption. Thus, access to a CHO-containing post-exercise beverage should consider an athlete's overall dietary goals (e.g. energy availability and body composition aspirations), ability to tolerate food and fluid pre-/post-exercise, taste preferences (i.e. beverage palatability) and immediate refuelling requirements. Put simply, sports practitioners and dietitians should bear in mind that, as long as athletes obtain an appropriate amount of CHO and fluid to promote recovery between exercise sessions, the source of nutrition is not critically important.

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Thesis Symbols, Abbreviations and Terminology

~	Approximately	HR	Heart rate
°C	Degrees Celsius	HR_{max}	Age-predicted HR maximum
%	Percent	ICF	Intracellular fluid
$\%_{\Delta}$	Percent change	K^{+}	Potassium
ACSM	American College of Sports Medicine	kg	Kilogram(s)
ADH	Anti-diuretic hormone	kJ	Kilojoule(s)
ANOVA	Analysis of variance	km	Kilometre(s)
AVAS	Adaptive visual analogue scales	J	Joule(s)
BM	Body mass	L	Litre(s)
BP	Blood pressure	mg	Milligram(s)
Ca^{2+}	Calcium	Mg^{2+}	Magnesium
CBF	Cerebral blood flow	min	Minute(s)
CHO	Carbohydrate	mL	Millilitre(s)
CI	Confidence interval	mm	Millimetre(s)
Cl^{-}	Chloride	mmol	Millimole(s)
CO	Cardiac output	mOsmo	Milliosmole(s)
d	Day(s)	MPO	Mean power output
dL	Decilitre(s)	MVC	Maximal voluntary contraction
DM	Dry mass	*n*	Number
EAT-26	Eating Attitudes Test-26	Nb.	Note
ECF	Extracellular fluid	Na^{+}	Sodium
eEE	Estimated energy expenditure	NMF	Neuromuscular function
EI_{Food}	Energy intake from food	NS	Not specified
$EI_{Beverage}$	Energy intake from beverage	P_{OSM}	Plasma osmolality
EI_{Total}	Energy intake from food and beverage	PPO	Peak power output
EMG	Electromyography	PSPO	Peak sustainable power output
g	Gram(s)	PV	Plasma volume
GI	Gastrointestinal	RH	Relative humidity
GIx	Glycaemic index	RML	Restricted maximum likelihood
h	Hour(s)	RPE	Rating of perceived exertion
H_2O	Water	s	Second(s)
Hb	Haemoglobin	SD	Standard deviation
Hct	Haematocrit	SEM	Standard error of the mean

Symbols and Abbreviations Continued:

SNS	Sympathetic nervous system	VAS	Visual analogue scale
SV	Stroke volume	VIF	Variance inflation factor
TBW	Total body water	VO_{2max}	Maximal aerobic capacity
TT	Time trial performance test	vs.	Versus
TTE	Time to exhaustion performance test	WI_{Food}	Water intake from food
U_{col}	Urine colour	$WI_{Beverage}$	Water intake from beverage
U_{OSM}	Urine osmolality	WI_{Total}	Water intake from food and beverage
U_{SG}	Urine specific gravity	y	Year(s)

Athletic Performance: For this thesis, the term "*athletic performance*" is used to describe a variety of abilities commonly used by athletes in sporting events, including endurance and anaerobic exercise performance, muscular strength (i.e. force production) and muscular endurance (i.e. fatigue resistance), sport-specific technical skills (e.g. kicking or passing a ball) and cognition. As many of these are also used by individuals undertaking manual tasks (e.g. labourers, fire fighters, military personnel) there is some discussion of these populations. However, trained athletes (i.e. adult males and females) are the primary population of interest.

Fluid balance: For this thesis, the term "*euhydration*" is used to describe a state of fluid balance (i.e. where the quantity of water input to, and output from, the body is equal). The terms "*hypohydration*" and "*hyperhydration*" refer to fluid deficits and excesses below and above euhydration respectively; whereas "*dehydration*" is the dynamic process of fluid loss (i.e. the transition from a euhydrated to hypohydration state). "*Hydration*" is the point at which the body currently resides among the states of euhydration, hyperhydration and hypohydration. Fluid that is consumed to replenish losses is termed "*rehydration*" (or, alternatively, "*fluid recovery*" or "*fluid replacement*").

Publications in Support of this Thesis

Published Journal Articles:

1. 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; 3(13).
2. McCartney, D., Desbrow, B., Irwin, C. Post-exercise ingestion of carbohydrate, protein and water: A systematic review and meta-analysis for effects on subsequent athletic performance. *Sports Medicine*, 2018; 48(2): 379-408.
3. McCartney, D., Irwin, C., Cox, GR., Desbrow, B. Fluid, energy and nutrient recovery via *ad libitum* consumption of different commercial beverages and food in female athletes. *Applied Physiology Nutrition and Metabolism*, 2019; 44(1): 37-46.
4. McCartney, D., Irwin, C., Cox, GR., Desbrow, B. The effect of different post-exercise beverages with food on *ad libitum* fluid recovery, nutrient provision and subsequent athletic performance. *Physiology & Behavior*, 2019; 201: 22-30.
5. McCartney, D., Desbrow, B., Cox, GR., Irwin, C. The effect of acute aerobic exercise, dehydration and *ad libitum* fluid consumption on mood and choice reaction time in trained females: A distributional analysis. *Journal of Social Sciences and Humanities*, In Press.

Manuscripts Currently Under Review:

1. McCartney, D., Desbrow, B., Irwin, C. The effect of aerobic exercise duration and intensity on cognitive performance in trained individuals.

Conference Presentations:

1. McCartney, D., Desbrow, B., Irwin, C. Does oral fluid intake following dehydration influence subsequent athletic performance? A systematic review and meta-analysis. The American College of Sports Medicine (ACSM) Annual Meeting, Denver, USA (2017).
2. McCartney, D., Desbrow, B., Irwin, C. Post-exercise ingestion of carbohydrate, protein and water: A systematic review and meta-analysis for effects on subsequent athletic performance. Sports Dietitians Australia (SDA) Conference,

Melbourne, Australia (2017); Asia Pacific Conference on Clinical Nutrition, Adelaide, Australia (2017).

3. McCartney, D., Desbrow, B., Irwin, C. The effect of acute aerobic exercise, dehydration and *ad libitum* fluid consumption on choice reaction time in trained females: A distributional analysis. The Fédération Internationale d'Education Physique (FIEP) Asia Conference, Kuala Lumper, Malaysia (2018).
4. McCartney, D., Irwin, C., Cox, GR., Desbrow, B. Fluid, energy and nutrient recovery via *ad libitum* consumption of different commercial beverages and food in female athletes. Congress of the European College of Sport Science (ECSS), Dublin, Ireland (2018).
5. McCartney, D., Irwin, C., Cox, GR., Desbrow, B. The effect of different post-exercise beverages with food on *ad libitum* fluid recovery, nutrient provision and subsequent athletic performance. The ACSM Annual Meeting, Orlando, USA (2019).
6. Irwin, C., Desbrow, B., McCartney, D. The effect of aerobic exercise duration and intensity of on cognitive performance in trained individuals. The ACSM Annual Meeting, Orlando, USA (2019).

Other Publications Co-Authored During Candidature

Published Journal Articles:

1. McCartney, D., Desbrow, B., Irwin C. Using alcohol intoxication goggles (Fatal Vision® goggles) to detect alcohol related impairment in simulated driving. *Traffic Injury Prevention*, 2017; 18(1): 19-27.
2. Irwin, C., Iudakhina, E., Desbrow, D., McCartney, D. Effects of acute alcohol consumption on measures of simulated driving: A systematic review and meta-analysis. *Accident Analysis and Prevention*, 2017; 102: 248-266.
3. Brickley, B., Desbrow, B., McCartney, D., Irwin, C. Effects of consuming a low dose of alcohol with mixers containing carbohydrate or artificial sweetener on simulated driving performance. *Nutrients*, 2018; 10(4): 419.
4. Irwin, C., McCartney, D., Khalesi, S., Desbrow, B. Caffeine content and perceived sensory characteristics of pod coffee: Effects on mood and cognitive performance. *Current Research in Nutrition and Food Science*, 2018; 6(2): 329-345.
5. McCartney, D., Rattray, M., Desbrow, B., Khalesi, S., Irwin, C. Smoothies: Exploring the attitudes, beliefs and behaviours of consumers and non-consumers. Curr*ent Research in Nutrition and Food Science*, 2018; 6(2): 425-436.
6. McCartney, D., Langston, K., Desbrow, B., Khalesi, S., Irwin, C. The influence of a fruit smoothie or cereal and milk breakfast on subsequent dietary intake: A pilot study. *International Journal of Food Sciences and Nutrition*, 2019; 70(5): 612-622.
7. Irwin, C., Desbrow, D., Khalesi, S., McCartney, D. University student's challenges following a personalised diet adhering to the Australian Dietary Guidelines. *Nutrition and Health*, In Press
8. Desbrow, B., Barnes, K., Cox, GR., Iudakhina, E., McCartney, D., Skepper, S., Young, C., Irwin, C. Providing calorie-containing recovery drinks to recreational runners increases voluntary energy and carbohydrate intake but has minimal impact on fluid recovery. *International Journal of Sport Nutrition and Exercise Metabolism*, In Press

9. Rogers, E., Irwin, C., McCartney, D., Desbrow, B. Tattoos do not affect exercise-induced localised sweat rate of sodium concentration. *Journal of Science and Medicine in Sport*, In Press

Manuscripts Currently Under Review:

1. Sayer, L., Rodriguez-Sanchez, N., Rodriguez-Giustiniani, P., Irwin, C., McCartney, D., Cox, GR., Galloway, S., Desbrow, B. Effect of drinking rate on retention of milk or water following exercise-induced dehydration.

Conference Presentations:

1. Sayer, L., Rodriguez-Sanchez, N., Rodriguez-Giustiniani, P., Irwin, C., McCartney, D., Cox, GR., Galloway, S., Desbrow, B. Effect of drinking rate on retention of milk and water following exercise-induced dehydration. Congress of the ECSS, Dublin, Ireland (2018).
2. Rodriguez-Sanchez, N., Rodriguez-Giustiniani, P., Sayer, L., Irwin, C., McCartney, D., Cox, GR., Desbrow, B., Galloway, S. The effect of slow, moderate and fast water drinking on fluid retention following exercise-induced hypohydration. Congress of the ECSS, Dublin, Ireland (2018).
3. Desbrow, B., Barnes, K., Cox, GR., Iudakhina, E., McCartney, D., Skepper, S., Young, C., Irwin, C. The influence of water, sports drink or low-alcohol beer on voluntary fluid and nutrient intake following exercise. Congress of the ECSS, Dublin, Ireland (2018).
4. Irwin, C., Desbrow, D., Khalesi, S., McCartney, D. Nutrition student's behaviours and experiences following a self-determined dietary plan adhering to the Australian Dietary Guidelines. Asia Pacific Conference on Clinical Nutrition, Adelaide, Australia (2017).
5. Brickley, B., Desbrow, B., McCartney, D., Irwin, C. Effects of consuming a low dose of alcohol with mixers containing carbohydrate or artificial sweetener on simulated driving performance. Asia Pacific Conference on Clinical Nutrition, Adelaide, Australia (2017).
6. McCartney, D., Langston, K., Desbrow, B., Irwin, C. Consumption of smoothies or traditional breakfast foods: Impact on subsequent energy intake - A pilot study.

Nutrition Society of Australia (NSA) Annual Scientific Meeting, Melbourne, Australia (2016).

7. McCartney, D., Rattray, M., Desbrow, B., Irwin, C. Perceived health benefits of smoothie consumption: Investigating consumers' attitudes, beliefs and behaviours. NSA Annual Scientific Meeting, Melbourne, Australia (2016).
8. Rogers, E., Irwin, C., McCartney, D., Desbrow, B. Skin tattoos do not affect exercise-induced sweat rate of sodium concentration. The ACSM Annual Meeting, Orlando, USA (2019).

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Chapter 1: Introduction

1.1 Background

1.2 Research Aims

1.3 Thesis Structure

1.1. Background

Athletes undertaking frequent training or those involved in sporting competitions with multiple heats or games may be required to complete two (or more) high-quality exercise sessions with limited recovery time between bouts (e.g. ≤4 h). Depending on the circumstances involved (e.g. the type or nature of the activity and the environmental conditions), each session has the potential to induce fluid loss (i.e. dehydration), deplete endogenous substrate stores (i.e. muscle and liver glycogen) and/or damage skeletal muscle tissue [1,2]. If fluid, carbohydrate (CHO), protein and/or electrolytes are not adequately recovered between exercise sessions, these physiological disturbances could possibly compromise subsequent athletic performance (see pages xviii–xix *Thesis Symbols, Abbreviations and Terminology*) [3-5]. Thus, employing nutritional strategies to enhance short-term post-exercise recovery is important when a subsequent event requires optimal performance.

The American College of Sports Medicine (ACSM) [1] and the Academy of Nutrition and Dietetics [2] publish nutritional guidelines to aid short-term post-exercise recovery. Currently, the guidelines advise individuals to consume 1.0–1.2 g· kg^{-1}·h^{-1} of CHO for 4 h beginning ≤30 min post-exercise; 0.25–0.30 g·kg^{-1} of high-quality protein ≤2 h post-exercise; and fluid in volumes equal to 1.25–1.50 L·kg body mass (BM) $lost^{-1}$ (i.e. via sweating) to optimise recovery between consecutive bouts (i.e. separated by ≤8 h) of dehydrating and fuel-demanding activity [1,2]. These nutritional strategies should assist to restore fluid balance and maximise the rate of muscle glycogen and protein resynthesis [1,2]. If, however, recovery time is *very* limited (e.g. ≤4 h), such large quantities of fluid and nutrients may be difficult to ingest and could potentially cause gastrointestinal (GI) problems (e.g. abdominal pain, nausea and/or vomiting) that hinder subsequent athletic performance. It is, therefore, important to develop a better understanding of nutritional strategies that can be employed to support athletes in this

context. Seeing as considerable scientific research has investigated the interaction between diet and exercise, a comprehensive review of the evidence may provide valuable insight into how these constituents are typically tolerated and influence athletic performance when consumed between exercise sessions with limited recovery time. Practical strategies to maximise nutrient delivery and minimise GI issues also warrant consideration.

Beverages may be an ideal way in which to consume the fluid and nutrients required to accelerate short-term post-exercise recovery and enhance subsequent athletic performance. This is partly because individuals often conclude exercise feeling thirsty and are usually highly motivated to drink [6]. In addition, individuals may be reluctant to eat between exercise sessions when they have limited recovery time [7], due to concerns over potential GI problems and/or appetite suppression resulting from the previous bout of activity.

Different types of post-exercise beverages may be available to athletes, ranging from bottled or tap water, containing only trace amounts of nutrients, to liquid meals that provide many of the macronutrients and micronutrients required to support post-exercise recovery. Given their diverse nutritional compositions and hedonic characteristics, beverages may differ in the extent to which they either help (or possibly hinder) short-term post-exercise recovery and/or subsequent athletic performance.

Considerable scientific attention has been directed towards the capacity of different beverages to influence short-term post-exercise recovery and subsequent athletic performance. However, this research is limited in that the majority of studies lack ecological validity, or, in other words, they fail to recreate real-life post-exercise conditions. In particular, they often prescribe drinking and deny participants access to food. This means that, after exercise, participants are required to consume a fixed volume of fluid (i.e. regardless of whether they like or tolerate the beverage), and rest in the laboratory for hours without anything to eat. Given that athletes usually control the volume of fluid they consume and often have food available in the initial hours post-exercise, other personal and/or contextual factors might influence post-exercise recovery and subsequent athletic performance in a free-living environment. Thus, an exploration of post-exercise beverage effects when *ad libitum* consumption and access to food is permitted is warranted.

To date, only one study [8] has investigated the ability of different beverages to influence short-term post-exercise recovery when consumed *ad libitum* and with food. This study, which involved endurance-trained males, found that different beverages (i.e. water, a CHO-electrolyte sports beverage and a milk-based formulation) were equally effective at replenishing fluid losses but altered energy and nutrient (i.e. CHO and protein) provision in the initial hours post-exercise. Such differences could potentially affect muscle glycogen and protein resynthesis, and therefore, subsequent athletic performance. Given the preliminary nature of these findings, additional studies employing protocols that better reflect real-life post-exercise conditions are required to improve our understanding of the interaction between fluid, food and nutrients in post-exercise recovery. In particular, investigations are required to explore more diverse participant populations (e.g. females) and exercise contexts (e.g. between consecutive bouts of activity) – whom/which could potentially display/elicit different eating and/or drinking behaviours. Whether the nutrient differences associated with access to the different beverages influences subsequent athletic performance also remains unknown.

1.2. Research Aims

The overall objective of this thesis is to develop a better understanding of how different beverages affect fluid restoration, nutrient provision and subsequent athletic performance when consumed between exercise sessions with "limited" recovery time; specifically, ≤4 h. This timeframe was selected to reflect a short, but still realistic (i.e. likely to occur in a free-living environment), period of recovery. This thesis addresses three "core" questions and four research aims developed using principles of programmatic research [9]. The research questions align with the overall thesis objective and the research aims are met by four specific research studies, all presented as separate chapters in this thesis. The research questions and aims are as follows:

Question 1: How do common beverage constituents; namely, water, CHO and protein, affect subsequent athletic performance when consumed between exercise sessions with limited recovery time (Figure 1.1)?

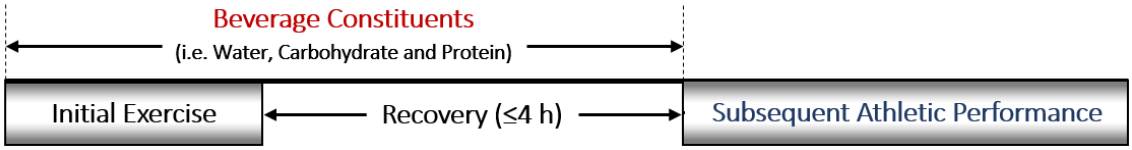

Figure 1.1. A schematic representation of research question 1. Red text is used to identify the independent variable and blue text is used to identify the dependent variable.

Aim 1: To explore the effect of fluid (water) consumption during or following dehydration on subsequent (i.e. ≤4 h recovery) athletic performance.

Aim 2: To explore the effect of consuming CHO and protein with water during or following exercise on subsequent (i.e. ≤4 h recovery) athletic performance.

Question 2: How do different post-exercise beverages affect fluid restoration and nutrient provision when consumed *ad libitum* and with food by females (Figure 1.2)?

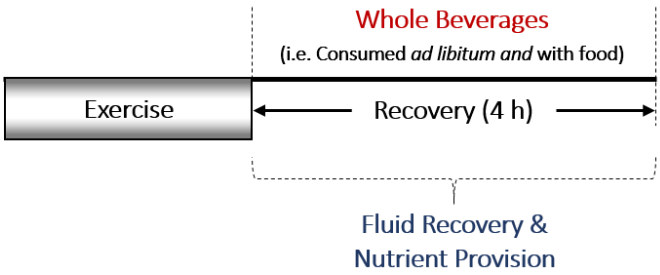

Figure 1.2. A schematic representation of research question 2. Red text is used to identify the independent variable and blue text is used to identify the dependent variables.

Aim 3: To determine the effect of consuming different post-exercise beverages *ad libitum* with food on short-term (i.e. 4 h) fluid recovery and nutrient provision in females.

Question 3: How do different post-exercise beverages affect fluid restoration, nutrient provision and subsequent athletic performance when consumed *ad libitum* with food between exercise sessions and with limited recovery time (Figure 1.3)?

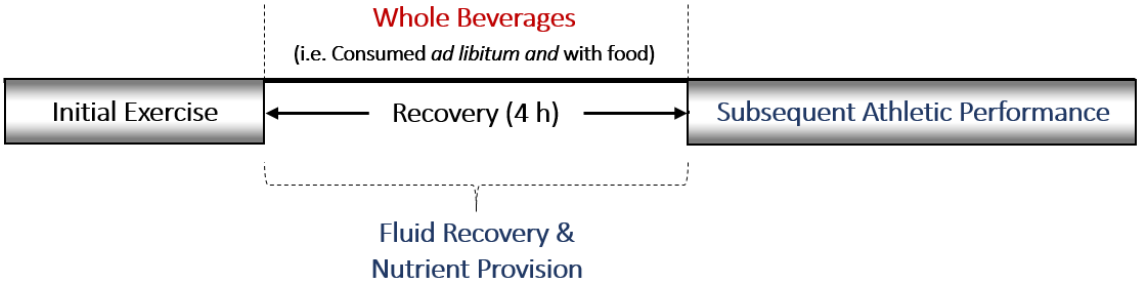

Figure 1.3. A schematic representation of research question 3. Red text is used to identify the independent variable and blue text is used to identify the dependent variables.

Aim 4: To determine the effect of consuming different post-exercise beverages *ad libitum* with food on short-term (i.e. 4 h) fluid recovery, nutrient provision and subsequent athletic performance

1.3. Thesis Structure

This thesis is presented in two parts. The first, titled '*Beverage Constituents: The effect of water, carbohydrate and protein consumption on subsequent athletic performance*', addresses Question 1 (see *1.2. Research Aims*). It includes an initial literature review (Chapter 2) outlining the manner in which common beverage constituents (i.e. water, CHO and protein) influence short-term post-exercise recovery; two systematic and meta-analytic reviews (Chapters 3 & 4) that address Aims 1 and 2 respectively (see *1.2. Research Aims*); and a summary of Thesis Part I (Chapter 5). The second part of this thesis, titled '*Whole Beverages: The effect of different post-exercise beverages with food on ad libitum fluid recovery, nutrient provision and subsequent athletic* performance' addresses Questions 2 and 3 (see *1.2. Research Aims*). Whereas Part I focuses on specific beverage constituents, Part II considers how whole beverages influence post-exercise recovery and subsequent athletic performance. It includes a literature review (Chapter 6) summarising research on the rehydration potential of different beverages, and their ability to influence nutrient provision and athletic performance when consumed with food; two experimental studies (Chapters 7 & 8)

addressing Aims 3 and 4 (see *1.2. Research Aims*) are then presented, followed by a summary of Thesis Part II (Chapter 9). The thesis concludes with a general discussion, including recommendations for future research (Chapter 10). The thesis structure is summarised in Figure 1.4. The reader should note that all four of the studies presented in this thesis (i.e. Chapters 3, 4, 7 & 8) have been published in peer-reviewed journals (see pages xx–xxi *Publications in Support of this Thesis*). Manuscripts for two "sub-studies" (completed using additional data collected during the major laboratory experiments) are also published/under-review; however, these will not be discussed further as they are not directly related to the specific aims addressed in this thesis.

Thesis Objective:

To develop a better understanding of how different beverages affect fluid restoration, nutrient provision and subsequent athletic performance when consumed between exercise sessions with limited recovery time (i.e. ≤4 h).

↓

Thesis Part I: Beverage Constituents: The effect of water, carbohydrate and protein consumption on subsequent athletic performance

Q1: How do common beverage constituents (water, CHO and protein) affect subsequent athletic performance when consumed between exercise sessions with limited recovery time?

Chapter 2: Literature Review I

AIM 1	AIM 2
To explore the effect of fluid consumption during or following dehydration on subsequent (i.e. ≤4 h recovery) athletic performance.	To explore the effect of consuming CHO and protein with water during or following exercise on subsequent (i.e. ≤4 h recovery) athletic performance.
CHAPTER 3 (Study 1)	**CHAPTER 4 (Study 2)**
The effect of fluid intake during or following dehydration on subsequent athletic and cognitive performance: A systematic review and meta-analysis	The effect of consuming carbohydrate and protein during or following exercise on subsequent athletic performance: A systematic review and meta-analysis.

CHAPTER 5: Thesis Part I Summary

↓

Thesis Part II: Whole Beverages: The effect of different post-exercise beverages with food on ad libitum fluid recovery, nutrient provision and subsequent athletic performance

Q2: How do different post-exercise beverages affect fluid restoration and nutrient provision in females when consumed *ad libitum* and with food?
Q3: How do different post-exercise beverages affect fluid restoration, nutrient provision and subsequent athletic performance when consumed *ad libitum* and with food between exercise sessions with limited recovery time?

Chapter 6: Literature Review II

AIM 3	AIM 4
To determine the effect of consuming different post-exercise beverages *ad libitum* with food on short-term (i.e. ≤4 h) fluid recovery and nutrient provision in trained females	To determine the effect of consuming different post-exercise beverages *ad libitum* with food on short-term (i.e. ≤4 h) fluid recovery, nutrient provision and subsequent athletic performance.
CHAPTER 7 (Study 3)	**CHAPTER 8 (Study 4)**
Fluid energy and nutrient recovery via *ad libitum* intake of different commercial beverages and food in female athletes.	The effect of different post-exercise beverages with food on *ad libitum* fluid recovery, nutrient provision and subsequent athletic performance.

CHAPTER 9: Thesis Part II Summary

↓

CHAPTER 10: Thesis Discussion and Conclusion

Figure 1.4. Thesis structure; including the overall thesis objective, core research questions, aims and thesis chapters.

THESIS PART I

“Beverage Constituents”

The Effect of Water, Carbohydrate and Protein Consumption on Subsequent Athletic Performance

Chapter 2: Literature Review I

2.1. Preface

2.2. The Physiology of Human Hydration

2.3. Measuring Hydration Status

2.4. Dehydration and Hypohydration

2.5. Recommendations for Hydration and Rehydration

2.6. The Role of Dietary Carbohydrate (CHO) and Protein in Short-Term Post-Exercise Recovery

2.7. Chapter Summary

2.1. Preface

This chapter summarises key aspects of exercise metabolism and describes how common beverage constituents (i.e. nutrients); namely, water, CHO and protein, influence short-term exercise recovery. Given the research candidate's interest in nutritional strategies to optimise subsequent athletic performance, particularly when limited recovery time exists between bouts of activity, the review concentrates on research examining recovery in the initial hours post-exercise (e.g. ≤4 h); studies employing longer (though still relatively short) recovery periods (i.e. up to 6 h) are, however, considered where appropriate.

The review focuses heavily on the importance of hydration – since water is the primary constituent of beverages. The major topics discussed are the physiological effects of fluid loss, their impact on the different components of athletic performance and current recommendations for maintaining and restoring fluid balance during- and post-exercise. In its final stages, the hydration section of the review discusses the challenges individuals might face when attempting to replenish fluid losses between exercise sessions with limited recovery time and the current state of the evidence regarding the impact of different post-exercise fluid consumption strategies on subsequent athletic performance. Thereafter, the review examines how dietary CHO and protein might assist to accelerate short-term exercise recovery. Please note that studies directly investigating the effect of post-exercise fluid, CHO and protein

consumption on subsequent athletic performance will not be discussed in this chapter, as findings are summarised in Chapters 3 & 4.

Peer-reviewed research articles were identified by searching the online databases PubMed (MEDLINE), Web of Science (via Thomas Reuters), Google Scholar and Scopus using a combination of terms, including fluid, water, dehydration, hypohydration, carbohydrate, glycogen, protein, amino acid, muscle, sport, exercise and athletic performance. Cross-matching of citation reference lists and forward citation searches were completed to ensure relevant articles were captured. Research has only been considered if performed on human subjects and published prior to March 2019.

2.2. The Physiology of Human Hydration

2.2.1. The Role of Water in the Human Body

Water is a multifunctional constituent of the human body [10]. It serves as a chemical solvent and reactant; a transport medium for nutrients and metabolic waste products; a shock-absorbent; lubricant, and; a structural component [10]. In fact, almost every biological process within the human body is dependent on maintenance of total body water (TBW) balance [10]. However, it is the cardiovascular and thermo-regulatory functions of water that are imperative to athletic performance [11]. The energetic demands of muscular activity (primarily, demand for oxygen), are met via circulating blood – the major component of which is water [12]. Circulating blood also aids in thermoregulation, transporting excess heat that is generated via substrate oxidation from the body's core to the surface of the skin, where it can dissipate into the environment [13]. The water molecule itself even has unique physical and chemical properties that assist to protect against changes in body temperature, including high specific heat (4.2 $J \cdot g\ °C^{-1}$) and thermal conductivity (0.6 $Wm^{-1}\ K^{-1}$) [14]; characteristics that facilitate the rapid absorption and distribution of heat across the entire body [10]. Finally, if heat loss via radiation and convection is insufficient, the evaporation of water from the surface of the skin (i.e. sweating) is an effective way to dissipate heat, owing to water's high heat of vaporisation (40.7 $kJ \cdot mol^{-1}$) [13,15].

2.2.2. Total Body Water (TBW) Distribution

Water accounts for ~50–70% of BM [16], although this proportion varies in accordance with body composition, as lean tissue has a higher water content than fat tissue (i.e. ~73 vs. 10%) [16]. Thus, water typically accounts for ~60–70% of BM in males and ~50–55% of BM in females [16]. The body's water is distributed among the intracellular (ICF; major electrolytes: potassium [K^+] and magnesium [Mg^{2+}]) and extracellular (ECF; major electrolytes: sodium [Na^+], chloride [Cl^-] and calcium [Ca^{2+}]) fluid compartments, which contain ~65% and ~35% of TBW respectively [16]. The ECF compartments are the interstitial (~28% TBW) and plasma (~7% TBW) spaces [10]. It is important to recognise that these are not "fixed" volumes, but represent the average effect of dynamic exchange between fluid compartments [16].

2.2.3. Determinants of Total Body Water (TBW) Balance

Fluid balance (i.e. '*euhydration*') is achieved when the volume of water input to, and output from, the body is equal [16]. Each day, ~2–3 L of water is lost via obligatory avenues, including via the kidney (~1600 mL urine), skin (~450 mL perspiration), respiratory tract (~300 mL in expired air) and GI tract (~200 mL faeces) [10]. However, heat stress (i.e. due to physical exertion and/or environmental factors, such as high ambient temperature) inducing sweat secretion can increase water loss considerably [17]. Compensatory water "input" occurs via drinking water and other beverages, consumption of food (~675 mL) and metabolic substrate oxidation (~300 mL) [10,15]. The National Health and Medical Research Council of Australia [18] recommend that male and female adults consume ~3.4 and 2.8 $L \cdot d^{-1}$, respectively. However, individuals undertaking heavy exercise or exposed to warm environments may require additional dietary water [17].

2.2.4. Regulation of Total Body Water (TBW) Balance

Fluid balance is maintained via homeostatic mechanisms that promote water input to, and output from, the body in response to changes in TBW content (Figure 2.1) [16]. Negative TBW balance increases plasma osmolality (P_{OSM}), stimulating secretion of anti-diuretic hormone (ADH) from the posterior pituitary gland via hypothalamic osmoreceptors [10]. Together, elevated P_{OSM} and ADH elicit the sensation of thirst and

increase water reabsorption at the distal convoluted tubule and collecting duct of the kidney [10]. When water intake exceeds the body's requirements, a reduction in P_{OSM} inhibits ADH secretion, increasing urine (water) output [10]. Importantly, fluid that is consumed rapidly, in large volumes and/or without other dietary constituents can also induce diuresis (i.e. "fluid-induced diuresis") [19]. Under "normal" circumstances (i.e. mild environmental conditions and moderate physical activity), homeostatic mechanisms maintain TBW content within reasonably narrow limits (±0.2 $L \cdot d^{-1}$) [20]. However, a TBW deficit (i.e. '*hypohydration*') can occur with excessive fluid loss [21]. As the evaporation of sweat is the primary avenue of heat loss during exercise, the risk of dehydration is significant to athletes and individuals working in physically active occupations [6,22,23].

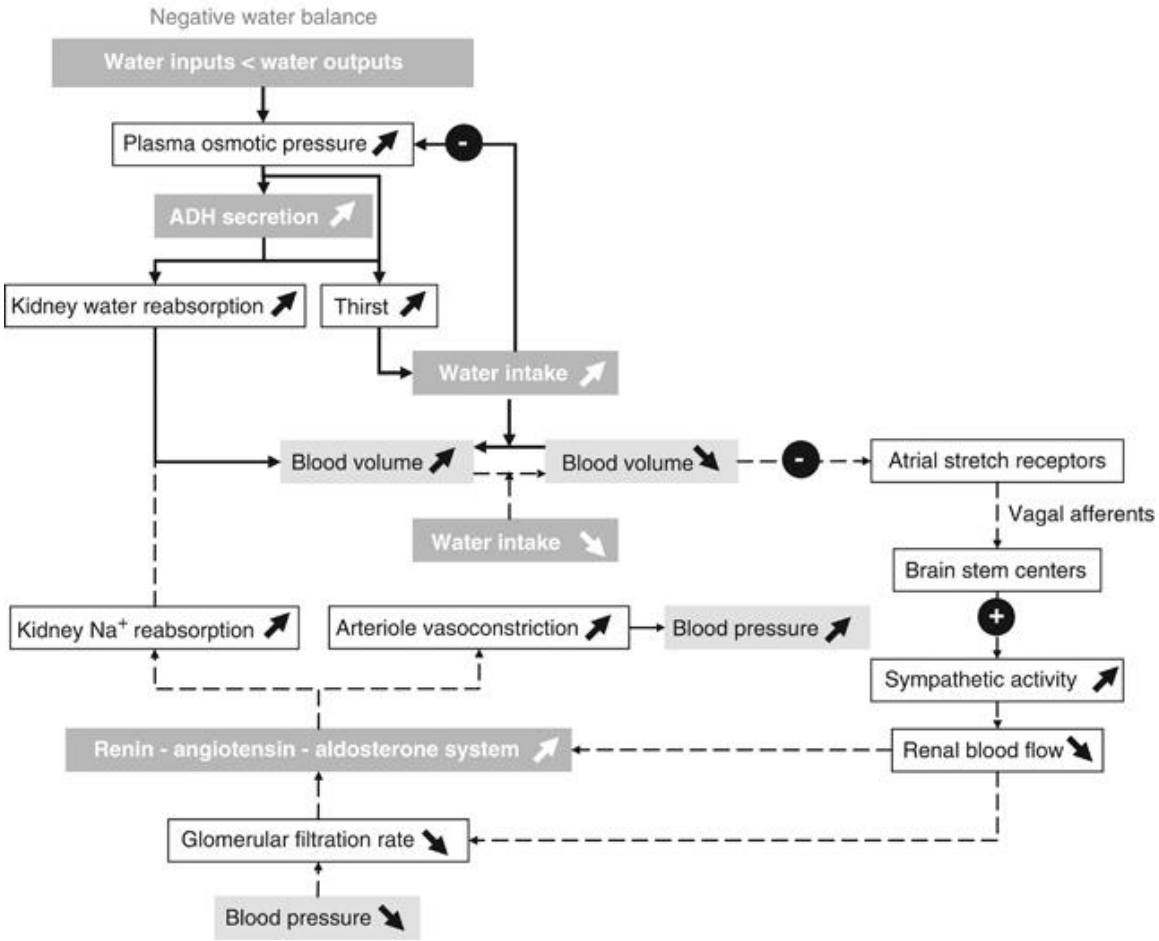

Figure 2.1. Homeostatic mechanisms that regulate TBW balance: main perturbations and physiological responses to TBW deficit. Solid arrows represent responses induced by increased P_{OSM} via osmoreceptors. Dashed arrows represent responses induced by insufficient water intake and decreased blood volume to restore blood volume and pressure. Figure reproduced from Jequier*, et al.* [10]

2.2.5. The Sweat Mechanism

Sweat is secreted onto the surface of the skin during exercise to dissipate excess heat [13]. Initially, cholinergic innervation of the eccrine sweat glands stimulates secretion of a precursor fluid that is drawn predominantly from the ECF compartment [13]. As the precursor advances through the duct segment of the sweat gland, Na^+ and Cl^- are reabsorbed and the osmolality of the fluid decreases [13]. The resulting fluid (i.e. sweat) is therefore hypotonic with respect to plasma (i.e. Na^+: ~10–70 $mmol \cdot L^{-1}$; Cl^-: ~5–60 $mmol \cdot L^{-1}$; K^+; Ca^{2+} and Mg^{2+} are present in smaller quantities) [13]. The loss of hypotonic fluid from the ECF compartment creates an osmotic gradient, initiating a transmembrane flow of water from the ICF to the ECF compartment [3]. As such, the water component of sweat is thought to be derived from both the ICF and ECF compartments [24]. Dehydration resulting from excessive water loss via sweating is termed as *hypertonic* [3]. It contrasts *isotonic dehydration*, in which water is lost exclusively from the ECF compartment, typically due to vomiting, diarrhoea and/or diuretic medication. Hypertonic dehydration is characterised by a reduction in plasma volume (PV) and elevated P_{OSM}; whereas isotonic dehydration decreases PV to greater extent and does not affect P_{OSM} [3]. Thus, the physiological effects of hypohydration differ depending on the method of fluid loss. This review is written in the context of *hypertonic dehydration*.

2.3. Measuring Hydration Status

The absolute volume and compartmental distribution of TBW content is in a constant state of flux, posing a challenge to accurate hydration assessment [16,21]. Indeed, no completely adequate method for determining an individual's hydration status is currently described [21,25]. That said, a number of assessment techniques and biological markers (each with their own strengths and limitations) are commonly used to approximate hydration status in the field and research laboratory (Table 2.1). The selection of a suitable measurement technique is dependent on several factors. The precision, accuracy and reliability of the method are of utmost importance in research and should be prioritised, within the limitations of technical expertise and financial resources. Whereas hydration assessment during day-to-day activities (or in the field) demands an approach that does not require technical expertise or sophisticated instrumentation, yet still demonstrates a reasonable degree of accuracy.

Table 2.1. Hydration assessment techniques.

Measure	Purpose	Practicality					Accuracy	Validity
		Cost	Time Efficiency	Technical Expertise	Portability	Overall Practicality		
Isotope Dilution	TBW Content	H	L	H	L	L	H	A & C
Neuron Activation	TBW Content	H	L	H	L	L	H	A & C
Plasma Osmolality	Fluid Concentration	M	M	M	L	M	H	A & C
Haematocrit & haemoglobin	Plasma Volume Change	M	M	M	L	M	H	A
Urine Specific Gravity	Fluid Concentration	M	M	L	H	M/H	M	C
Urine Osmolality	Fluid Concentration	M	M	M	L	M	M	C
Urine Colour	Fluid Concentration	L	H	L	H	H	M	C
Body Mass Change	TBW Change	L	H	L	H	H	M	A
Rating of Thirst	TBW Change	L	H	L	H	H	L	A

Table modified from Sawka*, et al.* [1] and Armstrong [26]. The components of practicality, overall practicality and accuracy of each assessment technique is rated as 'Low' (L), 'Moderate' (M) or 'High' (H). A: Acute; C: Chronic.

2.3.1. Isotope Dilution and Neutron Activation Analysis

Isotope dilution and neutron activation are generally regarded as "gold standard" techniques for assessing the volume of a fluid compartment and TBW content [21,26]. In isotope dilution, body fluid or expired air are sampled prior to the administration of a tracer substance (i.e. deuterium or deuterium oxide) [26]. A second sample is obtained once the tracer has equilibrated throughout the entire fluid space [26]. Provided the quantity of tracer is known, the baseline and equilibrated tracer concentrations of the biological samples can be used to derive a measurement of TBW content [26]. However, internal isotope equilibration may require 3–5 h and TBW is not usually stable for this length of time [21,26]. Hence, the technique has received criticism due to its low applicability and impracticality during daily activities [21,26]. Isotope dilution is also costly, laborious and has the potential to elicit adverse health effects when large doses of the tracer substance are administered (e.g. nystagma, nausea, dizziness) [27]. In neutron activation, the spectra of radioactive emissions are analysed to determine the quantity of water present in a sample [26]. While a single scan can provide an accurate non-invasive assessment of body fluid compartments, the approach requires a nuclear

reactor and extensive technical knowledge [26]. Thus, isotope dilution and neutron activation analysis are seldom used to assess hydration status in research.

2.3.2. Haematological Indices

PV and P_{OSM} are widely used haematological markers of hydration status [26]. P_{OSM} is a measure of total plasma solute content and is obtained using a freezing point- or vapour pressure-depression osmometer [26]. When body fluids are lost as sweat, P_{OSM} increases [28]. However, because P_{OSM} is itself a potent stimulator of ADH secretion, values are typically controlled within very narrow limits (i.e. ~280–295 mOsmo·kg^{-1}), making small deviations difficult to detect [21]. Change in PV is derived from blood haemoglobin (Hb) and haematocrit (Hct) concentrations, where a baseline value of each parameter is known [29]. Dehydration causes protein-free filtrate to leave the blood, decreasing PV [29]. This is characterised by an increase in the protein concentration of the remaining plasma [29]. While this assessment technique is very reliable [30], the blood collection procedures must be standardised to ensure valid measurements are obtained [30], e.g. the use of a tourniquet for drawing blood and postural differences during blood collection can alter Hb and Hct concentrations, influencing measurements of PV [30]. Despite these limitations, haematological indices are still widely utilised and accepted as markers of hydration status.

2.3.3. Urinary Indices

In addition to regulating TBW content via a change in urine excretion, the kidney also functions to eliminate metabolic and dietary waste products from the body [31]. For this reason, a change in TBW content, and thus, a change in urine volume, produces a measurable shift in urinary solute concentrations (e.g. osmolality [U_{OSM}], specific gravity [U_{SG}] and colour [U_{col}]) that can be used to determine hydration status [31]. U_{SG} is a measure of the density of urine compared to pure water and is assessed using refractometry [26]. The technique can be performed quickly and with relatively little expense or expertise [26]. In healthy adults, normal urine specimens have U_{SG} values of ~1.013–1.029 [26]. First-morning U_{SG} values ≤1.024 appear to be consistent with euhydration, whereas values >1.024 or 1.030 are suggested to indicate significant and serious hypohydration, respectively [32]. U_{SG} values <1.012 typically only occur when

water intake exceeds the body's requirements [26]. U_{OSM}, another indicator of urinary solute concentration, is highly correlated with U_{SG} [33,34]. Normal urine specimens have U_{OSM} values of ~600–800 mOsmol; values >800 mOsmol may indicate hypohydration [35]. The relationship between U_{col} and other markers of hydration status has also been investigated. The colour of urine is caused by urochrome (or urobilin); a urinary solute derived from the degradation of haem [28]. Using an eight-point colour scale, it was demonstrated that both U_{SG} and U_{OSM} were significantly correlated with measures of urine colour [36]. Therefore, this assessment technique may provide a crude indication of hydration status during day-to-day activities. While there are several advantages to using urinary indices to monitor hydration status, a change in TBW content constitutes just one of several factors known to affect urine concentration [37]. Dietary factors, acute fluid consumption, metabolism, physical exertion and psychological stressors all have the potential to influence urine concentration independent of TBW content [37]. Urine concentration has also demonstrated lower sensitivity and a delayed response to acute changes in TBW content in comparison to haematological indices [34]. However, many potentially confounding effects are attenuated overnight. Therefore, a first morning void provides the most reliable assessment of hydration status [37].

2.3.4. Change in Body Mass (BM)

Acute (i.e. ≤4 h) changes in BM can be used as a proxy measure of TBW loss and/or gain when food and fluid intake and urine or faecal losses are accounted for [25,26,28,30]. This approach assumes that a 1 kg change in BM is equivalent to a 1 L change in TBW content, since no other constituent of the human body can be lost at such a comparatively high rate [28]. However, this method overlooks the small (but not insignificant) contribution other factors make to total BM change over time [25] (e.g. substrate oxidation, that is, the loss of non-water mass through expiration of carbon dioxide). Therefore, BM measurements separated by an interval ≥4 h should be adjusted to account for each of these effects [26]. Water contained within the bladder and GI tract may also influence BM, although not technically contributing to the ICF or ECF compartments. The measurement of these parameters is complex, requiring an ultra-sound device [25]. Despite these limitations, change in BM constitutes an

inexpensive, time efficient and non-invasive method of acute hydration assessment that is widely utilised within controlled research settings [25].

2.4. Dehydration and Hypohydration

2.4.1. Sweat Loss During Exercise

Whole-body sweat rates during field-based endurance/ultra-endurance exercise [38-48], team/racket sports [22] and some physically active occupations [23] have previously been quantified (Tables 2.2–2.4). Overall, results indicate that endurance/ ultra-endurance exercise commonly elicits average sweat rates between ~0.8–2.0 $L \cdot h^{-1}$ [38-48], but can induce losses >2.4 $L \cdot h^{-1}$ [43]. Sweat rates reported during team/racket sports (i.e. soccer, rugby, American football, tennis, basketball) appear to vary widely, e.g. ~0.5–2.5 $L \cdot h^{-1}$ [22], which may reflect the contextual diversity of these events. Physically active occupations seem to elicit lower average sweat rates (i.e. ≤1 $L \cdot h^{-1}$) than most other structured sporting activities [23]. Of course, environmental conditions (e.g. ambient temperature, relative humidity [RH]) and individual characteristics (e.g. age, environmental acclimation, body surface area, aerobic fitness) will influence reported sweat rates [49]. It should also be noted that while relatively few studies have isolated whole-body sweat rates in females undertaking field-based exercise [40,44], laboratory studies suggest that females typically exhibit lower average sweat rates than their male counterparts [50]. Under low and moderate heat loads, these "sex differences" appear to be due to variations in body morphology (e.g. body size, body surface to BM ratio) [50]. However, other physiological factors (e.g. sex differences in sweat gland structure and function) may influence the sweat response under heat-stress conditions [51].

Table 2.2. Whole-body sweat rates during field-based endurance and ultra-endurance exercise.

Citation	Activity	Subjects	$VO_{2\,max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Environmental Conditions (Temperature; RH)	Sweat Rate ($L \cdot h^{-1}$)	
					Mean ± SD	Range
Endurance Exercise						
Magazanik, *et al*. [38] (1974)	Marathon Run	6 M	61.5	21 – 26°C; 50 – 60%	1.13	0.81 – 1.43
Myhre, *et al*. [39] (1982)	Marathon Run	3 M	NS	15.5 – 24.5°C	1.24	1.06 – 1.17
Millard-Stafford, *et al*. [40] (1995)	40 km Run	6 M	69.2 ± 3.9	25 – 32°C; 70 – 82%	1.71 ± 0.83	NS
		6 F	60.0 ± 7.5		1.25 ± 0.11	NS
Burke, *et al*. [41] (2005)	Half-Marathon Run	12 M	71.1 ± 3.3	9 – 21°C; 26 – 48%	1.49	0.75 – 2.23
Godek, *et al*. [42] (2005)	50 min Run	5 M	71.3 ± 6.2	28.4°C; 64.9%	1.56 ± 0.40	NS
				34.5°C; 43.0%	1.97 ± 0.28	NS
Lee, *et al.* [43] (2010)	Half-Marathon Run	31 M	59.1 ± 4.2	26.4°C; 81%	1.45 ± 0.32	0.83 – 2.42
O'Neal, *et al*. [44] (2012)	60 min Run	18 M	61.2 ± 8.8	25 – 29°C; 72 – 86%	1.80 ± 0.45	NS
		17 F	52.3 ± 7.2		1.16 ± 258	NS
Ultra-Endurance Exercise						
Rogers, *et al*. [45] (1997)	Triathlon Event (21 km Canoe; 97 km Cycle; 42 km Run)	13 M	54.6 ± 2.4	28.0°C; 48.3%	0.94 ± 0.16	NS
Fallon, *et al*. [46] (1998)	100 km Ultra-Marathon Run	7 M	NS	2 – 17°C; 45%	0.86 ± 0.15	NS
Speedy, *et al*. [47] (2001)	Triathlon Event (3.8 km Swim; 180 km Cycle; 42 km Run)	18 (11 M)	NS	21°C; 91%	Cycle: 0.81 Run: 1.02	NS
Armstrong [48] (2012)	164 km Cycling	20 M	NS	34.5°C; 53%	1.13	NS

F: Female subjects; M: Male subjects; NS: Not specified; RH: Relative humidity; VO_{2max}: Maximal aerobic capacity.

Table 2.3. Whole-body sweat rates during team sports.

Sport	Number of reviewed studies (*n*)	Subjects	Range in Mean Environmental Conditions (Temperature; RH)	Range in Mean Sweat Rate ($L \cdot h^{-1}$)
Soccer	21	415 M; 82 F	5 – 43°C; 7 – 96%	0.3 – 2.5
Rugby	7	116 M	7 – 27°C; 19 – 88%	0.4 – 2.0
American Football	13	225 M	22 – 35°C; 43 – 92%	0.6 –2.9
Tennis	10	98 M; 26 F	17 – 37°C; 32 – 62%	0.6 – 2.6
Basketball	9	189 M; 41 F	17 – 30°C; 20 – 60%	0.7 – 2.7

Table modified from Nuccio*, et al.* [22]. F: Female subjects; M: Male subjects; RH: Relative humidity.

Table 2.4. Whole-body sweat rates within different occupational settings.

Occupation	Number of reviewed studies (*n*)	Subjects	Range in Mean Environmental Conditions (Temperature; RH)	Range in Mean Sweat Rate ($L \cdot h^{-1}$)
Construction	2	16 M	33 – 38°C; 50%	0.47 – 1.03
Defence	1	14 M	32°C; 48%	0.84
Emergency Services	5	74 M; 6 F	26 – 34°C; 4 – 50%	0.54 – 1.37
Maintenance	6	53 M	30 – 39°C; 9 – 79%	0.16 – 0.56
Mining	4	98 M	32 – 39°C; 21 – 57%	0.30 – 0.38

Table modified from Jay [23]. F: Female subjects; M: Male subjects; RH: Relative humidity.

2.4.2. Fluid Intake During Exercise

Fluid consumption during exercise will assist to prevent or delay the development of hypohydration. The upper-limit for fluid intake is set by the maximal gastric emptying rate; ~1.0–1.5 $L \cdot h^{-1}$ for an adult male [14]. A comprehensive review [6] of self-selected drinking practices during competitive sporting activities found that trained athletes typically consume ~0.3–0.8 $L \cdot h^{-1}$ during endurance events and often conclude exercise in fluid deficit (~1.0–3.1% BM). Of course, as drinking must occur "on the move", the time lost in stopping or slowing down to obtain and ingest fluid may deter some individuals from hydrating in a "competition" environment [6]. Still, Passe*, et al.* [52] found that fluid intakes were similarly inadequate (i.e. *n*=18 runners replaced ~30% of their sweat losses) during a 16 km *self-paced* outdoor run (~21°C; 70% RH). The potential for GI problems (e.g. abdominal pain, vomiting and nausea) resulting from drinking during exercise, underestimation of sweat losses and limited access to fluid are proposed to explain this 'voluntary dehydration' [6,52]. Formal breaks, substitutions and informal stoppages of play may provide opportunities for fluid consumption during team/racket sports. Nonetheless, the aforementioned review reported that average fluid intakes during soccer, tennis and basketball (~0.4–1.5 $L \cdot h^{-1}$) were inadequate to prevent hypohydration (e.g. ~0.5–3.4% BM) [6]. While the drinking practices of workers in physically active occupations (e.g. construction, defence, emergency services, maintenance and mining) have not been well researched, studies have noted that these individuals often experience hypohydration (e.g. ~0.5–2.4% BM) [23]. Collectively, the available evidence indicates that voluntary fluid consumption is generally inadequate to offset sweat losses during exercise, often resulting in significant hypohydration.

2.4.3. Dehydration and Hypohydration: Effects on Athletic Performance

While severe hypohydration (e.g. >8% BM loss) inevitably induces a marked decline in overall functioning [53], the physiological effects of mild to moderate hypohydration (e.g. ~1–5% BM loss) are more challenging to detect. Over the past ~15 years, several articles have summarised evidence for the effect of hypertonic dehydration on different components of athletic performance (i.e. endurance and anaerobic exercise, muscular strength and endurance, sport-specific skills and cognitive function) [3,22,54-63]. This section of the review provides a brief overview of this evidence. (Nb. The mechanisms proposed to explain to the performance impairments are discussed in section *2.4.4. Dehydration and Hypohydration: Mechanisms of Performance Impairment*).

2.4.3a. Endurance Exercise Performance

The effect of dehydration on *endurance* or aerobic exercise performance has received considerable scientific attention [3,54-57]. In 2003, Cheuvront*, et al.* [54] proposed that "modest dehydration", or fluid loss ≥2% BM, was sufficient to elicit detectable levels of impairment in endurance performance under temperate and hot environmental conditions. While this initial interpretation was based upon a narrative synthesis of 13 studies, an updated review [3] incorporating 34 studies has since strengthened support for the hypothesis, demonstrating that dehydration ≥2% BM impaired 41 of the 60 performance outcomes assessed (68%). Some researchers do, however, contend that this interpretation is limited because studies often utilise performance tests with low ecological validity (i.e. fixed-power time to exhaustion [TTE] tests) [64]. They argue that time trial (TT) tests are of greater relevance because they allow athletes to implement specific pacing strategies (i.e. as they might in real-world racing situations) [64]. A meta-analysis [55] of 5 studies (*n*=39 participants; 82% male) employing TT performance tests found no effect of dehydration (–2.2±1.0% BM) on endurance cycling performance (percentage change in mean power output [$\%_{\Delta}$ MPO]=0.1±2.7; 95% confidence intervals [Cis]: –1.4, 1.5) and concluded that fluid loss ≤4% BM was unlikely to affect endurance under "real-world" conditions [55]. Given, however, the small number of studies reviewed, the analysis could potentially be confounded by other variables and should be interpreted with caution (e.g. differences in the average duration or intensity of exercise, training-status of participants, degree of

dehydration and/or environmental temperature). Thus, consensus on the level at which dehydration becomes detrimental to endurance performance is lacking.

Far less research has investigated the effect of pre-exercise hypohydration on subsequent endurance performance. Yet, this might occur if an individual fails to adequately rehydrate between exercise sessions, e.g. because the BM deficit is large and/or there is limited time or opportunity to replenish fluid losses. Indeed, studies indicate that some athletes commence training and competitive events in a hypohydrated state [65-68]. A meta-analysis [56] of 10 studies found that pre-exercise hypohydration (–3.9±0.9% BM) induced significant decrements in endurance performance ($\%_{\Delta}$ MPO=3.2; 95% CI's: 2.4, 4.0%) and maximal aerobic capacity (VO_{2max}) (–2.4%; 95% CI's: 1.1, 3.8%). It is, however, important to acknowledge that 6 of the reviewed investigations [69-74] did not subject participants to the hypohydration protocol during control (i.e. 'euhydrated') trials (i.e. the remaining studies dehydrated participants but administered fluid to counteract sweat losses on these trials). Thus, residual effects of the physiological stressors used to induce dehydration (e.g. fatigue, elevated core temperature) could potentially exaggerate the impact of pre-exercise hypohydration in some of these studies. Nonetheless, it is generally agreed that individuals should not commence endurance exercise in a state of fluid deficit [1,2,64].

2.4.3b. Anaerobic Exercise Performance

The effects of fluid loss on anaerobic exercise performance have also been reviewed [3,22,58-60]. A recent meta-analysis [58] identified significant decrements in muscular endurance (fatigue resistance)[a], muscular strength (force production)[b] and anaerobic power[c] due to hypohydration; anaerobic capacity[d] also tended to decline, however, this effect was not statistically significant. In contrast, hypohydration led to a small, non-significant improvement in vertical jump height[e] [58]. Earlier systematic reviews have documented similar effects [22,59,60]. Thus, it appears that mild to moderate hypohydration impairs non-BM dependent muscular performance [58].

[a] 16 effect estimates (–3.1±0.9% BM); –8.3%; 95% CI's: –12.8, –3.9%.

[b] 39 effect estimates (–2.9±1.0% BM); –5.5%; 95% CI's: –7.4, –3.6%.

[c] 9 effect estimates (–3.4±1.2% BM); –5.8%; 95% CI's: –10.3, –1.3%.

[d] 9 effect estimates (–3.4±1.2% BM); –3.5%; 95% CI's: –8.0, 1.0%.

[e] 12 effect estimates (–2.8±1.1% BM); 0.9%; 95% CI's: –0.4, 2.2%.

Whereas, BM dependent muscular performance may be maintained, presumably because the fluid deficit (i.e. reduction in BM) offsets any decline in muscular force [58]. Still, there is some evidence to suggest that anaerobic performance is more resistant to the effects of hypohydration than endurance performance. For instance, Yoshida*, et al.* [75] demonstrated that aerobic performance on the Harvard step test deteriorated at a lower level of fluid loss (–2.4% BM, *n*=7) than anaerobic performance on a 10 s maximal cycling test (–3.9% BM, *n*=9) using a dose-response study protocol (–0.7, –1.7, –2.5, –3.9% BM). Thus, the level of hypohydration required to degrade anaerobic performance is generally thought to be ~3–4% BM loss [22,59,60]. However, the magnitude the of performance decrement is likely to depend on a number of contextual factors, including the mode of physical testing employed (including their sensitivity and reliability), method of dehydration, length of anaerobic activity, length of recovery during intermittent anaerobic activities, and participants' training status [3,22,58-60].

2.4.3c. Sport-Specific Technical Skills

Decrements in endurance and/or anaerobic exercise performance will likely contribute to poorer performance during team/racket sports [22]. However, hypohydration might also affect an athlete's ability to execute sport-specific technical skills (e.g. kicking or passing a ball) [22], with studies have identifying significant impairment of basketball- [76], cricket- [77,78], hockey- [79], golf- [80] and surfing- [81] related skills due to hypohydration. Indeed, an exemplar study by Baker*, et al.* [82], in which participants (*n*=17 M basketball players) completed six experimental trials involving exercise-induced hypohydration with: (1) fluid replacement to counteract sweat losses (CHO-electrolyte sports beverage); (2) fluid replacement to counteract sweat losses (CHO-free placebo electrolyte beverage); or fluid to achieve a residual BM deficit of (3) 1%; (4) 2%; (5) 3%; or (6) 4%; identified a progressive decline on all timed and shooting drills as the level of hypohydration increased (Nb. The performance decrement was statistically significant at –2% BM). However, results are not entirely consistent with some other investigations finding no effect of fluid loss on tennis-, soccer-, field hockey- and basketball-related skills [22]. These inconsistencies may reflect differences in the research methodology employed (i.e. the degree of experimental control), the small number of studies completed (particularly, within

discrete sporting disciplines), and the fact that skilled performance can be difficult to measure, as relatively few reliable, valid and sensitive tests exist [83]. It should also be noted that, in addition to being "physical", many sport-specific technical skills have a cognitive component [22]. While the cognitive effects of hypohydration are outlined below (see *2.4.3d. Cognitive Function*), it is important to recognise that acute (≥20 min), aerobic (i.e. moderate intensity, ~65–75% age-predicted heart rate maximum [HR_{max}]) exercise has demonstrated a cognitive-enhancing effect [84]. Importantly, some evidence suggests that this cognitive benefit might outweigh any adverse effects of mild to moderate hypohydration [85]. Thus, the impact of hypohydration on sport-specific technical skills that are performed within a bout of aerobic exercise could conceivably be attenuated.

2.4.3d. Cognitive Performance and Mood State

Considerable scientific research has investigated the cognitive effects of hypohydration [61-63]. Overall, it appears that while a detrimental effect of hypohydration on mood (e.g. increased fatigue and tension; decreased alertness and concentration) and cognitive performance (e.g. short-term memory and perceptual discrimination) has been reported in some studies, results are inconsistent in the literature [61-63]. This inconsistency may be partly due to residual effects of the physiological stressors used to induce dehydration. Indeed, studies that use exercise and/or heat exposure to induce fluid loss, often fail to provide enough "recovery time" for the effects of these stressors to dissipate (i.e. before cognitive testing is completed) [86-88]; this is problematic as acute exercise has been demonstrated to enhance cognition [84] and elevated core body temperature (T_C) appears to increase cognitive burden [89]. The wide variety of different testing instruments used to measure cognitive performance in these studies might also contribute to the inconsistency [62,90]. To avoid such issues, some researchers have recommended the use of neuropsychological tests that have previously demonstrated sensitivity to other nutritional interventions [62,90]. Of course, as discussed previously (see *2.4.3c. Sport-Specific Technical Skills*), it is important to recognise that any detrimental effects of dehydration or hypohydration on cognition are likely to be attenuated during aerobic exercise [85].

2.4.3e. Modifying Factors

Several factors appear to modify (i.e. attenuate or exacerbate) the effects of dehydration and hypohydration on athletic performance. First, studies indicate that fluid loss becomes more detrimental to athletic performance as ambient temperature increases [91,92] (Figure 2.2); other environmental factors that increase heat storage (e.g. high RH and decreased air velocity [93]) could possibly elicit similar effects. The "timing" of fluid loss might also be influential. Indeed, it appears that studies of 'hypohydration' (i.e. fluid loss is incurred prior to the performance test) detect performance decrements more frequently than those of 'dehydration' (i.e. fluid loss is incurred throughout the performance test) [22]. Some evidence also suggests that certain individuals, such as those who are aerobically-trained [58] or accustomed to dehydration [94], are more resistant to the effects of fluid loss. For instance, Fleming*, et al.* [94] found that the completion of four familiarisation sessions designed to habituate individuals (n=10 M; VO_{2max}=54.5±6.2 $mL \cdot kg^{-1} \cdot min^{-1}$) to hypohydrated exercise significantly attenuated the impairment observed on a 5 km treadmill running TT performance test. Participants' ratings of perceived exertion were similarly reduced, suggesting that the mechanisms underpinning the effect were more psychological than physiological in nature. In regard to aerobic training, Caterisano*, et al.* [95] demonstrated that hypohydration (–3% BM) decreased isokinetic quadriceps muscular endurance (i.e. the ability to maintain ≥50% peak torque) in sedentary individuals and power athletes, but not endurance athletes. The authors proposed that the haemodynamic adaptations associated with aerobic training (i.e. primarily increased PV) might assist to offset the fluid shifts associated with dehydration. Findings from Schoffstall*, et al.* [96] supported this hypothesis indicating a significant inverse relationship between lean body mass (and hence, total body water) and loss of strength following hypohydration (–3% BM). Finally, it is important to acknowledge that while differences in age and sex do not appear to influence the effect of hypohydration, relatively few investigations have included female participants or masters athletes (i.e. >40 y), making comparisons difficult [22].

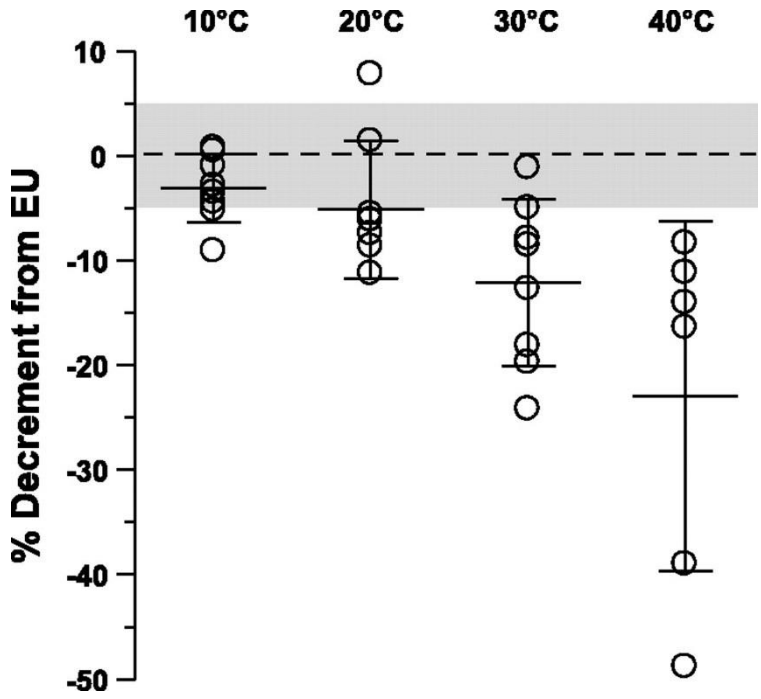

Figure 2.2. The percent decrement in total work performed on a 15 min TT cycling test when hypohydrated (–4% BM) relative to a euhydrated (EU) condition at 10°C, 20°C, 30°C and 40°C. Data are mean and 95% confidence intervals. Shaded area represents the coefficient of variation (±5%) based on variability measured during familiarisation sessions. Figure reproduced from Kenefick*, et al.* [91]

2.4.3f. Study Blinding

Studies examining the effect of fluid loss on athletic performance typically manipulate hydration status overtly. It has therefore been suggested that the detrimental effects reported might partly depend on participants' negative expectations (i.e. a 'nocebo' effect). A small number of studies have recently investigated the effect of hypohydration on athletic performance using "blinded" experimental protocols [97-101]. Two initial studies [97,98] in which participants (*n*=10 M, VO_{2max}=61.7 mL·kg^{-1}·min^{-1} [97] and *n*=11 M, VO_{2max}=55.2 mL·kg^{-1}·min^{-1} [98]) were dehydrated to – 3% BM via prolonged exercise in the heat (~35˚C), before receiving intravenous saline and completing a cycling TT test (25 km [97]); 20 km [98]), found no effect of hypohydration on endurance performance. However, it is important to recognise that intravenous rehydration does not accurately mimic the physiological effects of oral rehydration. Specifically, it does not reduce P_{OSM}; this is problematic because plasma hyperosmolality is an important regulator of thermoregulatory and cardiovascular functioning (see 2.4.4a *Thermoregulatory Mechanisms* and 2.4.4b *Cardiovascular Mechanisms*). The results of the three subsequent studies employing comparable methodology, but instead using *nasogastric rehydration* [99-101] (i.e. where the

physiological and perceptual consequences of hypohydration are better replicated), consistently indicate a detrimental effect of hypohydration on endurance performance. Of particular note is that the decrement in performance was similar between two blinded and unblinded participant groups in one investigation [101]), suggesting that knowledge of hydration status may not actually exacerbate the detrimental effects of hypohydration on performance.

2.4.4. Dehydration and Hypohydration: Mechanisms of Performance Impairment

Several mechanisms have been proposed to explain the performance impairments observed with dehydration and hypohydration, including: (1) increased thermal strain; (2) increased cardiovascular strain and drift; (3) altered cellular metabolism; (4) altered neuromuscular function; and (5) altered function of the neurotransmitter endocrine systems. While this section of the review considers each of the proposed mechanisms independently, it is important to acknowledge that these mechanistic changes do not occur in isolation. Hence, performance impairments resulting from fluid loss may be due to a combination of the following mechanisms.

2.4.4a. Thermoregulatory Mechanisms

Plasma hyperosmolality, hypovolemia (i.e. due to reduced PV) and/or hypernatremia appear to increase the threshold temperature at which sweating and cutaneous vasodilation are initiated [102-107] and reduce sweat rate [108,109]. These changes attenuate heat loss and increase T_C during exercise in temperate and hot environments [110]. Elevated T_C may, in turn, promote the development of "central fatigue" (i.e. a reduction in neural drive to the exercising muscles), altered CHO metabolism (e.g. increased muscle glycogen utilisation, decreased exogenous CHO oxidation and decreased intestinal CHO absorption), altered cardiovascular function (i.e. cardiovascular strain and drift), negative psychological state (e.g. decreased motivation and increased ratings of perceived exertion [RPE]), and increase the risk of heat illness [111-113], with these effects more commonly observed under heat-stress (i.e. than temperate) conditions [110]. This increase in "thermal strain" is thought, in part, to explain the detrimental effect of fluid loss on endurance performance [112]. Some researchers have also theorised that thermal stimuli (e.g.

elevated Tc and/or skeletal muscle temperature) activate central pathways that inhibit performance during repeated-bouts of anaerobic exercise [111,114]. Thus, anaerobic exercise performed within a sustained bout of activity (e.g. team sports) could potentially be impacted by these mechanisms [114]. However, evidence demonstrating that hypohydration (specifically) can mediate such a response is lacking.

2.4.4b. Cardiovascular Mechanisms

Hypovolemia increases cardiovascular strain and cardiovascular drift during prolonged exercise. *Cardiovascular strain* refers to the reduction in skeletal muscle oxygen delivery that occurs when blood flow is redirected to the cutaneous vasculature (i.e. the skin) to facilitate cooling during exercise under environmental heat-stress [111,112]. This effect appears to be exacerbated in hypohydrated individuals, since blood volume is reduced and T_C elevated to an even greater extent (see *2.4.3a. Thermoregulatory Mechanisms*) [112]. For instance, Gonzalez-Alonso*, et al.* [115] observed a ~13% (–2.0±0.6 $L \cdot min^{-1}$, $p<0.05$) reduction in lower limb blood flow due to hypohydration (–3.9 vs. –0.0% BM) in individuals exercising under heat-stress conditions (35°C; 40–50% RH). In addition to increasing cardiovascular strain, fluid loss has an effect to increase *cardiovascular drift* – the progressive rise in heart rate (HR) and fall in stroke volume (SV) that occurs during prolonged exercise in temperate and hot environments [116]. The loss of blood pressure (BP) (i.e. caused by hypovolemia) activates the sympathetic nervous system (SNS), triggering an increase in HR and concomitant reduction in SV; this, in turn, reduces cardiac output (CO) and VO_{2max} [116]. Indeed, Ganio*, et al.* [117] observed a ~7% reduction in VO_{2max} (56.8±6.0 vs. 52.9±6.6 $mL \cdot kg^{-1} \cdot min^{-1}$, $p<0.05$) due to hypohydration (–3.7 vs. –0.7% BM) under heat-stress conditions (30°C; 40% RH). Increased SNS activity also induces vasoconstriction, reducing cutaneous blood flow and increasing heat storage [116]. Thus, dehydration-induced cardiovascular alterations are likely to reduce skeletal muscle oxygen delivery, increase relative exercise intensity and exacerbate thermal stress during aerobic exercise [116]. Given, of course, that anaerobic exercise occurs almost independently of the cardiovascular system, these mechanisms are unlikely to explain the observed impairment of muscular strength or power [59]. However, these alterations may influence muscular endurance and/or performance during repeated-bouts of anaerobic

exercise by increasing reliance on the anaerobic energy pathways (i.e. increasing lactate and hydrogen ion accumulation) [59].

2.4.4c. Metabolic Mechanisms

Hydration-induced changes in cell volume have been reported to regulate a number of intracellular metabolic pathways [118,119]. For instance, *in vitro* cell culture studies suggest that hyperosmotic "cell shrinkage" promotes protein catabolism, glycolysis and glycogenolysis [118,119]. Therefore, these (and/or other intracellular metabolic effects) could conceivably impact aerobic and/or anaerobic exercise performance [59]. However, relatively few studies have investigated the effect of fluid loss on whole-body substrate use and skeletal muscle metabolism, and of these, most have only investigated CHO metabolism. Early *in vivo* research suggested that pre-exercise hypohydration might reduce muscle glycogen utilisation and plasma lactate accumulation during endurance exercise [120]. However, more recent studies, in which the duration and intensity of exercise were more tightly-controlled, have observed increased muscle glycogen utilisation (e.g. ~45%) [121] and plasma lactate accumulation (e.g. ~53%) [121] during prolonged, dehydrating activity under temperate and hot environments (i.e. broadly in keeping with *in vitro* evidence) [121-124]. While dehydration and hypohydration have also been suggested to influence lipid metabolism, anaerobic metabolism (i.e. intramuscular adenosine triphosphate and creatine phosphate stores), cellular buffering and mitochondrial function, research examining these mechanisms is limited and inconclusive [118,121-123,125].

2.4.4d. Neuromuscular Mechanisms

It has been suggested that dehydration might impair some aspects of neuromuscular function (NMF), thus reducing the muscle's ability to develop and/or maintain force or power [59]. However, differences in the electrical activity of contacting skeletal muscles (i.e. measured via electromyography [EMG]) are not usually observed as a result of fluid loss [125-132]. The absence of a clear NMF effect could potentially relate to the assessment techniques employed, as most studies measure electrical activity at a "global level", rather than attempting to isolate effects within specific "regions" of the neuromuscular system [59,133]. Indeed, when utilising the

interpolated twitch technique (i.e. in which an electronically evoked twitch is superimposed on a maximal voluntary contraction [MVC]) to evaluate central NMF *specifically*, Judelson*, et al.* [126] found that the ability of the central nervous system to maximally stimulate the musculature decreased as the degree of hypohydration increased (–0.2, –2.4, –4.8% BM). This study suggests that hypohydration impairs some aspect of central NMF (e.g. neural drive, signal propagation, motor unit activation and/or subject motivation) [133]. However, more recent studies employing comparable methodology failed to replicate these central NMF effects [130,131]. Studies investigating peripheral NMF (e.g. membrane excitability) have also failed to detect a significant impact of hypohydration [134,135]. Thus, while altered NMF is an appealing explanation for the reported deficits in muscular strength and endurance, evidence indicating that hypohydration impairs NMF is limited.

2.4.4e. Mechanisms of Cognitive Impairment

The mechanism(s) by which dehydration and hypohydration impair cognitive performance are not well understood [3,22]. Some researchers have attributed the impairment to reductions in cerebral blood flow (CBF) [136]. Indeed, in addition to restricting blood flow to skeletal muscles (see *2.3.4b. Cardiovascular Mechanisms*), studies indicate that cardiovascular strain limits CBF during exercise under heat-stress conditions [136]. However, mild to moderate hypohydration (e.g. –3% BM) does not appear to impact CBF at rest, the state in which most cognitive assessments are conducted [3]. Plasma hyperosmolality has also been suggested to degrade cognition by increasing blood–brain barrier permeability (i.e. shrinking endothelial cells and widening tight junctions) [3]. However, as barrier function is considered to be "near-normal" up to ~385 $mOsmol \cdot kg^{-1}$, this mechanism seems unlikely [137]. Some neurotransmitter and endocrine systems have also been found to behave differently as a result of hypohydration, potentially mediating the cognitive deficits [63]. For instance, the secretion of stress hormones, namely, cortisol, as a result of SNS activation, has been demonstrated to degrade memory and information processing [3,63], while dopaminergic and noradrenergic signalling (i.e. important in controlling attention, motivation and fatigue) are attenuated with fluid loss [63]. An alternative explanation is that other symptoms of dehydration and hypohydration, including thirst, headache

and/or mood disturbances, distract or demotivate participants during the cognitive assessment(s), leading to poorer performance [22].

2.5. Recommendations for Hydration and Rehydration

The ACSM [1] and the Academy of Nutrition and Dietetics [2] support the following recommendations to facilitate achievement and maintenance of fluid balance during and following dehydrating exercise. Please note that while recommendations for fluid replacement during exercise are introduced in this section, the remainder of the review will focus on post-exercise fluid and nutrient recovery.

2.5.1. Hydration and Athletic Performance

Individuals are advised to consume fluid in volumes sufficient to limit BM loss to <2% during exercise, if it is anticipated that they might otherwise become "excessively" dehydrated (i.e. ≥2% BM loss) [1,2]. By routinely observing BM changes that occur pre- to post-activity, active individuals can estimate their sweat losses and customise an appropriate drinking regimen to meet their specific needs [1,2]. This personalised approach is necessary as sweat rate and electrolyte concentrations vary markedly among individuals [49].

It is important to acknowledge that there is considerable scientific debate as to whether a "prescribed drinking regimen" intended to limit TBW loss offers a performance advantage, compared to "*ad libitum* drinking" or "drinking to thirst" [138], where the quantity of fluid consumed is often inadequate to prevent excessive dehydration [6]. Indeed, two recent studies documented non-significant improvements in 20 km trail running (01:44:09 vs. 01:44:39; *n*=13; 28°C) [139] and half marathon (1:29:36 vs. 1:29:48; *n*=10; 30°C) performance [140] when fluid consumption was *ad libitum* or "to thirst", instead of being prescribed, despite participants incurring significantly greater fluid losses under this condition (i.e. –3.1 vs. –1.3% [140]; –2.6 vs. –1.3% [139]). Two meta-analyses [55,141] have also failed to indicate a benefit of drinking above thirst sensation. In light of this evidence, some individuals may themselves elect (or be encouraged) not to adhere to a prescribed drinking regime. Particularly, if excessive time is likely to lost in obtaining and consuming the fluid, or there is a high likelihood for GI problems. Consequently, the size of the fluid deficit

incurred post-exercise may be increased. That said, even a prescribed drinking regimen is not intended to compensate completely for sweat loss during physical activity. In circumstances where dehydration cannot be prevented, rehydration following exercise is of critical importance.

2.5.2. Rehydration and Subsequent Athletic Performance

Individuals are advised to re-establish euhydration following exercise. If recovery time and opportunity permits (e.g. >12 h), a usual diet should be sufficient to replenish TBW content [1]. However, if the fluid deficit is large and there is limited time and/or opportunity to rehydrate (e.g. ≤8 h) before recommencing exercise, individuals are advised to consume fluid in volumes equal to 1.25–1.50 L·kg BM $lost^{-1}$ to promote rapid and complete recovery of TBW content [1,2]. The volume of fluid ingested must exceed the size of the TBW deficit to compensate for the ongoing fluid losses via sweating, obligatory urine production and fluid induced diuresis [142,143] (i.e. whereby ingested fluid rapidly decreases P_{OSM} and stimulates urinary output [144,145]). Approximately 2–4 h before recommencing exercise individuals are also advised to "pre-hydrate" by consuming ~5–7 mL·kg BM^{-1} [1]. If the subsequent urine output is concentrated (or absent), an additional intake of 3–5 mL H_2O·kg BM^{-1} is recommended [1].

The consumption of 1.50 L·kg BM $lost^{-1}$ is generally sufficient to replenish exercise-induced fluid losses (Chapter 6) [142]. However, individuals preparing to undertake a subsequent bout of exercise may have limited time between sessions to consume fluid. In this context, such large volumes have the potential to *enhance* or *inhibit* athletic performance, either by facilitating rapid initial gastric emptying and fluid absorption to restore PV and P_{OSM} [146] or by inducing GI problems (particularly if beverages with higher calorie loads and hence slower rates of gastric emptying are consumed, e.g. milk/milk-based formulations [146,147]). The demands of a subsequent activity (i.e. mode, duration and intensity) and/or the environmental conditions (i.e. ambient temperature) might also influence the suitability of the aforementioned guidelines. Despite this, relatively few studies have investigated the impact of fluid intake volume on subsequent athletic performance [148-152], and the majority of these have employed a prolonged (e.g. overnight) rehydration protocol [148-150], decreasing the likelihood that GI problems will impact performance. Thus, a comprehensive review of the evidence is required to better understand the impact of different post-exercise

fluid ingestion strategies on subsequent athletic performance in situations where limited recovery time exists. Understanding how to maximise the benefits of fluid consumption under these circumstances will inform the development of future fluid replacement guidelines.

2.6. The Role of Dietary Carbohydrate (CHO) and Protein in Short-Term Post-Exercise Recovery

From this point forward, the review will consider how other nutrients often consumed in beverages (i.e. CHO and protein), might influence short-term exercise recovery. While other beverage constituents (e.g. caffeine) have also demonstrated ergogenic effects [153], these will not be discussed in the review. Given the research candidates' interest in nutritional strategies to optimise *subsequent* athletic performance, particularly when limited recovery time exists between bouts of exercise, the review will concentrate on research that examines recovery in the initial hours post-exercise (e.g. ≤4 h). The longer-term effects of post-exercise CHO and protein supplementation are beyond the scope of this review.

2.6.1. Carbohydrate (CHO) and Exercise Recovery

CHO is an important fuel for exercise performance, yet our ability to store this substrate is limited. Thus, rapid restoration of muscle glycogen stores is of critical importance for individuals intending to undertake two or more exercise sessions with limited recovery time between bouts.

2.6.1a. Carbohydrate (CHO) Storage and Metabolism

Most of the body's CHO is stored as glycogen in cells of the liver (e.g. ~100 g) and muscle (e.g. ~350–700 g) (other tissues and cells, including the kidney, red blood cells and brain also store small amounts of glycogen); a small amount of glucose (e.g. ~4 g) derived from hepatic glycogenolysis and gluconeogenesis as well as the GI tract (i.e. dietary CHO) also circulates in the blood [154]. Muscle glycogen and blood glucose are the primary substrates for contracting skeletal muscle during exercise at moderate or greater intensities (e.g. >60% VO_{2max}) [154,155]. The amount of glycogen stored in skeletal muscle depends on an individual's training and nutritional status; however,

typical concentrations in fed, rested, aerobically-trained males (i.e. VO_{2max} ~60 mL·kg^{-1}·min^{-1}) are ~530±140 mmol·kg^{-1} dry mass (DM) (Figure 2.3) [156], with studies suggesting that ~8–11% is deposited as subsarcolemmal glycogen (i.e. below the sarcolemma), ~77–84% as intermyofibrillar glycogen (i.e. between the myofibrils) and ~3–13% as intramyofibrillar glycogen (i.e. within the myofibrils) [157,158]. While several factors influence the rate at which glycogen is utilised during exercise (e.g. exercise intensity and duration, the pre-exercise diet, substrate availability, training status, environmental conditions), typical concentrations are generally sufficient to maintain ~120 min of activity at ~70% VO_{2max} (Figure 2.4) [154,155]. Recent evidence indicates that the majority of glycogen utilised during endurance (i.e. ~1 h) and supramaximal exercise (i.e. repeated 4 min "sprints") is drawn from the intra-myofibrillar [157] and intermyofibrillar [159] glycogen compartments, respectively. When skeletal muscle glycogen concentrations fall below ~250–300 mmol·kg^{-1} DM, Ca^{2+} release from the sarcoplasmic reticulum becomes impaired and the intensity of the exercise must be reduced to a level where the greater proportion of energy needs can be met by fat oxidation [154]. Exhaustion of hepatic glycogen stores also results in hypoglycaemia, which impairs physical and cognitive function [154]. It therefore follows that glycogen depletion is a major cause of fatigue in both aerobic exercise and activities involving repeated-bouts of anaerobic exercise [154,155].

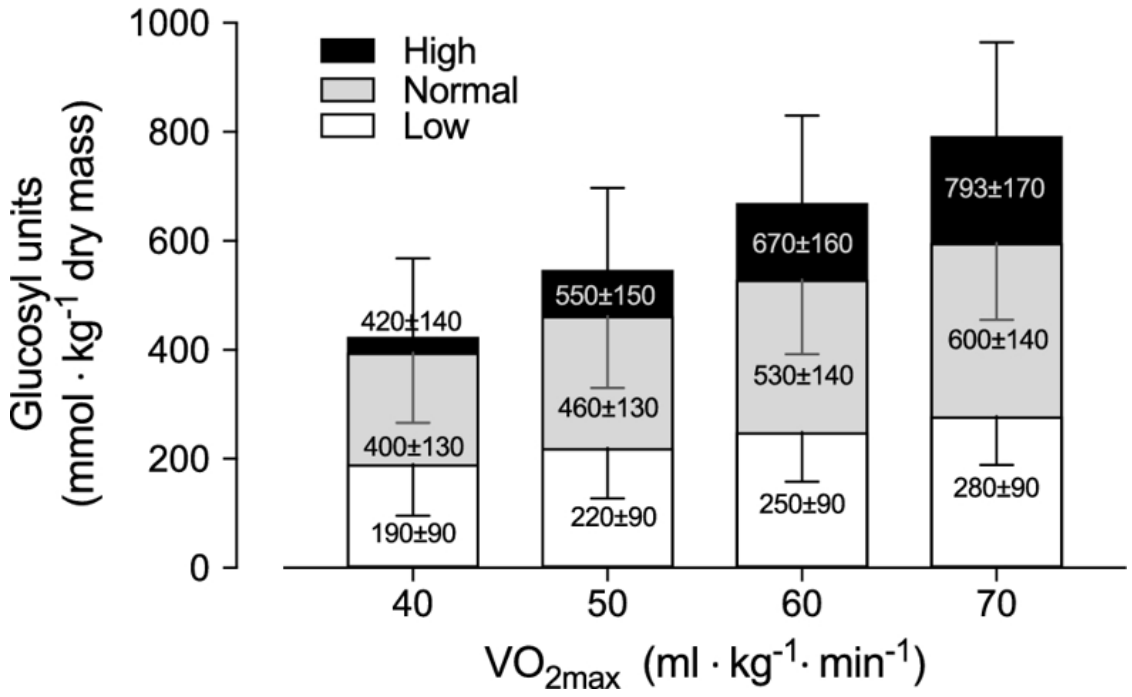

Figure 2.3. Predicted resting glycogen concentration in the vastus lateralis of males with a VO_{2max} of 40–70 mL·kg^{-1}·min^{-1} in conditions of 'Low' (glycogen depletion and a low-CHO diet), 'Normal' (neither high nor low CHO-diet) and 'High' (>6 g CHO·kg^{-1}·d for ≥3 d or (>7 g CHO·kg^{-1}·d for ≥2 d) CHO availability. Data are predicted means and standard deviations. Figure reproduced from Areta*, et al.* [156].

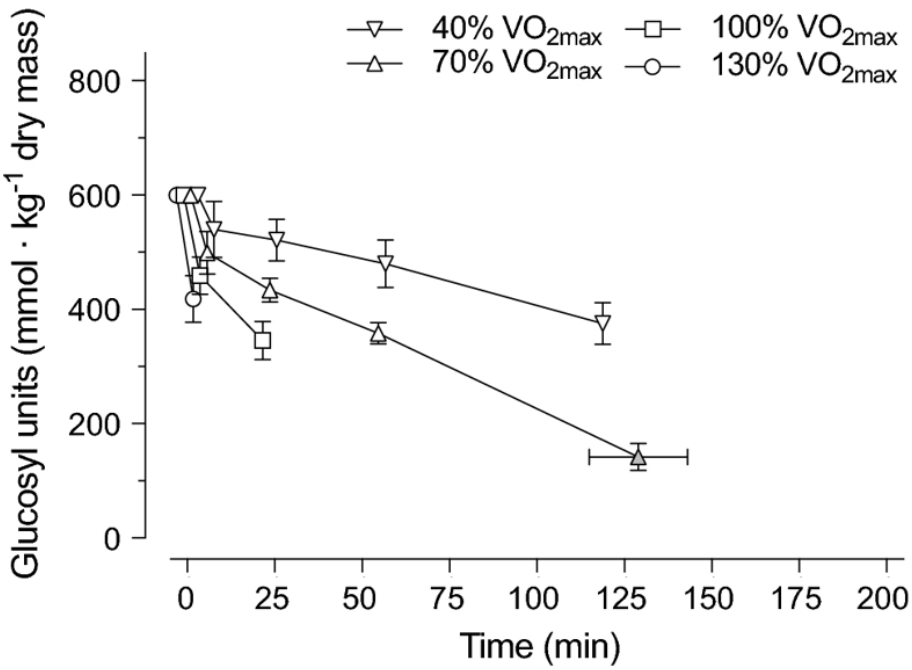

Figure 2.4. Predicted glycogen concentration in the vastus lateralis of males with a VO_{2max} of 60 mL·kg^{-1}·min^{-1} during continuous cycling exercise at 40, 70, 100 and 130% VO_{2max} (where the baseline concentration is 600 mmol·kg^{-1} dry mass). The grey-shaded timepoint represents predicted TTE. Bars are 90% confidence intervals. Figure reproduced from Areta*, et al.* [156].

2.6.1b. The Mechanism of Glycogen Resynthesis

Muscle glycogen resynthesis is a biphasic process [154,160]. Initially, there is a "rapid" or "insulin-independent" phase in which glycogen is resynthesised at ~50–130 mmol·kg^{-1} DM·h^{-1} [154,160]. This phase usually lasts ~30–60 min and is thought only to occur when glycogen stores are heavily depleted (i.e. <150 mmol·kg^{-1} DM) [154,160]. The second "insulin-dependent" phase is characterised by a prolonged (i.e. up to ~48 h) increase in the sensitivity of muscle glucose uptake and glycogen synthesis to insulin [154,160]. The magnitude of this increase can be extremely high and result in muscle glucose uptake and activation of glycogen synthase at insulin concentrations that normally have no discernible effect on either process [154,160]. Glycogen resynthesis during the second phase will occur very slowly (i.e. ~9–13 mmol·kg^{-1} DM·h^{-1}), unless CHO is consumed (i.e. ~20–50 mmol·kg^{-1} DM·h^{-1}) [154,160]. If the glycogen deficit is modest (e.g. ~175 mmol·kg^{-1} DM·h^{-1}) and CHO intake is ~1.0–1.2 g·kg^{-1}·h^{-1}, stores can usually be replenished within ~4–5 h [154]. However, if the glycogen deficit is large, complete restoration usually takes >24 h because the rate of storage declines by ~50% beyond ~4 h post-exercise [154]. For instance, Starling et al., [161] demonstrated that a high CHO diet (~10 g·kg^{-1}·d^{-1}) replenished ~93% of the muscle glycogen oxidised (i.e. 330 mmol·kg^{-1} DM·h^{-1}) during 2 h of cycling at 65% VO_{2max} in 24 h. That said, other factors, including training status, muscle fibre type composition and muscle damage will also influence the rate of post-exercise muscle glycogen resynthesis [160].

2.6.1c. Dietary Strategies to Maximise Glycogen Resynthesis

Strategies for increasing skeletal muscle glycogen concentrations and maintaining "normal" glycogen levels on a day-to-day basis have been described. Briefly, a high CHO diet (e.g. 2–3 d of >6 g CHO·kg^{-1}·d) and a reduced exercise load is reported to increase muscle glycogen stores to "supercompensated" levels (e.g. ~670±160 mmol·kg^{-1} DM) (Figure 2.3) [156]; guidelines for daily CHO consumption based on an individual's exercise load (and BM) also exist [2]. However, strategies to maximise short-term muscle glycogen storage between exercise sessions with limited recovery time are also required [160]. The following section of this review will describe factors that may affect the rate of muscle glycogen storage in the initial hours following exercise, including the timing of CHO intake, the amount, type and form (i.e. solid vs. liquid) of CHO consumed, and presence of other co-ingested nutrients [160].

Only a few studies have systematically investigated the relationship between CHO intake dose and rate of muscle glycogen resynthesis in the initial hours after exercise [162-164]. Blom*, et al.* [162] initially suggested that 0.35 g CHO·kg^{-1}·h^{-1}, ingested at 2 h intervals, maximised the rate of muscle glycogen resynthesis. Specifically, the study indicated a significant benefit of increasing CHO consumption from 0.18 to 0.35 g·kg^{-1}·h^{-1} (i.e. 9 vs. 25 mmol·kg^{-1} DM·h^{-1}) but found no further advantage of consuming ≥0.70 g CHO·kg^{-1}·h^{-1} [162]. Ivy*, et al.* [163] also failed to demonstrate a difference in the rate of muscle glycogen resynthesis when 0.75 vs. 1.50 g CHO·kg^{-1}·h^{-1} was administered at 2 h intervals (i.e. 20 vs. 22 mmol·kg^{-1} DM·h^{-1}). In contrast, however, a more recent investigation [164] observed higher rates of muscle glycogen storage when consuming 1.2 compared to 0.8 g CHO·kg^{-1}·h^{-1} (i.e. 17 vs. 35 mmol·kg^{-1} DM·h^{-1}) [164]. The authors attributed their result to the fact that CHO was supplemented at (regular) 30 min, rather than (infrequent) 2 h, intervals, which assisted to maintain elevated blood glucose and insulin concentrations throughout the recovery period [164]. Indeed, several other studies have observed high post-exercise muscle glycogen resynthesis rates (e.g. ≥40 mmol·kg^{-1} DM·h^{-1}) with frequent CHO supplementation (i.e. every ~15–60 min) [160]. Reported rates of glycogen resynthesis at different CHO intake doses are summarised in Figure 2.5. Based on these data, Jentjens*, et al.* [160] concluded that of ~1.0–1.2 g CHO·kg^{-1}·h^{-1} is likely to maximise muscle glycogen storage in the initial hours post-exercise. Consuming >1.2 g CHO·kg^{-1}·h^{-1} does not appear to accelerate this process any further [160].

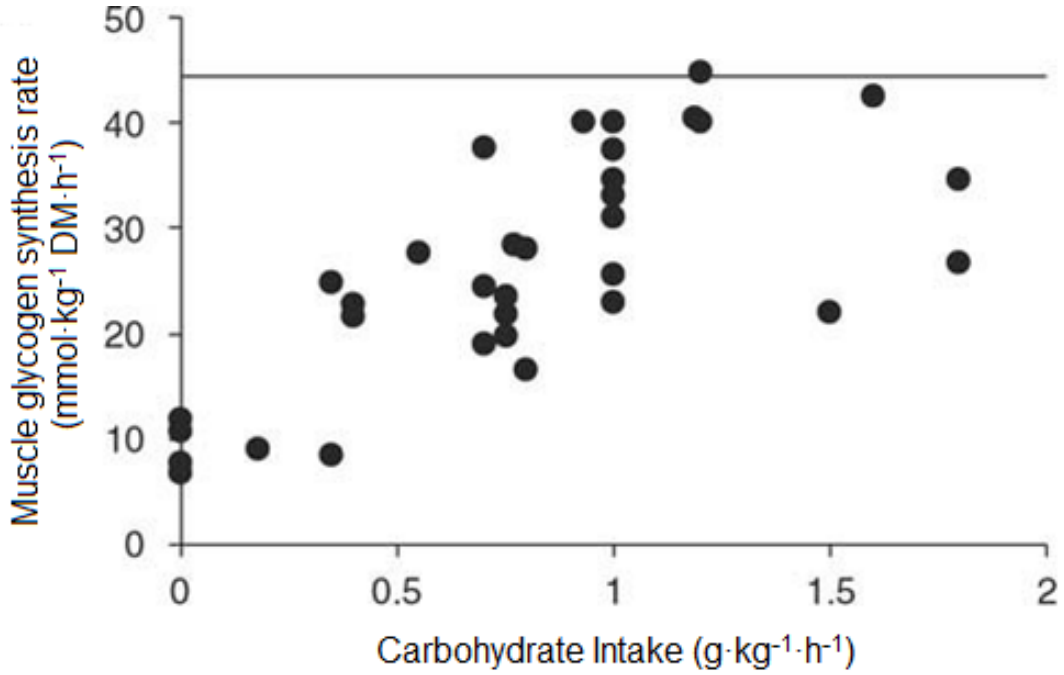

Figure 2.5. Reported rates of post-exercise (i.e. ≤4 h) muscle glycogen resynthesis with different amounts of ingested CHO. The horizontal line depicts the highest observed rate of muscle glycogen resynthesis. Figure reproduced from Jentjens*, et al.* [160].

The 'timing' of CHO ingestion has also been suggested to impact muscle glycogen storage in the initial hours post-exercise [160,165]. Indeed, Ivy et al., [166] observed a significant reduction in the rate of muscle glycogen resynthesis when CHO supplementation (i.e. 1.0 $g \cdot kg^{-1} \cdot h^{-1}$) was delayed by 2 h, rather than occurring immediately post-exercise (i.e. 18 vs. 33 $mmol \cdot kg^{-1}$ $DM \cdot h^{-1}$). Studies have since demonstrated that a 2 h delay in CHO ingestion attenuates the usual post-exercise increase in muscle glucose uptake [167,168]; an effect that could conceivably limit muscle glycogen storage. It is also important to recognise that early CHO supplementation increases the overall opportunity for post-exercise muscle glycogen storage to occur, since the rate of synthesis will remain very low until CHO feeding is initiated [166]. Thus, individuals are generally advised to consume CHO <30 min post-exercise to rapidly replenish exercise-induced losses [2].

Several studies have investigated the impact of single CHOs and CHO-containing mixed-meals that differ in their glycaemic index (GIx) on post-exercise muscle glycogen storage [160,165]. This research is based on the premise that the different blood glucose and insulin responses to higher and lower GIx sources of CHO have the potential to influence the rate of muscle glycogen resynthesis [160,165]. Indeed, lower rates of glycogen storage have been reported with the consumption of fructose (GIx=19) and

sucrose (GIx=65) than glucose (GIx=100) [160]. Thus, glucose is thought to be an ideal post-exercise CHO source in situations that require rapid glycogen restoration. That said, Bowtell*, et al.* [169] failed to observe a difference between sucrose and glucose when these CHOs were ingested in large amounts (i.e. 1.2 $g \cdot kg^{-1} \cdot h^{-1}$). Some studies have also identified a benefit of consuming high-GIx CHO-containing mixed-meals [170,171]. For instance, Kiens*, et al.* [170] found that the provision of a "high" GIx (i.e. ≥70) meal increased plasma insulin concentrations and the rate of muscle glycogen storage compared to an energy- and CHO-matched "low" GIx (i.e. <55) meal (i.e. 24 vs. 40 $mmol \cdot kg^{-1}$ DM$\cdot h^{-1}$). Unfortunately, these data are difficult to interpret as neither the amount of CHO consumed, nor the GIx of the meals were reported. Burke*, et al.* [171] also identified a significant improvement in post-exercise muscle glycogen storage after 24 h of consuming a high GIx diet (i.e. 10 g CHO$\cdot kg^{-1} \cdot d^{-1}$; 463 vs. 576 $mmol \cdot kg^{-1}$ DM). While the short-term (e.g. <6 h) effects of this dietary intervention were not assessed directly, it seems likely that, with a considerable proportion of glycogen resynthesis occurring in the initial hours post-exercise, GIx CHO could be of benefit when recovery time is limited. Nonetheless, further research is required to clarify the influence of GIx on short-term post-exercise muscle glycogen storage.

To the researcher's knowledge, only two studies have investigated the effect of CHO "form" (i.e. liquid vs. solid) on muscle glycogen storage in the initial hours post-exercise [172,173]. The most recent of these studies demonstrated that high-GIx CHO (i.e. 0.75 g CHO$\cdot kg^{-1} \cdot h^{-1}$) ingested in the form of a glucose polymer solution ('liquid') or a rice/banana cake ('solid') post-exercise resulted in similar levels of muscle glycogen restoration (i.e. 23 vs. 24 $mmol \cdot kg^{-1}$ DM$\cdot h^{-1}$) [173]. This result was unexpected as gastric emptying of liquids typically occurs faster than solids [174]. However, the same study indicated that an equivalent dose of CHO administered intravenously (i.e. bypassing the GI system altogether) also had a comparable effect on muscle glycogen restoration (i.e. 24 $mmol \cdot kg^{-1}$ DM$\cdot h^{-1}$) [173], suggesting that gastric emptying rate does not limit glycogen resynthesis when moderate amounts of CHO are consumed [173]. While the earlier study [172] also observed similar levels of muscle glycogen storage with solid vs. liquid CHO (i.e. 25 vs. 25 $mmol \cdot kg^{-1}$ DM$\cdot h^{-1}$ over 5 h), these findings must be interpreted with caution as the solid meal contained ~10% more CHO than the liquid meal; the test meals also differed in their fat and protein content [172]. Thus, while the available evidence suggests that different "forms" of CHO are equally effective at replenishing

muscle glycogen post-exercise, further research is required to verify these effects with larger doses of CHO (e.g. 1.0–1.2 $g \cdot kg^{-1} \cdot h^{-1}$).

2.6.1d. Other Physiological Effects of Carbohydrate

Briefly, in addition to promoting skeletal muscle glycogen resynthesis, there are several other mechanisms by which CHO ingested between exercise sessions could enhance subsequent athletic performance. For instance, ongoing absorption of CHO ingested during the latter stages of a recovery period might assist to maintain blood glucose levels during exercise. Some evidence also suggests that the presence of CHO in the oral cavity enhances central drive and/or motivation during exercise [175]. While studies investigating these oral receptor-mediated effects typically administer CHO *during* the activity (i.e. as a 'mouth rinse'), CHO consumption prior to exercise could conceivably elicit similar effects [175]. CHO-sensitive receptors located in other regions of the GI tract have also been suggested to modulate exercise performance, however, their role is still poorly understood [175].

2.6.2. Protein and Exercise Recovery

Unlike CHO, protein contributes minimally to the energetic demands of exercise. However, other attributes of this nutrient, including its ability to accelerate muscle damage repair and glycogen resynthesis, may be of benefit to individuals undertaking two or more exercise sessions with limited recovery time between bouts.

2.6.2a. Protein and Repair of Exercise-Induced Muscle Damage

Acute exercise, *particularly* if it is strenuous, unfamiliar and/or involves an eccentric (i.e. lengthening) component, can damage skeletal muscle, resulting in soreness, stiffness, swelling, and impaired muscle function (e.g. reduced force-generating capacity) [5]. While soreness usually appears within ~8 h and peaks ~1–3 d post-exercise, pronounced decrements in muscle function are detectable immediately post-exercise (Figure 2.6) [5]. These decrements have been attributed to failure of excitation-contraction coupling, redistribution of sarcomere lengths, damage to the contractile machinery and altered metabolism [176]. Given that post-exercise protein consumption promotes skeletal muscle anabolism [177], the ingestion of this nutrient has been suggested to accelerate muscle damage repair, and thus, the restoration of

muscle function [178]. Indeed, *regular* (i.e. daily) protein supplementation has been shown to improve indices of muscle damage (i.e. plasma creatine kinase levels), soreness and function during exercise recovery [178]. However, a recent systematic review [178] concluded there were insufficient experimental data to demonstrate that the acute ingestion of protein either before, during or after a single bout of exercise reduced muscle damage or improve muscle function within ≤24 h.

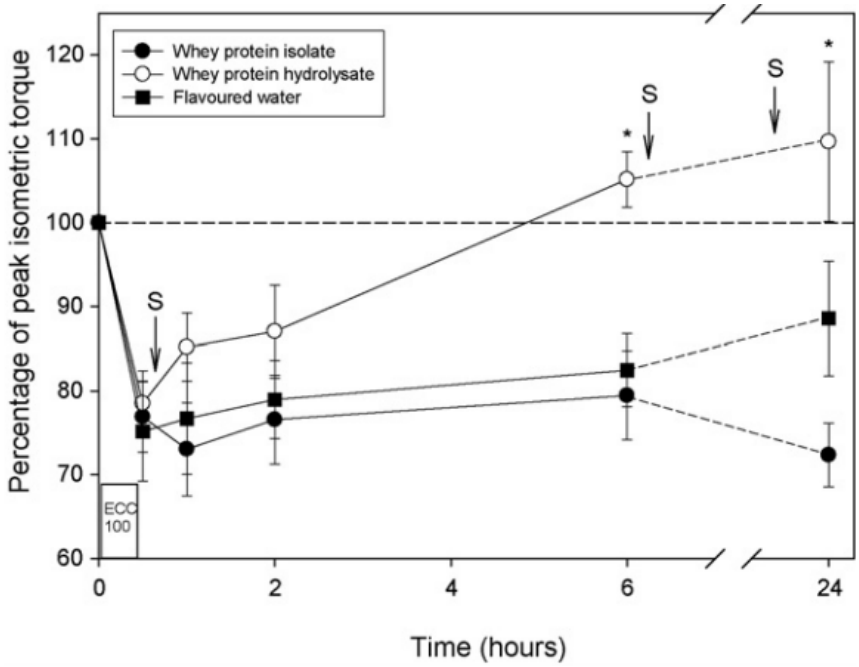

Figure 2.6. Percentage of peak isometric torque of the knee extensors pre- and post-supplementation with a water placebo, whey protein isolate, or hydrolyzed whey protein isolate treatment. Values are Mean±SEM. ECC100: 100 maximal eccentric contractions of the knee extensors (i.e. exercise-induced muscle damage); S: Supplement administered. *Significantly different from immediately post-ECC100 for hydrolysed whey protein isolate. Figure reproduced from Buckley*, et al.* [179].

To the research candidate's knowledge, only Buckley*, et al.* [179] has investigated the short-term effects of protein supplementation. In this study of sedentary males, the consumption of a fast-absorbing hydrolysed whey protein supplement (*n*=6) facilitated a more rapid recovery of muscle function after eccentric exercise than a placebo (*n*=11) or non-hydrolysed (*n*=11) form of the same protein supplement (Figure 2.6) [179]. That said, muscle function was similar (and remained below baseline levels at 6 and 24 h post-exercise) when the intact whey protein and placebo were consumed [179], suggesting that 'normal' dietary proteins (i.e. derived from food and beverages) are unlikely to confer a benefit within this limited timeframe. It is also important to recognise that findings from this investigation may not be generalisable to endurance- and/or

resistance-trained individuals who are accustomed to exercise and unlikely to experience the same degree of muscle damage as the sedentary participants that completed this study [178]. Thus, it appears that while protein ingestion has the potential to accelerate skeletal muscle damage repair during recovery from exercise [180], the magnitude of this effect within ~4–6 h might be too small to improve muscle function.

2.6.2b. Protein and Muscle Glycogen Resynthesis

The co-ingestion of CHO with intact protein, a protein hydrolysate and/or some free amino acids (i.e. in particular, leucine and phenylalanine) seems to potentiate the usual post-prandial increase in pancreatic insulin secretion [160,181]. For example, Kaastra*, et al.* [182] observed a 108±17% increase in insulin release when 0.8 g CHO·kg^{-1}·h^{-1} was consumed with (i.e. compared to without) a casein hydrolysate (0.4 g·kg^{-1}·h^{-1}) in the post-exercise period; the addition of free leucine (0.1 g·kg^{-1}·h^{-1}) to the protein-containing supplement increased insulin secretion even further (+190±33%). Similar, though less pronounced, insulinemic effects have also been observed with the administration of intact proteins [183] and proteins that have a lower leucine and phenylalanine content (e.g. wheat protein) [184]. Given insulin's ability to stimulate muscle glucose uptake and glycogen synthase (see *2.6.1b The Mechanism of Glycogen Resynthesis*), the co-ingestion of CHO and protein has been suggested to accelerate muscle glycogen resynthesis [181].

Several studies have reported higher rates of post-exercise muscle glycogen storage with co-ingestion of CHO and protein than with CHO ingestion alone [185-189]. That said, a number of these [185-187] employed *isocarbohydrate* (i.e. rather than *isoenergetic*) controls, such that the effect observed could be due to the additional energy delivered (i.e. increased substrate availability) and not the protein per se [160,181]. Indeed, van Loon*, et al.* [164] found that 0.8 g CHO·kg^{-1}·h^{-1} consumed with a leucine-/phenylalanine-enriched wheat hydrolysate (0.4 g·kg^{-1}·h^{-1}) increased the rate of muscle glycogen resynthesis compared to an isocarbohydrate (i.e. 17 vs. 35 mmol·kg^{-1} DM·h^{-1}), but not isoenergetic (i.e. 45 mmol·kg^{-1} DM·h^{-1}) treatment. Still, other studies have reported significant glycogenic effects of co-ingested protein in the initial hours post-exercise when utilising isoenergetic controls [188,189].

Findings from several other studies suggest that the co-ingestion of CHO with protein *does not* accelerate muscle glycogen resynthesis [190-197]. Interestingly,

however, the majority of these investigations [190-193] administered a relatively large amount of CHO (i.e. ~1.0–1.2 $g \cdot kg^{-1} \cdot h^{-1}$, as is recommended to maximise muscle glycogen resynthesis); leading some researchers [181,198] to theorise that the glycogenic effect of co-ingested protein might be contingent on the quantity of CHO consumed. Reported rates of muscle glycogen resynthesis in the presence and absence of co-ingested protein at different CHO intake doses are summarised in Figure 2.7. On the basis of these data, Alghannam*, et al.* [198] concluded that a CHO intake of ~1.0–1.2 $g \cdot kg^{-1} \cdot h^{-1}$ is likely to maximise muscle glycogen storage, whether or not additional protein is provided. But, that co-ingested protein might accelerate muscle glycogen resynthesis when CHO intake is 'sub-optimal' (i.e. <1.0 $g \cdot kg^{-1} \cdot h^{-1}$) [198]. The amount of protein required to elicit a glycogenic effect also warrants consideration. Indeed, some studies have failed to detect a benefit of co-ingested protein when administering a very small dose (i.e. ≤0.2 $g \cdot kg^{-1} \cdot h^{-1}$) [196,197] – an observation consistent with evidence indicating that protein intakes ≤0.2 $g \cdot kg^{-1} \cdot h^{-1}$ are not usually sufficient to potentiate insulin secretion [198]. Thus, while there are some inconsistencies in the literature, the collective evidence suggests that if CHO intake is ≤1.0 $g \cdot kg^{-1} \cdot h^{-1}$, the co-ingestion of ~0.3–0.4 $g \cdot kg^{-1} \cdot h^{-1}$ protein may assist to maximise muscle glycogen storage in the initial hours post-exercise.

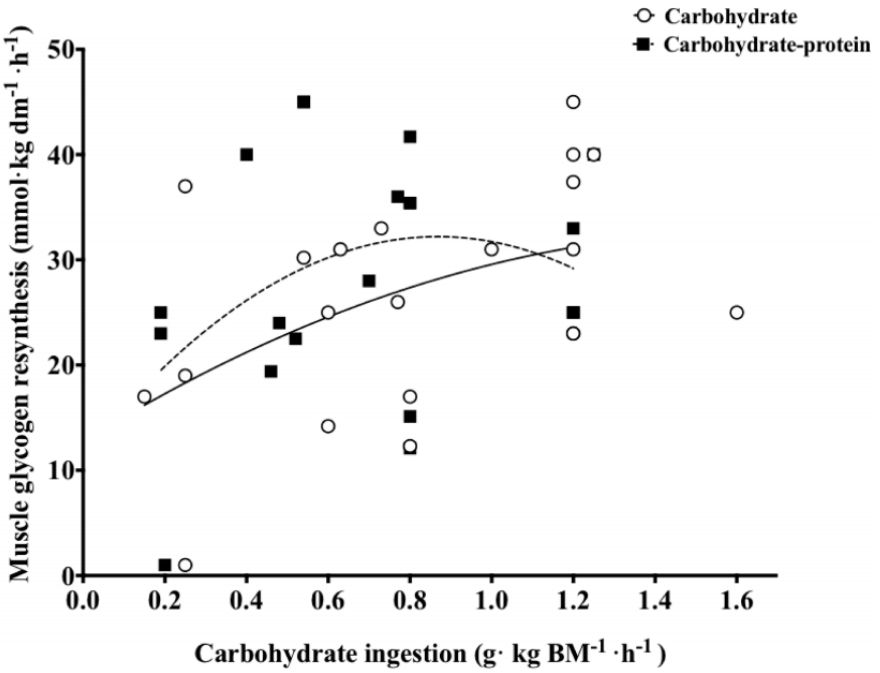

Figure 2.7. Reported rates of post-exercise (i.e. 2–6 h) muscle glycogen resynthesis with different amounts of ingested CHO (± dietary protein). The trend lines depict the suggested patterns of muscle glycogen resynthesis with each treatment; the solid and broken lines represent CHO ingestion and CHO co-ingested with protein, respectively. Figure reproduced from Alghannam*, et al.* [198].

2.6.3. Carbohydrate and Protein for Subsequent Athletic Performance

Collectively, the available evidence suggests that both CHO and protein are important nutrients to maximise recovery in the initial hours following exercise. However, a review of trials involving consecutive exercise sessions is needed to determine whether these nutrients can convey meaningful performance enhancements in a context where recovery time between exercise sessions is limited. Under these circumstances, it may not be possible to completely restore substrate losses or promote significant muscle-damage repair and attempting to do so may produce negative side-effects (e.g. GI problems) that hinder athletic performance.

2.7. Conclusion

2.7.1. Summary of the Literature Review

Water is multifunctional constituent of the human body that plays a role in virtually every biological process. Fluid consumption to balance losses is therefore essential to health and life. While homeostatic mechanisms are reasonably effective at maintaining TBW content within narrow limits (i.e. ±0.2 $L \cdot d^{-1}$), individuals participating in heavy physical exercise, particularly under warm–hot and/or humid

environmental conditions, often fail to adequately replenish sweat losses and develop significant hypohydration (i.e. ≥2% BM loss). Evidence suggests that mild to moderate hypohydration (i.e. ~1–5% BM loss) is sufficient to impair aerobic and anaerobic exercise performance (including muscular strength and endurance), as well as sport-specific technical skills. While a detrimental effect of hypohydration on mood and cognitive performance is less consistent, a decline in memory and perceptual discrimination has been demonstrated in some studies. Considering these findings, individuals are recommended to rehydrate between exercise sessions and avoid commencing a subsequent bout of activity in a hypohydrated state. Specifically, the ACSM and Academy of Nutrition and Dietetics advise individuals consume fluid in volumes equivalent to ~1.25–1.50 L·kg BM $lost^{-1}$ to promote rapid and complete recovery of TBW content. However, individuals preparing to undertake a subsequent bout of exercise may have limited time or opportunity between sessions to consume fluid. In this context, the impact of different fluid consumption practices is less clear.

Other nutrients often consumed in rehydration beverages (i.e. CHO and protein), might also assist to accelerate recovery in the initial hours after exercise. Indeed, CHO, particularly, if it is high GIx and consumed in reasonably large quantities (i.e. ~1.0–1.2 $g \cdot kg^{-1} \cdot h^{-1}$) immediately post-exercise, has been demonstrated to accelerate muscle glycogen storage. This is important, because glycogen depletion is a major cause of fatigue in both aerobic exercise and activities involving repeated bouts of anaerobic exercise. Dietary protein also has the potential to accelerate muscle damage repair (i.e. this is necessary for the restoration of optimal muscle function), as well as glycogen resynthesis, if CHO intake is sub-optimal (i.e. <1.0 $g \cdot kg^{-1} \cdot h^{-1}$). In both cases, however, a comprehensive review of trials involving consecutive exercise sessions is needed to determine whether these nutrients can enhance performance in a context where recovery time between exercise sessions is limited.

2.7.2. Thesis Part I Research Framework

The research framework of this thesis has been described in Chapter 1 (see *1.3 Research Aims*). Briefly, Thesis Part I addresses one research question and two main research aims. The research aims are as follows:

Aim 1: To explore the effect of fluid (water) consumption during or following dehydration on subsequent (i.e. ≤4 h recovery) athletic performance.

Aim 2: To explore the effect of consuming CHO and protein with water during or following exercise on subsequent (i.e. ≤4 h recovery) athletic performance.

The two research aims are met by two specific research studies presented in Chapters 3 & 4, respectively.

Chapter 3: The Effect of Fluid Intake During or Following Dehydration on Subsequent Athletic and Cognitive Performance: A Systematic Review and Meta-Analysis.

Reader's note:

This chapter includes a co-authored paper. The bibliographic details of the co-authored paper, including all authors are as follows:

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; 3(13).

* Please note that supplementary materials related to this research can be found at the Journal's webpage: https://sportsmedicine-open.springeropen.com.

The research candidate has made the following contributions to this study:

- Developed the study design and registered the research methodology
- Completed the literature search, quality assessment, data extraction, data synthesis and statistical analyses
- Prepared the manuscript for submission to a peer-reviewed journal
- Presented the research at an international conference

(Signed) _____ (Date: 20.02.2019)

Danielle McCartney

(Countersigned) _____ . (Date: 20.02.2019)

Supervisor: A/Prof Ben Desbrow

(Countersigned) _____ (Date: 20.02.2019)

Supervisor: Dr Christopher Irwin

3.1. Abstract

Background: The detrimental effects of dehydration and hypohydration on athletic and cognitive performance have been well documented. As such, individuals are advised to consume fluid in volumes equal to 1.25–1.50 L·kg BM lost^{-1} to promote rapid and complete restoration of TBW content. However, individuals preparing to perform further physically- or cognitively demanding activities may have limited time to consume fluid. Within this context, the impact of fluid intake practices is unclear. This systematic review explored the effect of fluid consumption during or following dehydration on subsequent athletic and cognitive performance. **Method:** PubMed (MEDLINE), Web of Science and Scopus databases were searched to identify studies that measured athletic performance (categorised as: *continuous*, *intermittent, resistance*, *sport-specific skills* and *balance* exercise) or cognition following dehydration of participants under control (no fluid or negligible fluid ≤200 mL) or intervention (fluid intake >200 mL) conditions. Both water and non-water beverages were eligible for inclusion, provided that macronutrient intake was matched on control and intervention trials. Random-effects meta-analyses and meta-regression analyses were conducted to evaluate intervention efficacy for continuous exercise performance. **Results:** 64 trials (*n*=643 participants, 93% male) derived from 42 publications were reviewed. Dehydration decreased BM by 1.3–4.2% and the fluid intake volume was equal to 0.4–1.55 L·kg BM lost^{-1}. Fluid consumption significantly improved continuous exercise performance (22 trials), Hedges' *g*=0.46, 95% CI's: 0.32, 0.61 (I^2=80.5). Differences in environmental temperature (*p*<0.001) and exercise duration (*p*=0.071) tended to influence the magnitude of the performance change, with fluid demonstrating greater efficacy when exercise was performed in hotter environments and over longer durations. The volume and timing of fluid consumption did not significantly influence the magnitude of this effect (*p*'s>0.05). Research investigating the effect of fluid intake on intermittent (10 trials), resistance (9 trials), sport-specific (6 trials) and balance (2 trials) exercise and on cognition (15 trials) was relatively limited, and a narrative synthesis of the available data failed to indicate a clear improvement. **Conclusion:** Fluid consumption during or following dehydration is likely to improve continuous exercise performance, even if the volume is inadequate for complete rehydration.

3.2. Introduction

The detrimental effect of hypohydration on athletic and cognitive performance have been extensively researched. Recent meta-analyses detected meaningful decrements in aerobic [56] and anaerobic [58] exercise performance and muscular strength and endurance [58] when participants commenced activity in an already dehydrated state. Experimental studies have also demonstrated motor-skill impairment on sport-specific exercise tests (e.g. cricket [78], basketball [76,82], golf [80], field hockey [79], surfing [81]) following fluid loss. While evidence indicating a detrimental effect of hypohydration on cognition is less consistent [199], a decline in memory, perceptual discrimination and mood have been documented in some studies [61]. Dehydration is common among athletes [200-203] and manual workers (e.g. military, fire fighters and labourers) [204], who rely on their physical and mental proficiencies to

compete and/or train at elite levels and remain productive in the workforce. This evidence has provided rationale for fluid replacement recommendations.

The ACSM [1] and the Academy of Nutrition and Dietetics [2] recommend that hypohydrated individuals consume fluid in volumes equivalent to 1.25 to 1.50 L·kg BM lost^{-1} to restore euhydration if the fluid deficit is large and recovery time is limited (i.e. <8 h). While the importance of returning to euhydration over a period of a day(s) is not in dispute, many individuals are required to undertake repeated bouts of activity, where limited time exists between tasks or the demands of a subsequent activity (i.e. type, duration and/or intensity) or the environment (e.g. conflict zone) may influence the utility of the aforementioned guidelines. Within this context, fluid intake has the potential to enhance or inhibit performance. Thus, determining rehydration strategies that counteract the detrimental effects of fluid loss, while optimising performance on subsequent tasks are important.

Ingesting large volumes of fluid may cause GI problems, impeding athletic performance. Particularly if the amount of time available to consume fluid is limited and/or fluids with higher calorie loads (e.g. milk/milk-based formulations) and hence slower rates of gastric emptying are ingested [146,147]. The nature of the subsequent activity, e.g. 'bouncing' action caused by running, may also impact GI tolerance [205]. Conversely, drinking large fluid volumes promotes rapid initial gastric emptying [146], facilitating fluid absorption, and may convey greater benefit than drinking smaller volumes. To date, relatively few studies have investigated the impact of fluid intake volume on subsequent athletic performance [148-152], and the majority of these have employed a prolonged (e.g. overnight) rehydration period [148-150], reducing the probability of GI disturbance affecting subsequent performance. Thus, the importance of ingested fluid volume and its impact on performance remains unclear. The aim of the present systematic review and meta-analysis was to explore the effect of fluid consumption during or following a period of dehydrating sweat loss on subsequent athletic and cognitive performance. Understanding how to maximise the benefits of fluid consumption under these circumstances will inform the development of future fluid replacement guidelines.

3.3. Methods

The methodology of this review was devised in accordance with specifications outlined in the *Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols PRISMA-P 2015 Statement* [206] and registered at the International Prospective Register of Systematic Reviews (PROSPERO) ahead of the formal study selection process (ID: CRD42016036560).

3.3.1. Literature Search

Potential research studies were identified by searching the online databases PubMed (MEDLINE), Web of Science (via Thomas Reuters) and Scopus from inception until April 2016 using the terms exercise, athletic, performance, mood and cognit* (the star-symbol was used to capture all words beginning with "cognit", e.g. cognitive, cognition, etc.) each in combination with "fluid replacement" (the enclosed quotation marks were used to search for an exact phrase), "fluid ingestion", "fluid intake", "fluid consumption", "fluid administration", rehydrat* and euhydrat*. Records that contained irrelevant terms (e.g. patient, rat, mouse, aged care, reaction, disease, illness, bacteria, children and elderly) were excluded from the literature search using the Boolean search operator 'NOT'. Two investigators (D.M. and C.I.) independently screened the potential research studies to identify relevant texts. Initially, all irrelevant titles were discarded. The remaining studies were then systematically screened for eligibility by abstract and full text, respectively. The final decision to include or discard research studies was made between two investigators (D.M. and C.I.), with any disagreement resolved in consultation with a third investigator (B.D.). The reference lists of all included studies were then hand searched for missing publications. Full details of the screening process are presented in Figure 3.1.

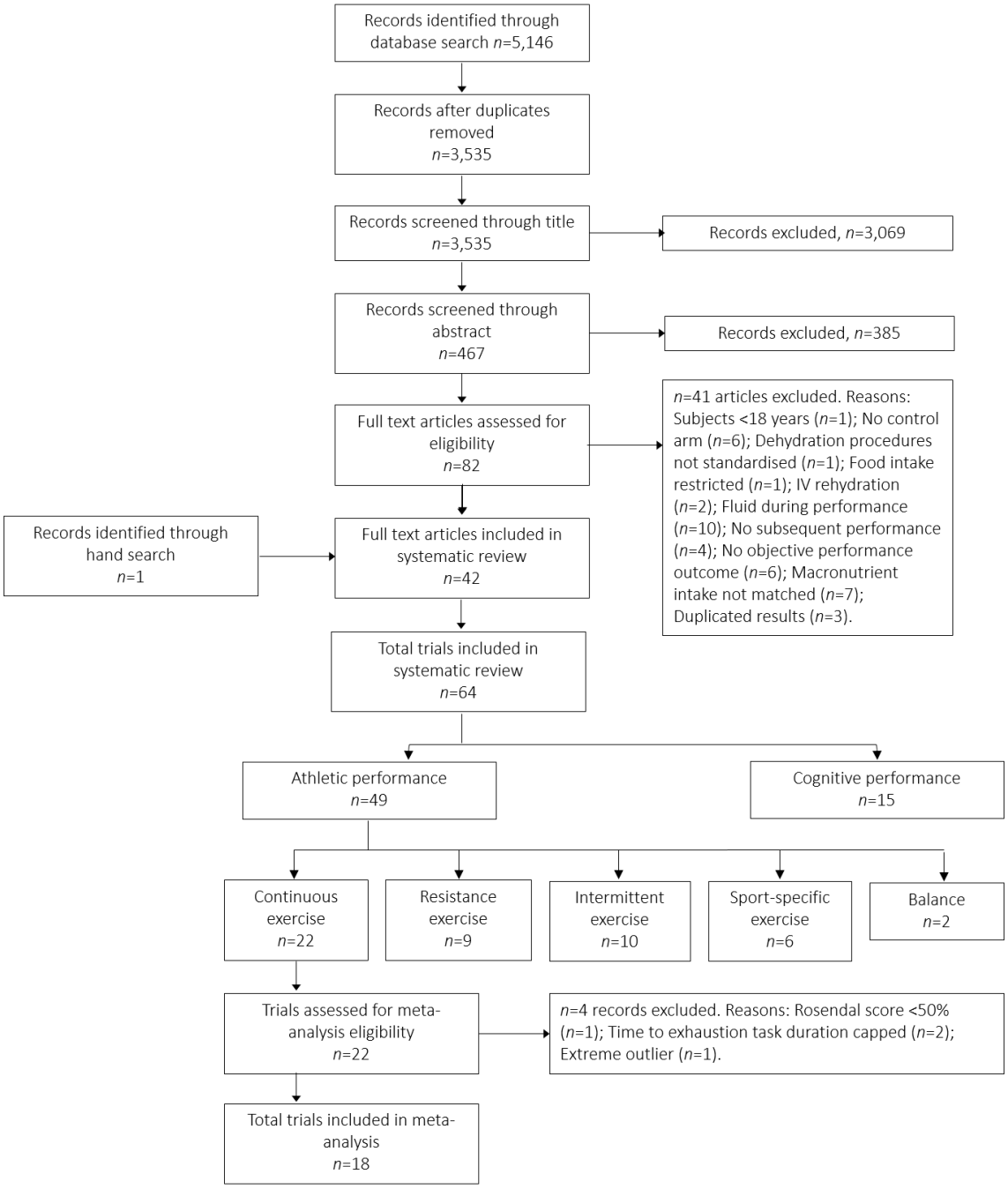

Figure. 3.1. PRISMA Flow Chart (study selection methodology). Where a study contained >1 intervention-arm that was eligible for inclusion (i.e. paired against a suitable control condition), these were treated as separate 'studies' termed *trials*.

3.3.2. Inclusion and Exclusion Criteria

Research studies that fulfilled the following criteria were eligible for inclusion:

1) Controlled trials (random or non-random participant allocation) employing repeated-measures experimental designs;
2) Human studies on adult (≥18 years of age) male or female participants with no known medical conditions or co-morbidities;

3) Athletic or cognitive performance (see *Primary and Secondary Research Outcomes* below for full description) was measured under control and intervention conditions. The control condition was dehydration with no fluid or negligible fluid intake, where 'negligible' fluid intake was accepted as ≤200 mL. This threshold was intended to increase data capture and statistical power, since this was the first systematic review to examine the effect of fluid consumption following dehydration on subsequent athletic and cognitive performance. The intervention condition was dehydration with concurrent and/or subsequent fluid intake >200mL;
4) The mode of dehydration was standardised; in other words, participants were subjected to the same dehydration protocol with or without fluid consumption on control and intervention trials);
5) Hydration status was manipulated prior to the performance test; in other words, dehydration and fluid ingestion occurred prior to, not during, the performance assessment. A schematic representation of this experimental protocol is displayed in Figure 3.2;
6) There was "limited" time or opportunity to consume fluid, defined as: ≤4 h between the conclusion of the dehydration protocol and the subsequent performance test, unless the performance an overnight rest (i.e. where sleep reduced the opportunity for fluid consumption) (Figure 3.2);
7) An objective measurement of hydration status (e.g. BM, U_{SG}, U_{OSM}, P_{OSM} or PV) was used to indicate the level of dehydration attained;
8) Accessible full text articles written in English.

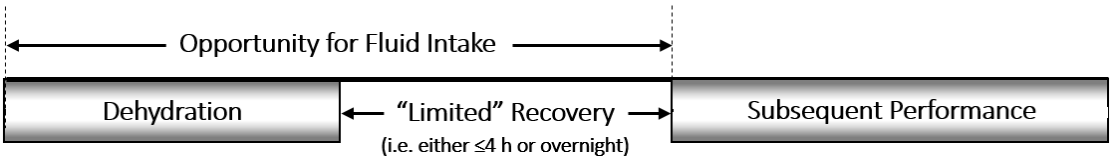

Figure. 3.2. A schematic representation of the experimental protocol employed in studies eligible for inclusion in the present review.

Studies were excluded if: (1) dehydration involved restriction of food intake; (2) fluids were not administered orally (e.g. intravenous infusions), or (3) were co-administered with another experimental treatment (e.g. glycerol, L-alanyl-L-glutamine,

external cooling); (4) participants consumed >200 mL of fluid or an unspecified volume of fluid on the control trial (e.g. Bardis*, et al.* [207] and Baker*, et al.* [208]); (5) macronutrient intake was not matched on control and intervention trials; or (6) performance data were not adequately reported (i.e. values were not quantified, descriptive terms were not used or appropriate intervention-control comparisons were not conducted). It should be noted that both water and non-water beverages were eligible for inclusion, provided that macronutrient intake was matched on control and intervention trials.

For the purpose of this systematic review, research studies containing multiple intervention-arms that were eligible for inclusion (i.e. each paired against a suitable 'no fluid' control) were treated as separate experimental 'studies' termed '*trials*' (e.g. McConell*, et al.* [152] investigated performance under two different fluid conditions; Hillman*, et al.* [92] investigated performance under different environmental conditions). Separate trials derived from a single study are identifiable by the addition of letters (e.g. a–d) to the citation.

3.3.3. Methodological Quality Assessment

All eligible studies were examined for publication bias using the Rosendal Scale [209]. Excellent methodological quality is indicated by a Rosendale Score ≥60% [210]. Items 7, 8 and 9 of the scale, which related to the use of blinding procedures, were omitted from the evaluation as oral fluid ingestion cannot be blinded. Scoring was determined by dividing the number of 'yes' responses by the total number of applicable items. Studies were ineligible for meta-analysis if they received a Rosendal score <50%.

3.3.4. Data Extraction and Synthesis

Data were extracted from relevant publications following the Cochrane Handbook for Systematic Reviews of Interventions *Checklist of Items to Consider in Data Collection or Data Extraction* [211] and entered into a Microsoft Excel spread sheet.

3.3.4a. Primary and Secondary Research Outcomes

Primary research outcomes were: 1) objective indicators of athletic performance; subjective measurements of performance (e.g. RPE) were not examined in this review.

The types of athletic performances studied were broadly classified as follows: (a) *continuous exercise*; (b) *intermittent exercise*; (c) *resistance exercise*; (d) *sport-specific exercise*; and (e) *balance tasks*. Performances that involved a coordinated motor-movement (i.e. resembling some skill involved in a particular sporting event) were categorised as "sport-specific" exercises, whereas non-specific sporting activities (e.g. sprint running) were sorted into one of the remaining groups (where appropriate). If more than one "type" of athletic performance was measured in a single study (e.g. Walsh*, et al.* [212] examined continuous and resistance exercise performance), the performances were presented in their respective categories and treated as separate trials; and 2) objective indicators of cognitive performance, including subjective measurements of mood state. The decision to include mood as a primary research outcome was based on previous suggestions that mood and symptom questionnaires may be more sensitive to subtle changes in hydration status than tests of cognitive ability [90]. Subjective ratings of GI discomfort and thirst following fluid consumption were intended as the secondary research outcomes. However, very few investigations evaluated GI tolerance [152] or thirst [130,213,214]. Thus, insufficient data were available to complete secondary analyses.

3.3.4b. Other Relevant Data

Other information extracted from relevant research studies included: (1) participant characteristics (age, euhydrated BM and VO_{2max}); (2) the dehydration protocol (mode of dehydration, temperature and RH, protocol duration, level of dehydration); (3) the rehydration protocol (fluid type and volume, drink time and time from finishing fluid consumption to commencing performance task); and (4) the performance task (task description and performance outcomes, temperature, RH, airflow, intensity and duration).

The percentage of BM loss was used to indicate the level of dehydration at the onset of performance and the fluid intake volume was expressed as a percentage of BM loss (i.e. L·kg BM $lost^{-1}$). If the volume of fluid consumed was unknown, the BM deficit post-rehydration was reported.

Time from completing the dehydration protocol to commencing the subsequent performance task (*recovery time*) and time from commencing fluid ingestion to commencing the subsequent performance task (*fluid assimilation time*) were

approximated from the experimental protocol, where adequate information was provided. If the necessary information was not reported in the article and it was published within the previous 10 years, authors were contacted via email with a request to provide missing data.

3.3.5 Statistical Analyses

Sufficient data were available to perform a meta-analysis examining the impact of fluid consumption during or following dehydration on subsequent continuous exercise performance. Meta-analyses were not performed on other types of athletic or cognitive performance because: (1) intermittent and sport-specific exercise performance trials were methodologically heterogeneous, particularly in regards to the exercise protocol and outcomes used; (2) few authors responded to an email request for raw data regarding resistance exercise performance (i.e. preventing computation of a correlation coefficient); and (3) cognitive performance data was rarely quantified (i.e. descriptive terms only).

3.3.5a. Meta-Analysis on Continuous Exercise Performance

All statistical procedures were performed using IBM SPSS Statistical Software, Version 22.0 and Comprehensive Meta-Analysis, Version 3.0. Repeated-measures intervention effect sizes were calculated as Hedges' *g* [215], where the mean difference between each intervention and control performance score was standardised against the standard deviation (SD) of the performance change and corrected for bias due to small sample size. The magnitude of effect was defined in accordance with Cohen [216]: Hedges' *g* ≤0.2 = small; 0.2–0.5 = medium; and ≥0.8 = large, where a positive value indicates a beneficial effect of fluid intake on continuous exercise performance. Where the SD of the performance change was not reported, the missing value was imputed using a correlation coefficient [211] calculated using the following formula:

$$SD_{\Delta} = \sqrt{(SD^2_{\text{No Fluid}} + SD^2_{\text{Fluid}}) - (2 \times R \times SD_{\text{No Fluid}} \times SD_{\text{Fluid}})}$$

Where SD_{Δ} is the missing standard deviation of change and R is the correlation coefficient. R was approximated as the mean correlation coefficient (R=0.84) calculated

using raw performance data from nine continuous exercise trials (derived from four separate publications). Sensitivity analysis was performed using *R*=0.50, 0.74 and 0.94 to test the robustness of the imputed value. The weighted mean treatment effect was calculated using random-effect models, where trials were weighted by the inverse variance for the standardised performance change. Statistical significance was attained if the 95% CI did not include zero. Data are described as Mean±SD, unless otherwise indicated; articles that reported the standard error of the mean (SEM) had their values multiplied by the square root of the sample size to convert to SD.

3.3.5b. Heterogeneity and Sensitivity Analyses

Heterogeneity was assessed using Cochran's Q and the I^2 index. Low, moderate and high heterogeneity was indicated by an I^2 value of 25, 50 and 75%, respectively [217]. A *p*-value <0.10 for Cochran's Q was used to indicate significant heterogeneity [211]. Sensitivity analyses were performed by removing individual trials and examining the effect of each study on the results of the weighted mean treatment effect.

3.3.5c. Meta-Regression Analysis

The research candidate identified the fluid intake volume (i.e. L· kg B M $lost^{-1}$) and fluid assimilation time as variables that might moderate the effect of fluid intake on athletic performance. However, environmental temperature, exercise duration, the ecological validity of the exercise protocol and the level of fluid loss (i.e. % BM loss) incurred may influence the effect of dehydration on athletic performance [55,57,91,92,218]. Therefore, the relationship between these variables and the magnitude of the treatment effect (Hedges' *g*) was investigated using a restricted maximum likelihood (RML) multiple meta-regression (random effects) model that controlled for potential confounders. RML simple meta-regression was also performed to explore the influence of environmental temperature on Hedges' *g* values. The ecologically validity of each continuous exercise protocol was defined as per Goulet [57], where fixed-power TTE protocols were considered "non-ecologically valid" and TT protocols were considered "ecologically valid". Exercise duration was taken as the mean exercise time (min) on control and intervention trials. One study did not report total exercise time [92], therefore exercise duration was approximated as per Stewart*, et al.*

[130], who performed a comparable performance test. As per Savoie*, et al.* [58], regression analyses were examined for influential cases and outliers (i.e. studentized residuals, Cook's distance and centred leverage values), normality of residuals (i.e. Shapiro-Wilk Test) and multicollinearity (i.e. the variance inflation factor [VIF]). Statistical significance was accepted as $p<0.05$.

3.3.5d. Systematic Review

All athletic and cognitive performances are presented in the systematic review investigating the effect of dehydration and fluid intake on subsequent athletic and cognitive performance. While it was our intention to calculate within-subject intervention effect sizes for all athletic performance outcomes, the majority of the publications reviewed did not provide the necessary data to complete a paired analysis. Further, the types of performances investigated varied widely among studies, such that the missing SD of change could not be reliably estimated from a known correlation coefficient. To enable comparison of effects across studies, effect sizes were approximated as Hedges' *g* for *independent* groups. The mean difference between each intervention and control performance score was standardised against a pooled SD and corrected for bias due to small sample size using the supplementary spreadsheet by Lakens [219]. This approach will likely underestimate the magnitude of the true effect. Cognitive performance outcomes are presented in descriptive terms only, since few publications quantified the effect of fluid intake numerically. Statistical significance was accepted as $p<0.05$ in all studies.

3.4. Results

3.4.1. Overview of Studies and Study Quality

Sixty-four repeated measures trials (n=643 participants, 93% male, excluding Del Coso*, et al.* [220] where sex was not specified, NS) derived from 42 original publications were included in the present systematic review. Methodological quality assessment yielded a median Rosendale Score of 58%. Two trials received a Rosendale Score <50% [86,221]. While these studies are presented in the systematic review, they were not deemed eligible for inclusion in the subsequent meta-analysis. The highest Rosendal

Score of 83% was calculated for Rodrigues*, et al.* [222]. Complete results of the quality assessment are published in the online supplementary material.

3.4.2. Study Characteristics

Characteristics of included studies are summarised in Table 3.1 and Tables 3.3–3.6 (full details are presented in the online supplementary material). Dehydration and rehydration protocols were heterogeneous. In 58 out of the 64 trials reviewed, dehydration was accomplished via passive heat exposure (*n*=11) [96,125,218,221,223-227] or physical exercise (*n*=47), either conducted in a thermoneutral laboratory ≤25°C (*n*=16)[a], heated chamber (*n*=28)[b] or environmental conditions NS (*n*=3) [240,241]. The remaining trials reduced body water content via water immersion (*n*=2) [242,243] or moderate-intensity exercise in combination with ~24 h dietary fluid restriction (*n*=2) [244,245]. In 46 trials (74%)[c], dehydration produced BM losses ≥2%. Of these, 27 trials (43%) dehydrated participants by ≥3.0% of their initial BM[d]. Mean BM losses ranged from 1.3 [240] to 4.2% [91]. Ingested fluids were predominantly water or saline; two included studies failed to indicate the type of fluid consumed [218,227]. Studies administering CHO-containing fluids were often excluded due to unequal provision of macronutrients on control and intervention trials. Except for Maxwell*, et al.* [148] (i.e. in which sleep reduced the opportunity for fluid consumption), all of the studies allowed ≤4 h between the conclusion of the dehydration protocol and the subsequent performance test. The volume of fluid administered ranged from 0.40 [240] to 1.55 L·kg BM lost^{-1} [235]. In 20 trials (33%), participants ingested a volume of fluid <100% of BM losses[e]. Only two trials [148,235] provided a volume of fluid that complied with current recommendations for restoring fluid loss (1.25–1.50L·kg BM lost^{-1}) [1,2]. In 14 trials, participants ingested a small volume of non-nutritive fluid (≤200mL)[f] or mouth-rinse [212] on the control trial.

[a] [86,88,92,151,152,213,226,228-231]

[b] [77,87,91,92,130,148,212,214,220,222,232-239]

[c] [77,88,91,92,125,130,148,152,214,218,220-229,231-233,236,237,241-245]

[d] [91,92,125,130,148,151,218,220,223,225,227,231,232,236,237,242-245]

[e] [77,87,88,151,152,212,213,221,228,229,232,239,240,244,245]

[f] [77,218,226,227,232,233,236]

3.4.3. Athletic Performance

3.4.3a. Continuous Exercise Performance

Twenty-two trials (*n*=170 participants, 98% male) measured the effect of fluid consumption on continuous exercise performance (Table 3.1). The majority of testing was completed on trained individuals (mean $VO_{2\ max}$: 57.5–68.4 $mL \cdot kg^{-1} \cdot min^{-1}$) (*n*=13 trials)[a]. In *n*=11 trials, exercise was performed in a warm or hot environment (30–40°C) by acclimated (*n*=2) [233,245] and unacclimated (*n*=5) [92,214,221,232,244] participants, where environmental adaptation was specified. The remaining trials were completed under thermoneutral (18–25°C)[b] (*n*=7) or cold (2–10°C) [91,218] (*n*=2) conditions, where environmental temperature was specified. Fluid consumption significantly improved continuous exercise performance in 13 of the trials reviewed.

3.4.3b Meta-Analyses and Meta-Regression Analyses

Eighteen trials (*n*=139 participants, 97% male) were included in the meta-analysis examining the effect of fluid consumption on continuous exercise performance. Four continuous exercise trials included in the review were omitted from the meta-analysis because: (1) duration of the TTE performance test was capped (*n*=2) [214,232]; (2) Rosendal score <50% (*n*=1) [221]; and (3) extreme outlier, exceeding the average effect estimate by >3 SD with a studentized residual of 2.82 (*n*=1) [234], with the results possibly confounded by fatigue. In this study [234], untrained participants completed 1 h of exercise at 32°C before commencing a TTE test at 80% $VO_{2\ max}$, without any recovery. All other investigations completed on participants with a $VO_{2\ max}$ <50.0 $mL \cdot kg^{-1} \cdot min^{-1}$ (i.e. untrained or physically active individuals) employed passive methods of dehydration or allocated ~2 h recovery after active dehydration [91,218]. Excluding this trial did not affect the result of the meta-analysis (Hedges' g = 0.48, 95% CI's: 0.33, 0.63).

[a] [92,151,152,212,214,221,223,232,233,244,245]

[b] [91,92,130,151,152,218]

Table 3.1. Characteristics of studies evaluating the effect of fluid consumption on continuous exercise performance (studies are presented by order of effect)

Citation	Participants	$VO_{2\,max}$	DH protocol (exercise intensity); temperature; duration	Total REC (min)	DH trial BM loss (%)	Fluid type	Fluid assimilation time (min)	Fluid intake ($L \cdot kg^{-1}$ BM lost)	Duration (min)	Performance test	Performance temperature; RH; airflow	Hedges' *g*
Kenefick et al. (2010a) [91]	8 M	43.6 ± 4.1	EX; 50°C; 3 h W/R	120	4.1	Saline	300	1.05	15	Cycle, TT (15 min)	10°C	0.05
Chevront et al. (2005a) [218]	8 (6M), physically active	48 ± 9	HT	210	3.0	NS	390	1.00	30	Cycle, TT (30 min)	2°C; 50%; 2.2m/s	0.11
Stewart et al. (2014) [130]	7 M, recreational cyclists	52.7 ± 7.9	EX (H); 37°C; 120 min	120	3.8	Water	120	1.15	7.2	Cycle, TT (5 km)	18-25°C; 20-30%	0.12
McConell et al. (1999a) [152]	8 M, well-trained cyclists	63.8 ± 1.2	EX (V); 21°C; 45 min	0	1.9	Water	45	1.00	15	Cycle, TT (15 min)	21°C; 41%; fan	0.15
McConell et al. (1999b) [152]	8 M, well-trained cyclists	63.8 ± 1.2	EX (V); 21°C; 45 min	0	1.9	Water	45	0.50	15	Cycle, TT (15 min)	21°C; 41%; fan	0.24
McConell et al. (1997a) [151]	7 M, well-trained cyclists	68.4± 2.5	EX (V); 21°C, 120 min	0	3.2	Water	120	0.50	3.5	Cycle, TTE (90% VO_{2maz})	21°C, 43%; fan	0.24
Kenefick et al. (2006) [214]	8 M, unacclimated	63.7 ± 10.2	EX (M); 36°C; 75 min	30	2.3	Saline + NNS	20	1.00	55	Run, TTE (49% VO_{2max})	37°C; 42%	0.28*
Hillman et al. (2011a) [92]	7 M, unacclimated, cyclists	NS	EX (V); 34°C; 90 min	15	3.0	Water	105	1.00	7.2	Cycle, TT (5 km)	23°C	0.32
Chevront et al. (2005b) [218]	8 (6M) physically active	48 ± 9	HT	210	2.9	NS	390	1.00	30	Cycle, TT (30 min)	20°C; 50%; 1m/s	0.35*
Paik et al. (2009) [223]	10 M, moderately active	53.6 ± 11.4	HT	120	3.0	Water	120	1.00	30	Run, TTE (80% VO_{2max})	NS	0.43
McConell et al. (1997b) [151]	7 M, well-trained cyclists	68.4± 2.5	EX (V); 21°C, 120 min	0	3.2	Water	120	1.00	4.2	Cycle, TTE (90% VO_{2maz})	21°C, 43%; fan	0.58*
Hillman et al. (2011b) [92]	7 M, unacclimated, cyclists	NS	EX (V); 34°C; 90 min	15	3.8	Water	105	1.00	7.2	Cycle, TT (5 km)	34°C	0.60*
Kenefick et al. (2010c) [91]	8 M	46.3 ± 5.2	EX; 50°C; 3 h W/R	120	4.0	Saline	300	1.05	15	Cycle, TT (15 min)	30°C	0.64*
Castellani et al. (1997) [232]	8 M, unacclimated	57.9 ± 4.5	EX (M); 33°C; 180 min	135	4.1	Saline + NNS	120	0.50	72	Walk, TTE (54% VO_{2max})	36°C; 47%; 2.3m/s	0.68*
Walsh et al. (1994a) [212]	6 M, endurance cyclists	61.4 ± 4.4	EX (V); 30°C; 60 min	0	1.8	Saline + NNS	50	0.90	8.2	Cycle, TTE (90% VO_{2maz})	30°C; 60%; 0.8m/s	0.74*
Kenefick et al. (2010b) [91]	8 M	45.3 ± 4.6	EX; 50°C; 3 h W/R	120	4.2	Saline	300	1.05	15	Cycle, TT (15 min)	20°C	0.79
Kavouras et al. (2006) [245]	8 M, acclimated cyclists	61.4±2.3	FR + EX (M); 120 min	0[a]	3.9	Water + NNS	80	0.75	23	Cycle, TTE (74% VO_{2max})	37°C; 48%; 2.54m/s	0.81*
Kenefick et al. (2010d) [91]	8 M	43.7 ± 7.0	EX; 50°C; 3 h W/R	120	4.1	Saline	300	1.05	15	Cycle, TT (15 min)	40°C	0.87*
Below et al. (1994) [233]	8 M, acclimated, trained	62.9 ± 2.8	EX (V); 31°C; 50 min	0	2.0	Saline + NNS	50	1.00	11	Cycle, TT[b]	31°C; 54%	0.89*
Casa et al. (2000) [244]	8 M, unacclimated cyclists	61.4 ± 2.3	FR + EX (M); 120 min	0[a]	3.9	Saline + NNS	35	0.50	27	Cycle, TTE (74% VO_{2max})	37°C; 2.3m/s	1.25*
Melin et al. (1994) [221]	6 M, unacclimated, trained	57.5 ± 4.2	HT	60	2.6	Water	NS	0.50	97	March, TTE (50% VO_{2max})	35°C; 20-30%; 0.8m/s	1.23*
Hasegawa et al. (2006) [234]	9 M, untrained	48.5 ± 4.5	EX (M); 32°C; 60 min	4	1.6	Water	65	1.00	4.4	Cycle, TTE (80% VO_{2max})	32°C; 80%	4.01*

DH: Dehydration; EX: Exercise; FR: Fluid restriction; HT: Heat; NNS: Non-nutritive sweetener; NS: Not specified; RH: Relative humidity; Total REC: Time from completing the dehydration to commencing the subsequent task; TT: Time trial; TTE: Time to exhaustion; TW: Total work; W/R: Work rest cycle. Exercise intensity is described as high (H), vigorous (V) or moderate (M), in accordance with classifications outlined by Norton*, et al.* [246]. Values are Hedges' *g* effect sizes. *Significant difference between performances undertaken with and without fluid replacement (p<0.05).

[a] Dehydrating exercise completed 24 h prior to the performance test; however, dietary fluid restriction applied up to the onset of the performance test

[b]Target work (J) = work completed during a 10 min period when the subject could maintain an intensity eliciting a VO_2 of 10% above the LT.

The weighted mean treatment effect suggests that fluid intake during or following dehydration significantly improves continuous exercise performance (Hedges' *g*=0.46, 95% CI's: 0.32, 0.61) (Figure 3.3). However, high heterogeneity was observed across trials (I^2=80.5, *p* <0.010). Subsequent analyses (see below) determined that 82% of the between-trial variation could be attributed to differences in the environmental temperature at which the exercise was performed. Thus, sensitivity analyses were completed with trials grouped by environmental temperature (≤25°C or >25°C). The magnitude and significance of the intervention effect was stable during sensitivity analysis where trials were sequentially removed, with Hedges' *g* ranging between 0.29–0.35 and 0.70–0.81 for ≤25°C and >25°C subgroups, respectively (CI's did not include zero). Findings were also comparable across different levels of correlation (i.e. *R*=0.50, 0.74, 0.84 and 0.94), suggesting that the meta-analysis was robust to the imputed correlation coefficient *R*=0.84 (see online supplementary material for full results).

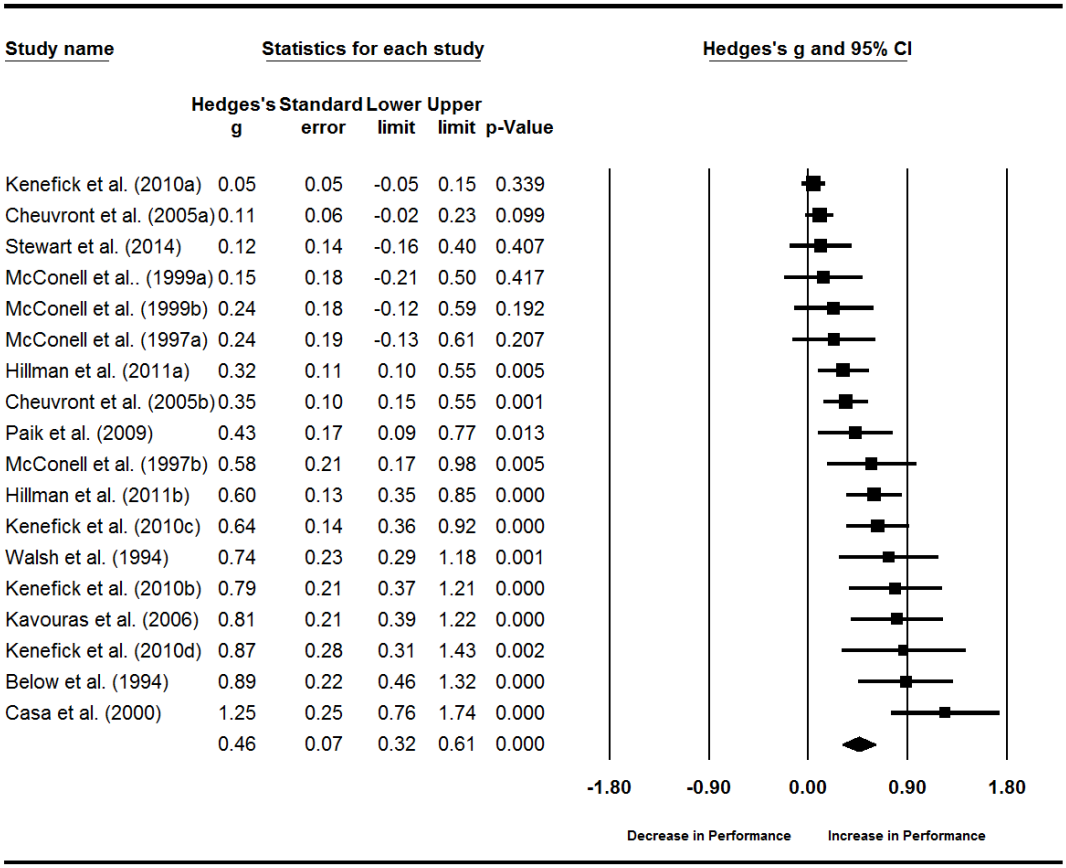

Figure. 3.3. Forest plot displaying the effect of fluid intake on continuous exercise performance (Hedges' *g*). The size of each square is proportional to the weight of the study.

One continuous exercise trial [223] was excluded from the simple meta-regression analysis to determine the relationship between changes in environmental temperature and the magnitude of the weighted mean effect after failing to report temperature at which exercise was performed. Analysis of the remaining 17 trials (*n*=129 participants, 97% male) indicated a strong significant correlation (R^2=0.82, *p*<0.001) (Figure 3.4), suggesting that fluid intake may enhance continuous exercise performance to a greater extent at higher environmental temperatures.

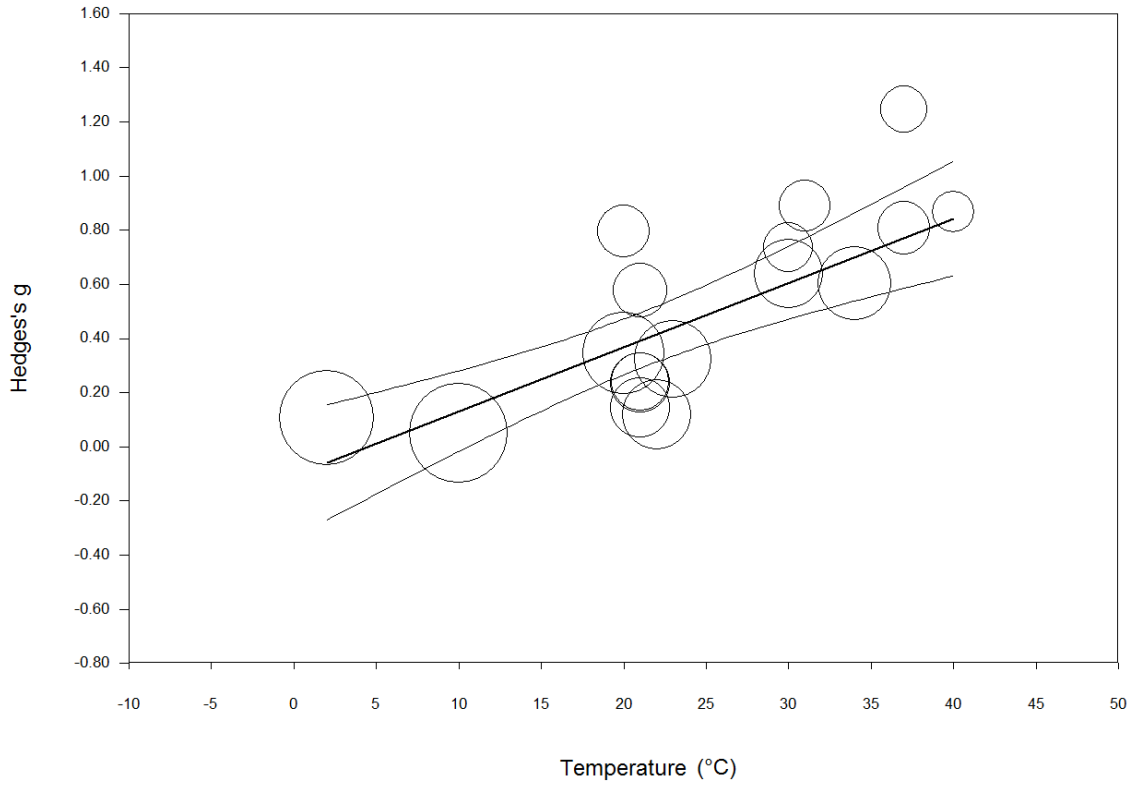

Figure. 3.4. Correlation between change in environmental temperature and continuous exercise performance (Hedges' *g* and 95% CIs) (R^2=0.82, *p* <0.001). Circle diameter corresponds to the weight of each study.

The influence of temperature, exercise duration, exercise protocol ecological validity and the level of dehydration were controlled in the modelling of the relationship between the volume of fluid consumed (i.e. L·kg BM $lost^{-1}$) and the weighted mean treatment effect (Figure 3.5; Table 3.2). The volume of fluid ingested ranged between 0.50–1.15 L·kg BM $lost^{-1}$. No correlation was observed between these parameters (*p*=0.625) (Table 4.7). Mean exercise duration ranged between 4–30 min (since no trials involving an exercise task >30 min were eligible for inclusion). There was a trend for fluid consumption to improve performance to a greater extent with increasing exercise

duration (p=0.071). The majority of trials (n=12) measured continuous exercise performance on an ecologically valid exercise protocol, e.g. total work completed within a fixed timeframe (n=10) [91,92,152,218] or time to complete a fixed distance (n=2) [130,233]. The remaining trials (n=5) employed a TTE protocol with lower ecological validity [151,212,244,245]. No significant correlation was observed between the ecological validity of exercise protocol and the magnitude of the weighted mean effect (p=0.188) or the level of dehydration and the magnitude of the weighted mean effect (p=0.845).

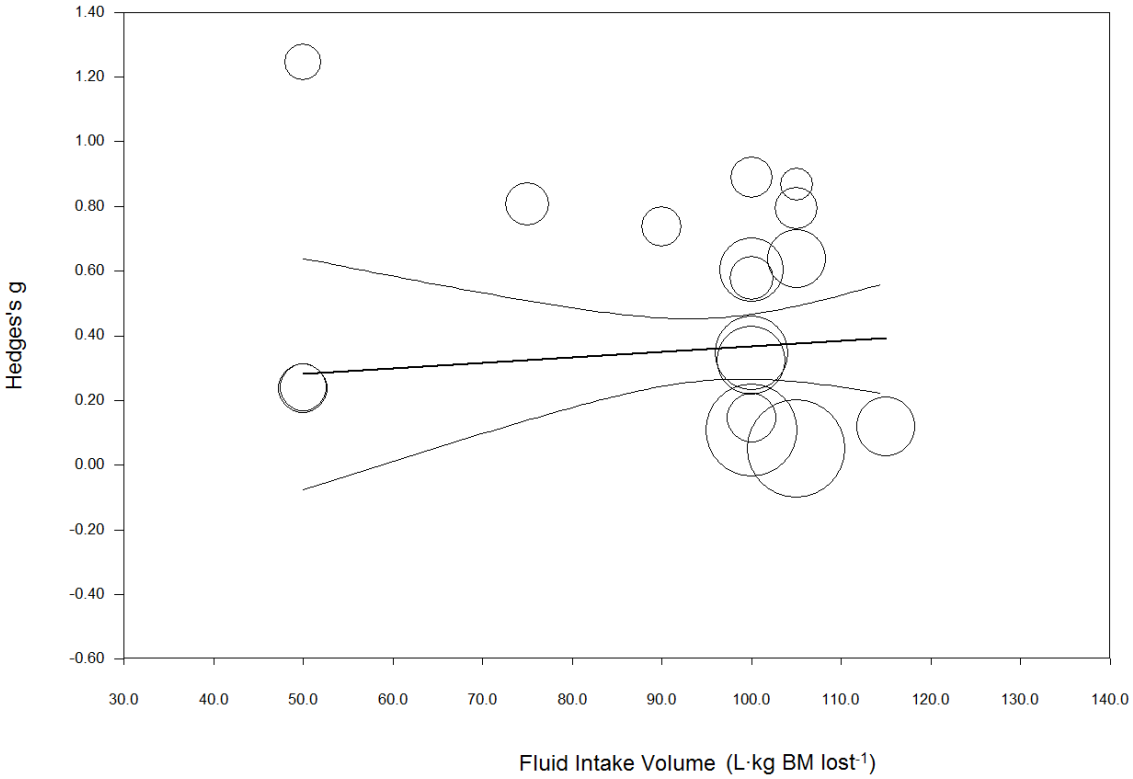

Figure 3.5. Correlation between change in fluid intake and continuous exercise performance (Hedges' *g* and 95% CIs) controlling for environmental temperature, exercise duration, level of dehydration and the ecological validity of the exercise protocol (p=0.625). Circle diameter corresponds to the weight of each study.

Table 3.2. Summary of moderator variables for the meta-regression analysis of the effect of fluid volume on the magnitude of the weighted mean treatment effect

Covariate	Coefficient (95% CI's)	*p* value	R^2
Fluid volume	0.002 (-0.006, 0.009)	0.625	
Temperature	0.025 (0.015, 0.036)	<0.001	
Exercise duration	0.011 (-0.001, 0.023)	0.071	0.91
Ecological validity	0.218 (-0.124, 0.561)	0.188	
Level of dehydration	0.013 (-0.126, 0.151)	0.845	

One trial [244] failed to report time from commencing fluid consumption to beginning the subsequent performance task and was excluded from the multiple regression analysis examining the relationship between fluid assimilation time and the weighted mean treatment effect. Modelling of this relationship controlled for the influence of environmental temperature and type of exercise protocol. Exercise duration was omitted from the model due to collinearity with fluid assimilation time (VIF=3.18, where all other analyses yielded VIFs ≤1.7). Fluid assimilation time ranged between 45–390 min. Analyses of the 16 eligible trials (*n*=121 participants, 94% male) did not indicate a significant effect of fluid assimilation time on the mean treatment effect (*p*=0.110).

3.4.3c. Intermittent Exercise

Ten trials (*n*=95 male participants) investigated intermittent exercise performance (Table 3.3). Exercise was undertaken in hot (32–36°C) [148,235], thermoneutral (19–22°C) [224,229] and cold (16°C) [77] conditions, where environmental temperature was specified. The majority of testing was completed using team sport participants (i.e. individuals who are accustomed to intermittent exercise) [77,88,148,229]. Participants in the remaining trials were untrained [235], physically active [224] or endurance cyclists [212], where the population was defined. Fluid intake (0.8–1.55L·kg BM lost^{-1}) significantly improved intermittent exercise performance on 4 trials [77,88,148,235]. The magnitude of improvement ranged from small to large (Hedges *g*=0.19–0.97).

3.4.3d. Resistance Exercise

Nine trials (*n*=83 male participants) evaluated resistance exercise performance (Table 3.4). Across the 8 trials reviewed, 22 separate performance tests were identified. The majority were knee extension or elbow flexion exercise tasks, at variable intensities (*n*=18 tasks) [125,220,222,225,228,236], although 2 trials measured performance via repetition lifts [96,242]. Individuals who were accustomed to performing resistance exercise were rarely studied [96,242]. Fluid intake (1.0–1.10 L· kg BM lost^{-1}) significantly improved performance on 7 of 22 resistance exercise tasks completed across 5 trials (Hedges' *g*=0.22–5.57) [247], and impaired performance on 1 task [236].

3.4.3e. Sport-Specific Exercise

Six trials (*n*=64 participants, 84% male) evaluated performance on exercise tasks that were specific to either cricket (*n*=1) [77], soccer (*n*=3) [213,229], squash (*n*=1) [240] or racehorse riding (*n*=1) [230] (Table 3.5). All participants were experienced on the sporting activity for which they were assessed. Fluid intake had no effect on soccer-specific ball-skills (e.g. passing and shooting) [229]. However, squash-specific movements, cricket bowling accuracy and racehorse riding indicated moderate to large performance improvements (Hedges' *g*=0.55–1.46) with fluid consumption (0.4–1.0 L·kg BM $lost^{-1}$).

3.4.3f. Balance Exercise

Two trials (*n*=49 males) investigated balance performance (Table 3.6). A significant positive effect of fluid intake was documented for 1 out of 8 balance tests completed across both trials.

Table 3.3. Characteristics of studies evaluating the effect of fluid consumption on intermittent exercise tasks

Citation	Participants	$VO_{2\,max}$	DH protocol (exercise intensity); temperature; duration	Total REC (min)	DH trial BM loss (%)	Fluid type	Fluid assimilation time (min)	Fluid intake ($L \cdot kg^{-1}$ BM lost)	Performance Test	Performance outcomes(s)	Performance temperature; RH	Hedges' *g*
Walsh et al. (1994b) [212]	6 M, endurance cyclists	61.4 ± 4.4	EX (V); 30°C; 60 min	15	1.8	Saline + NNS	65	0.90	IST[a]	Max. velocity; Lower limb force	NS	No effect
Maxwell et al. (1999) [235]	11 M, untrained	NS	EX (M); 32°C; 48 min	120	1.5	Saline + NNS	208	1.55	MART[b]	Sprint duration	32°C; 73%	↑ (0.19)
Devlin et al. (2001a) [77]	7 M, sub-elite cricketers	56 ± 6	EX; 28°C; 60 min	0	2.8	Water	60	0.80	MMRT[c]	20 m shuttle runs	16°C; 60%	↑ (0.30)
Cheuvont et al. (2006) [224]	8 M, physically active	52 ± 6	HT	Testing at 0, 30 & 60	2.7	Water	185–240	1.00	15 s WAnT	MPO; PPO; Rate of fatigue	22°C; 65%	No effect
Edwards et al. (2007a) [88]	11 M, soccer players	50.9 ± 4.0	EX; 19-25°C; 90 min	0	2.4	Water	90	0.80	Yo-Yo Test [d]	Distance covered	NS	↑ (ES unknown)
Maxwell et al. (2009a) [148]	8 M, unacclimated, game players	59.9 ± 8.0	EX (H/M); 36°C; 90 min	Overnight	3.9	Water	>12 h	1.50	IST[e]	TW; PPO (During RSB 1 & 2)	36°C; 49%	TW (0.97) & PPO (0.79) ↑ RSB 2.
Maxwell et al. (2009b) [148]	8 M, unacclimated, game players	59.9 ± 8.0	EX (H/M); 36°C; 90 min	Overnight	3.9	Water	>12 h	1.00	IST[e]	TW; PPO (During RSB 1 & 2)	36°C; 49%	No effect
Kraft et al. (2011) [243]	10 M	NS	WI	45	3.0	Water	158–178	1.00	IST[f]	MPO; PPO; Rate of fatigue	NS	No effect
Owen et al. (2013a) [229]	13 M, semi-professional soccer players	54 ± 3	EX; 19°C; 105 min	5	2.5	Water	110	0.89	Yo-Yo Test[d]	Distance covered	19°C; 59%	No effect
Owen et al. (2013b) [229]	13 M, semi-professional soccer players	54 ± 3	EX; 19°C; 105 min	5	2.5	Water	110	0.51	Yo-Yo Test [d]	Distance covered	19°C; 59%	No effect

DH: Dehydration; ES: Effect size; EX: Exercise; HT: Heat; IST: Intermittent Sprint Test; MART: Maximal Anaerobic Running Test; Max.: Maximum; MMRT: Maximal multistage running test; MPO: Mean power output; NNS: Non-nutritive sweetener; NS: Not specified; PPO: Peak power output; RH: Relative humidity; Total REC: Time from completing dehydration to commencing the subsequent task; TW: Total work; WAnT: Wingate Anaerobic Test; WI: Water immersion. Exercise intensity is described as high (H), vigorous (V) or moderate (M), in accordance with classifications outlined by Norton*, et al.* [246]. Values are Hedges' *g* effect sizes.

[a] The intermittent sprint test (IST) comprised of five 5 s sprints at 3 min intervals on a cycle ergometer

[b] The maximal anaerobic running test (MART) involved repeated 20 sec runs on a treadmill, at increasing intensities, with 100 sec passive recovery between runs until volitional exhaustion.

[c] The maximal multistage running test (MMRT) involved repeated 20 m runs between two points, at increasing intensity.

[d] The Yo-Yo Intermittent Recovery Test is a soccer-specific performance test that comprises of 20 m shuttle runs separated by 10 s jog recovery. Running speed during the test is incremental and maximal performance is indicated by total distance covered.

[e] The IST comprised of a 36 min of repeated sprint exercise divided into 2 min periods of a 4 sec sprint and 100 s of active recovery (35% $VO_{2\,max}$) and 16 sec passive rest. A repeated sprint bout (RSB) involving 5x2 sec sprints with 18 s active recovery was also completed after the 8th and 16th sprints (RSB 1 and RSB 2). All testing was completed on a cycle ergometer

[f] The IST comprised of a 3 min warm up followed by 6 x 15 s maximal sprints separated by 30 s active recovery on a cycle ergometer

Table 3.4. Characteristics of studies evaluating the effect of fluid consumption on resistance exercise tasks

Citation	Participants	DH protocol (exercise intensity); temperature; duration	Total REC (min)	DH trial BM loss (%)	Fluid type	Fluid assimilation time (min)	Fluid intake ($L \cdot kg^{-1}$ BM lost)	Performance Test	Performance outcomes(s)	Hedges' *g*
Montain et al. (1998) [236]	8 M, physically active	EX (M); 40°C; 2–3 h	3–4 h	4.0	Water	3–4 h	NS	Knee extension	ET >50% MVC; MVC pre-ET test, 30 s post-ET test; >30 s post-ET test	ET >50% MVC ↑ (2.70) MVC ↓ (ES unknown)
Greiwe et al. (1998) [225]	7 M, unacclimated	HT	120	3.8	Water	306	1.00	Knee extension; elbow flexion	Peak torque; ET 100% MVC	No effect
Bigard et al. (2001) [125]	11 M, unacclimated, active	HT	180	3.0	Water	120	1.00	Knee extension	MVC, ET 25% MVC; ET 75% MVC	ET 25% MVC ↑ (0.22)
Schoffstall et al. (2001) [96]	10 M, power lifters	HT	120	1.7	Water	120	1.10	Bench Press	1 RM	↑ (0.24)
Del Coso et al. (2008) [220]	7 (NS), acclimated, cyclists	EX (V); 36°C; 2 h	0	3.7	Water	120	0.90	Knee extension	MVC	No effect
Kraft et al. (2010) [242]	10 M, strength trained	WI	≥45	3.1	Water	165	1.00	[a]Full body protocol	Repetitions at 12 RM	↑ (0.87)
Ali et al. (2013) [228]	10 M, soccer players	EX (V); 22°C; 90 min	0	2.9	Water	90	0.50	Knee flexion/ extension (3.14 and 1.05 rad/s)	Peak torque; TW; MPO	No effect
								Knee extension; elbow flexion	Peak torque; mean torque	No effect
Wilson et al. (2014) [230]	8 M, licenced jockeys	EX; 20°C; 45 min	0	1.8	Water	35	1.00	Chest-press; knee flexion	Max. strength	↑ Chest (5.57) and leg (1.05) max. strength
Rodrigues et al. (2014) [222]	10 M, unacclimated, active	EX (V); 37°C; 91 min	30	2.0	Water	121	NS	Knee extension; elbow flexion	Peak torque	Knee extensor peak torque ↑ (0.85)

DH: Dehydration; ES: Effect size; ET: Endurance time; EX: Exercise; HT: Heat; MPO: Mean power output; MVC: Maximal voluntary contraction; NS: Not specified; RM: Repetition maximum; Total REC: Time from completing dehydration to commencing the subsequent task; TW: Total work; WI: Water immersion. Exercise intensity is described as high (H), vigorous (V) or moderate (M), in accordance with classifications outlined by Norton*, et al.* [246]. Values are Hedges' *g* effect sizes.

[a] The full body resistance exercise protocol measured total repetitions in 3 sets of bench press, lat pull down, overhead press, barbell curl, triceps and leg press exercise at 12 RM

Table 3.5. Characteristics of studies evaluating the effect of fluid consumption on sport-specific exercise tasks

Citation	Participants	$VO_{2\,max}$	DH protocol (exercise intensity); temperature; duration	Total REC (min)	DH trial BM loss (%)	Fluid type	Fluid assimilation time (min)	Fluid intake ($L \cdot kg^{-1}$ BM lost)	Performance Test	Performance outcomes(s)	Hedges' *g*
Devlin et al. (2001b) [77]	7 M, sub-elite cricketers	56 ± 6	EX; 28°C; 60 min	0	2.8	Water	60	0.80	Cricket bowling	Accuracy; velocity	↑ bowling accuracy for line (0.85) & length (0.85)
Ali et al. (2011) [213]	10 (0 M), soccer players	47 ± 4	EX; 17°C; 90 min	0	1.4	Water	90	1.07	LSPT[a]	Movement time; penalty time; performance time	No effect
Fritz et al. (2013) [240]	13 M, elite squash players	NS	EX	NS	1.3	Water	NS	0.40	Ghosting Test[b]	TT	↓ (0.55)
Owen et al. (2013c) [229]	13 M, semi-professional soccer players	54 ± 3	EX; 19°C; 105 min	5	2.5	Water	110	0.89	LSPT[a]	Movement time; penalty time; performance time	No effect
									LSST[c]	Time taken; shot speed; points per shot	No effect
Owen et al. (2013d) [229]	13 M, semi-professional soccer players	54 ± 3	EX; 19°C; 105 min	5	2.5	Water	110	0.51	LSPT[a]	Movement time; penalty time; performance time	No effect
									LSST[c]	Time taken; shot speed; points per shot	No effect
Wilson et al. (2014) [230]	8 M, licenced jockeys	NS	EX; 20°C; 45 min	0	1.8	Water	~35	1.00	Simulated ride	Pushing frequency	↑ (1.46)

DH: Dehydration; EX: Exercise; LIST: Loughborough Intermittent Sprinting Test; LSPT: Loughborough Soccer Passing Test; LSST: Loughborough Shooting Test; Max.: Maximum; NS: Not specified; Total REC: Time from completing dehydration to commencing the subsequent task; TT: Time trial. Values are Hedges' *g* effect sizes.

[a] During the Loughborough Soccer Passing Test (LSPT), participants completed a random sequence of eight short and long passes of a soccer ball towards a target, as quickly as possible with the fewest time penalties.

[b] The "Ghosting Test" is a squash-specific movement test. Participants were instructed to collect a half-ball that was placed on three racquets positioned around the court, move to the "T", and then to the next racquet at the opposite corner as quickly as possible.

[c] During the In the Loughborough Shooting Test (LSST), participants were required to sprint ~12 m, then pass, control and shoot the ball at targets within the goal area.

Table 3.6. Characteristics of studies evaluating the effect of fluid consumption on balance tasks

Citation	Participants	DH protocol (exercise intensity); temperature; duration	Total REC (min)	DH trial BM loss (%)	Fluid type	Fluid assimilation time (min)	Fluid intake ($L \cdot kg^{-1}$ BM lost)	Performance Test	Performance outcomes(s)	Hedges' *g*
Erkmen et al. (2010) [231]	17 M, physically active	EX (V); 21-24°C; 60 min	Testing at 0 and 20 min	3.3	Water	60 or 80	1.00	One-leg stand static balance test	Eyes closed/open [a]OSI (0 min post-DH) Eyes closed/open [d]OSI (20 min post-DH)	↓ Eyes open OSI 0 min post-Dh (1.12)
Ely et al. (2012a) [237]	32 M, unacclimated	EX; 50°C; 3 h W/R	90	4.1	Water	270	1.00	20 s dynamic balance test	[k]OSI; [b]mean deflection; time spent stable (at 10°C, 20°C, 30°C and 40°C)	No effect

DH: Dehydration; EX: Exercise; OSI: Overall Stability Index; Total REC: time from completing dehydration to commencing the subsequent task; W/R: Work rest cycle. Exercise intensity is described as high (H), vigorous (V) or moderate (M), in accordance with classifications outlined by Norton*, et al.* [246]. Values are Hedges' *g* effect sizes.

[a] The overall stability index (OSI) is an indicator of a subject's ability to balance on a platform. A higher OSI indicates poorer balance performance.

[b] Mean deflection was defined as the average position of the subject during the balance test. A higher mean deflection indicates more displacement and poorer balance performance

3.4.4 Cognitive Performance and Mood State Outcomes

Fifteen trials (*n*=182 participants, 90% male) investigated the effect of fluid intake on cognitive performance and/or mood state. Findings are summarised in Table 3.7 (full details are presented in the online supplementary material). Across the 15 trials reviewed, 49 separate neuropsychological tests were identified. Evidence indicating a beneficial effect of fluid intake on cognitive performance was observed on 5 cognitive tests completed across 5 trials [226,238,241]. The cognitive domains affected were memory, psychomotor function and processing speed. Four of the 6 trials evaluating the influence of fluid intake on mood state observed significant positive effects [226,238,241], as indicated by decreased ratings of fatigue, anger, depression, tension and confusion, and increased vigour.

Table 3.7. Characteristics of studies investigating the effect of fluid consumption on cognitive performance

Citation	Participants	DH protocol (exercise intensity); temperature; duration	Total REC (min)	DH trial BM loss (%)	Fluid type	Fluid assimilation time (min)	Fluid intake ($L \cdot kg^{-1}$ BM lost)	Cognitive domains assessed	Performance temperature; RH	Hedges' *g*
Cian et al. (2001a) [226]	7 M, unacclimated, endurance trained	EX (V); 25°C; 120 min	Testing at 140 & 240 min	2.7	CHO-electrolyte	80/180	1.00	Memory Perceptive discrimination Psychomotor processing speed Mood	NS	↑ Memory 2 h post-DH
Cian et al. (2001b) [226]	7 M, unacclimated, endurance trained	HT	Testing at 140 & 240 min	2.6	CHO-electrolyte	80/180	1.00	Memory Perceptive discrimination Psychomotor processing speed Mood	NS	↑ Memory 2 h post-DH; ↓ Fatigue
Grego et al. (2004) [86]	8 M, endurance cyclists	EX (V); 20°C; 180 min	5	4.1	Water	185	0.73	Perceptual discrimination Memory/processing speed	NS	No effect
Serwah et al. (2006a) [87]	8 M	EX (V); 31°C; 90 min	3	1.7	Water	9	1.00	Psychomotor processing speed	NS	No effect
Serwah et al. (2006b) [87]	8 M	EX (V); 31°C; 90 min	3	1.7	Water	90	0.50	Psychomotor processing speed	NS	No effect
Edwards et al. (2007b) [88]	11 M, soccer players	EX; 19-25°C; 90 min	0	2.4	Water	90	0.80	Visual scanning/processing speed	NS	No effect
Adam et al. (2008a) [227]	8 (6 M), active soldiers	HT	120	3.0	NS	300	NS	Psychomotor processing speed Psychomotor function Perceptive discrimination Visual scanning/vigilance	20°C; 50%;	No effect
Adam et al. (2008b) [227]	8 (6 M), active soldiers	HT	120	3.0	NS	300	NS	Psychomotor processing speed Psychomotor function Perceptive discrimination Visual scanning/vigilance	2°C; 50%	No effect
D'Anci et al. (2009a) [241]	16 M, university athletes	EX; 60 min	NS	2.0	Water	60	NS	Memory Psychomotor processing speed Arithmetic/processing speed Visual scanning/vigilance Spatial processing Mood	NS	↑ Psychomotor processing speed; ↓ Anger, fatigue, depression, tension and confusion; ↑ vigour
D'Anci et al. (2009b) [241]	13 (0 M), university athletes	EX; 60-75 min	NS	1.7	Water	60-75	NS	Memory Psychomotor processing speed Arithmetic/processing speed Visual scanning/vigilance Spatial processing Mood	NS	↑ Psychomotor processing speed; ↓ Anger, fatigue, depression, tension and confusion; ↑ vigour

Table 3.7. *(continued)*

Citation	Participants	DH protocol (exercise intensity); temperature; duration	Total REC (min)	DH trial BM loss (%)	Fluid type	Fluid assimilation time (min)	Fluid intake ($L \cdot kg^{-1}$ BM lost)	Cognitive domains assessed	Performance temperature; RH	Hedges' *g*
Ganio et al. (2011) [238]	24 M, physically fit	EX; 28°C; 40 min	20	1.6	Water	60	NS	Psychomotor processing speed Visual scanning/vigilance Memory/processing speed Learning/memory Logical reasoning/processing speed Mood	23°C	↑ Psychomotor processing speed and memory/processing speed; ↓ Fatigue and tension
Ely et al. (2012b) [237]	32 M, unacclimated	EX; 50°C; 3 h W/R	90	4.1	Water	270	1.00	Psychomotor processing speed Memory/processing speed Logical reasoning/ speed Mood	Testing at 10°C, 20°C, 30°C and 40°C	No effect
Wilson et al. (2014) [230]	8 M, licenced jockeys	EX; 20°C; 45 min	0	1.8	Water	~35	1.00	Response inhibition	NS	No effect
Wittbrodt et al. (2015a) [239]	12 M, recreationally active	EX (V); 32°C; 50 min	NS	1.5	Water	>50	1.00	Psychomotor processing speed Memory Perceptive discrimination Visual scanning/processing speed	32°C; 65%	No effect
Wittbrodt et al. (2015b) [239]	12 M, recreationally active	EX (V); 32°C; 50 min	NS	1.5	Water	>50	0.80	Psychomotor processing speed Memory Perceptive discrimination Visual scanning/processing speed	32°C; 65%	No effect

CHO: Carbohydrate; DH: Dehydration; EX: Exercise; HT: Heat; NS: Not specified; Total REC: Time from completing dehydration to commencing the subsequent task; W/R: work rest cycle. Exercise intensity is described as high (H), vigorous (V) or moderate (M), in accordance with classifications outlined by Norton*, et al.* [246]. Values are Hedges' *g* effect sizes.

3.5. Discussion

Individuals prone to dehydration (e.g. athletes and manual workers) may have limited opportunity to adequately rehydrate prior to performing further physically or cognitively demanding activities. The present systematic review and meta-analysis examines evidence for the effect of fluid intake on subsequent athletic and cognitive performance following dehydration. A beneficial effect for fluid intake was observed when athletic performance involved continuous exercise tasks. Further, the magnitude of improvement appeared greater when the continuous exercise was performed at elevated environmental temperatures and over longer exercise durations. While the volume of fluid consumed (i.e. relative to BM lost) did not significantly influence the size of the treatment effect, fluid intake at levels complying with current recommend-actions for completely replacing lost fluid (1.25–1.50 L·kg BM lost^{-1}) [1,2] are yet to be thoroughly investigated. Evidence for a beneficial effect of fluid intake on intermittent, resistance and sport-specific exercise performance and cognitive function or mood is less apparent and requires further elucidation.

The weighted mean effect suggests that fluid ingestion during or following dehydration significantly improves continuous exercise performance compared to control conditions (no fluid or negligible fluid intake). Individual studies all indicated a beneficial effect from fluid intake; however, the magnitude of the improvement was heterogeneous (I^2=80.5%), likely reflecting differences in the methodologies employed between studies. Simple meta-regression determined that 82% of variation between trials can be attributed to differences in the ambient environmental temperature at which subsequent exercise was performed, with fluid intake demonstrating greater efficacy under heat stress conditions. The decline in aerobic performance that occurs with dehydration has largely been attributed to circulatory strain, whereby reductions in blood volume limit oxygen transport to the exercising muscle [112,248]. Under elevated environmental temperatures, blood flow is also redirected to the skin facilitating evaporative cooling, augmenting circulatory strain and further impairing exercise performance [91]. These physiological perturbations are typically characterised by increased HR and T_C [91,112,248]. Hence, thermoregulatory parameters were monitored

in many of the studies reviewed[a]. The majority of reviewed studies reported that fluid consumption was associated with significant reductions in core or rectal temperature[b] and HR[c] at various time points during continuous exercise performance. Thus, fluid intake may offset the circulatory strain typically observed when exercise is undertaken in warm environments. The meta-regression analysis also suggests that differences in the duration of the continuous exercise performed may account for a proportion of the heterogeneity observed between experimental trials, with exercise performed over longer durations yielding greater benefit from fluid intake than short duration exercise. However, as the majority of performance tests included in the analysis were relatively short in duration (4–30 min), we cannot be certain that this relationship would hold true over longer exercise durations. Of final note is that, although not investigated in this analysis, some evidence suggests that aerobically trained individuals, who have a higher TBW content, are more resistant to the physiological effects of dehydration [95,96]. As such, these individuals, may derive less benefit (than untrained individuals) from fluid consumption.

Results of the meta-regression failed to indicate a statistically significant relationship between the volume of fluid consumed and continuous exercise performance. However, the majority of trials tested a quantity of fluid that was within a very narrow fluid intake range (i.e. 1.0–1.05 L·kg BM lost^{-1}, *n*=13 out of 18). Hence, the performance effects associated with ingesting a comparably small volume of fluid (e.g. ≤0.75 L·kg BM lost^{-1}) or an amount consistent with fluid replacement guidelines (e.g. 1.25–1.50 L·kg BM lost^{-1}) remains uncertain. Three experimental investigations have examined the dose-response effect of ingested fluid volume on continuous exercise performance following a period of dehydration with the results demonstrating inconsistent findings [150-152]. Unfortunately, the investigation with the greatest contrast in fluid volumes (i.e. 0.75 vs 1.50 L·kg BM lost^{-1} [150]) did not employ a 'no fluid' control and was unable to be included in the meta-analysis. Findings from previous studies suggest that fluid intake during exercise exceeding that dictated by thirst may not provide additional performance benefits [150]. However, only three of the

[a] [91,92,130,151,152,212,214,218,221,232,233,244,245]

[b] [91,214,218,221,232,233,244]

[c] [91,214,218,221,232,233,244,245]

publications reviewed measured subjective thirst within the investigation (and these studies did not test different fluid volumes, only one intervention vs. control). Thus, it is not clear whether the equivocal effect of fluid intake volume can be attributed to thirst sensation. Based on current evidence, prescribing fluid volumes required to optimise performance on a subsequent continuous exercise task requires clarification.

If relatively small and large fluid intakes elicit comparable treatment effects, individuals who have limited time to rehydrate prior to performing aerobic activities may opt to consume smaller fluid boluses, delaying complete rehydration until circumstances permit (e.g. overnight). This strategy may reduce the probability of the drinker experiencing volume-induced GI discomfort during the subsequent activity, which may occur when larger fluid volumes are ingested [152]. Only one of the 42 publications reviewed monitored GI symptomology [152]. In this study, subjective ratings of GI discomfort following different fluid intakes (0.5 vs. 1.0 L·kg BM lost^{-1}) were described as mild–moderate and moderate–high on each respective trial. This suggests that larger fluid volumes are likely to induce some degree of participant discomfort which may compromise performance. However, research examining continuous exercise performance following two volumes of fluid intake (i.e. 0.75 vs 1.50 L·kg BM lost^{-1}) demonstrated significantly faster (~3.0%) running performance with the larger bolus [150]. Importantly, this study employed a prolonged (i.e. overnight) rehydration period reducing the probability of severe GI disturbance and allowing ingested fluid to equilibrate throughout the body. Further research examining exercise performance (and GI symptoms) when large fluid volumes are ingested over short rehydration periods is therefore warranted.

The effect of fluid intake on intermittent, resistance, sport-specific and balance exercise types remains unclear. It appears that fluid ingestion during or following dehydration may improve performance on subsequent intermittent, resistance and sport-specific exercise tasks. However, methodological differences make comparison of results across trials challenging.

In regard to intermittent exercise, 4 of the 10 trials demonstrated a significant positive effect of fluid intake on performance, while no trial reported a significant performance decrement. Similar to the results from continuous exercise, beneficial

effects of fluid intake are apparent when intermittent exercise tasks have been completed in warm environments [148,235]. The impact of task duration may also influence the likelihood of observing performance effects, with longer duration tasks more regularly demonstrating a performance enhancement associated with fluid ingestion [148,235]. For instance, Maxwell*, et al.* [148] observed that fluid intake only benefited performance on a second repeated sprint bout completed in the latter stages of testing.

Concerning resistance exercise, 6 of the 9 trials observed a significant positive effect of fluid intake on performance. One trial reported that fluid intake was detrimental to performance [236]. However, results from this study should be interpreted with caution as the strength performance task was preceded by an endurance task that varied in duration. Evidence indicating a beneficial effect of fluid intake on resistance exercise performance appears stronger when tests of muscular endurance, rather than tests of muscular strength, are employed [125]. Findings from this systematic review demonstrate significantly improved performance on 3 out the 4 sub-maximal intensity resistance exercise tasks [125,236,242]. In contrast, performance on only 4 out of 15 maximal intensity tests demonstrated improvement with fluid intake [96,222,230]. The current data are inadequate to determine the influence of other variables (e.g. participant population, mode of dehydration, etc.) on the effect of fluid intake. Further research examining the effects of fluid intake on resistance exercise performance using standardised procedures is required.

The 6 that evaluated the effect of fluid intake on sport-specific exercise performance exhibited considerable heterogeneity, with tests of cricket [77], soccer [88,213,229], squash [240] and racehorse riding [230] performance all being employed. While the majority of sports-specific research has demonstrated no impact of fluid consumption on subsequent performance, the paucity of data and lack of replication studies makes it difficult to determine an overall effect of fluid intake on sport-specific exercise performance.

The present systematic review identified 15 trials examining the effect of fluid intake following dehydration on cognitive performance and mood state. Evidence indicating a beneficial effect of fluid intake on cognitive performance was only observed

in some studies [226,241] and there was no clear indication of greater treatment efficacy on a particular cognitive domain. However, some limitations to the current evidence exist. In four trials, the cognitive assessment was conducted ≤5 min after concluding the dehydration protocol [86-88]; a further four trials did not provide the necessary information to calculate the amount of time between the end of the dehydration protocol and commencement of the cognitive tests [88,239,241]. Prior research indicates that acute exercise has a small positive effect of on cognitive performance (typically dissipating within ~15 min of exercise cessation) [84], while elevated T_C via heat stress may provide additional cognitive burden [89]. Thus, residual effects of the physiological stressors used to induce dehydration in these trials may obscure any influence of fluid consumption on cognitive performance. Investigations examining the effect of dehydration on cognitive performance should also employ neuropsychological tests that have previously demonstrated sensitivity to nutritional interventions [62,90,249]. Yet, only two studies included in the present review selected cognitive tests on this basis [227,241]. The majority did not provide any rationale for their chosen method of assessment [86-88,226,237,239], increasing the likelihood of false-negative reports. Fluid consumption positively influenced mood state (measured as reduced anger, fatigue, depression, tension and confusion) in 4 out of the 6 trials where it was measured [226,238,241]. While this may suggest that self-reported mood state questionnaires are more sensitive to the effects of fluid intake than objective tests of cognitive function, subjective mood ratings were only influenced by fluid intake during trials where significant cognitive effects were also observed, in other words, effects on mood and cognition were not independent of each another. Collectively, it appears that the influence of fluid intake on mood and cognitive performance is still poorly understood and requires further research employing tasks with demonstrated sensitivity.

This review does contain a number of limitations. Firstly, only studies with accessible full text articles in English were included. Second, three of the studies reviewed [77,238,245] examined rehydration in combination with another placebo treatment (studies were excluded if fluids were co-administered with another experimental treatment). Thus, participants' perceptions regarding the expected treatment may have influenced these results. Third, as oral fluid replacement cannot be blinded, it is possible

that the placebo effect may account for a small amount of benefit observed with rehydration. However, it was necessary to exclude research studies that blinded participants to hydration status using intravenous methods because the infusion does not accurately mimic the physiological effects of oral rehydration. Fourth, the present review elected to compare against a "no fluid" or "negligible fluid" control condition, because a euhydrated control may be confounded by the effects of the dehydration protocol itself (i.e. hyperthermia or fatigue). However, using this comparison we cannot determine whether fluid intake fully or partially restored performance to euhydrated levels. Similarly, fluid ingestion may have minimal or no effect on athletic or cognitive performance if the outcome measured is not sensitive to the effects of modest fluid losses. Fifth, where fluid was administered at the time of dehydration (i.e. *concurrent* fluid intake), rather than following dehydration (i.e. *subsequent* fluid intake), different physiologic responses to the dehydration protocol may occur on control and intervention trials, e.g. decreased core temperature leading to reduced central fatigue [111]. This could have implications for subsequent athletic performance, and consequently, the magnitude of the treatment effect. Sixth, fluid intake ≤200mL was considered 'negligible' and included within the definition of control conditions. However, one study has reported a benefit of ingesting 100mL of fluid (increased TTE following exercise induced dehydration [250]. Thus, trials administering ≤200mL fluid to dehydrated control participants may underestimate the true magnitude of the treatment effect.

3.6. Conclusion

The results of this review suggest that individuals who have limited opportunity to adequately rehydrate prior to performing continuous exercise (particularly under heat-stress conditions) should consume fluid, even if the volume is inadequate for complete rehydration (i.e. <1.25–1.50 L· kg BM lost^{-1}). The influence of fluid consumption for those individuals performing intermittent, resistance and sport-specific exercise, or undertaking cognitively demanding activities is not as well understood and this review serves to highlight areas for future research.

Chapter 4: The Effect of Consuming Carbohydrate and Protein During or Following Exercise on Subsequent Athletic Performance: A Systematic Review and Meta-Analysis

Reader's note:

This chapter includes a co-authored paper. The bibliographic details of the co-authored paper, including all authors are as follows:

McCartney, D., Desbrow, B., Irwin, C. Post-exercise ingestion of carbohydrate, protein and water: A systematic review and meta-analysis for effects on subsequent athletic performance. *Sports Medicine*, 2018; 48(2): 379-408.

* Please note that supplementary materials related to this research can be found at the Journal's webpage: https://www.springer.com/medicine/journal/40279.

The research candidate has made the following contributions to this study:

- Developed the study design and registered the research methodology
- Completed the literature search, quality assessment, data extraction, data synthesis and statistical analyses
- Prepared the manuscript for submission to a peer-reviewed journal
- Presented the research at a national conference

(Signed) _____ (Date: 20.02.2019)

Danielle McCartney

(Countersigned) _____ (Date: 20.02.2019)

Supervisor: A/Prof Ben Desbrow

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4.1. Abstract

Background: Athletes may complete consecutive exercise sessions with limited recovery time between bouts (e.g. ≤4 h). Nutritional strategies that optimise post-exercise recovery in these situations are therefore important. This two-part systematic review investigated the effect of consuming carbohydrate (CHO) and protein with water during or post-exercise on subsequent athletic performance. **Method:** SPORTDiscus (EBSCOhost), PubMed (MEDLINE), Web of Science (via Thomas Reuters) and Scopus were searched to identify studies that measured either endurance (≥5 min duration) or anaerobic (≤60 s duration) performance ≤4 h after completing a standardised exercise bout under the following conditions: Part-1: Water (W) vs. CHO ingested with an equal volume of water (CHO+W); and, Part-2: CHO+W vs. protein ingested with CHO and an equal volume of water (PRO+CHO+W), where one of either CHO or energy intake was matched. Random-effects meta-analyses and meta-regression analyses were conducted to evaluate intervention efficacy. **Results:** Part-1: 45 trials (*n*=486 participants, 93% male) were reviewed for effects on endurance performance and 9 trials (*n*=134, 73% male) were reviewed for effects on anaerobic performance. CHO+W (102±50 g CHO; 0.8±0.6 g CHO·kg^{-1}·h^{-1}) improved endurance performance compared to W (1.6±0.7 L); %$_{\Delta}$MPO=4.0, 95% CI's: 3.2, 4.7 (I^2=43.9). Improvement was attenuated when participants were "Fed" (a meal ≤4 h prior to the initial bout) as opposed to "Fasted" (*p*=0.012). CHO+W (51±8 g CHO; 0.8±0.1 g CHO·kg^{-1}·h^{-1}) also improved anaerobic performance compared to W (0.5±0.3 L); %$_{\Delta}$ Peak Power Output (%$_{\Delta}$PPO)=2.548, 95% CI's: 1.114, 3.982 (I^2=0.00). Part-2: 13 trials (*n*=125 male participants) were reviewed. PRO+CHO+W (35±26 g PRO; 0.5±0.4 g PRO·kg^{-1}) did not affect endurance performance compared to CHO+W (115±61 g CHO; 0.6±0.3 g CHO·kg BM^{-1}·h^{-1}; 1.2±0.6 L); %$_{\Delta}$MPO=0.5, 95% CI's: -0.5, 1.6 (I^2=72.9). **Conclusion:** Athletes with limited time for recovery between consecutive exercise sessions should prioritise CHO and fluid ingestion to enhance subsequent athletic performance.

4.2. Introduction

Athletes undertaking frequent training or those involved in sporting competitions with multiple heats or games may be required to complete consecutive exercise sessions with limited recovery time between bouts (e.g. ≤4 h). The meta-analysis in Chapter 4 highlighted the importance of consuming fluid, even in volumes that are inadequate to completely restore sweat-induced losses, to facilitate performance on a subsequent exercise session. However, consideration for nutrition interventions that optimise repletion of endogenous substrate stores (e.g. muscle and liver glycogen) and promote the recovery of damaged and inflamed muscle is also required. Nutrition recommendations for post-exercise recovery highlight the importance of high CHO availability to maximise the rate of muscle glycogen resynthesis and also indicate that protein may assist in both glycogen restoration (via an insulin-mediated response) and muscle-damage repair (via supply of amino acids) [2]. However, trials involving consecutive exercise are needed to determine whether these nutrients can convey meaningful performance enhancements; particularly in a context where limited

recovery time exists between exercise bouts (e.g. ≤4 h). Under these circumstances, it may not be possible to completely restore substrate losses [160] or promote significant muscle-damage repair and attempting to do so (i.e. by consuming large quantities of nutrition) may produce negative side-effects (e.g. GI discomfort) that hinder athletic performance.

Considerable scientific research has investigated the effect of consuming CHO during and/or following an initial bout of activity on subsequent endurance exercise performance and some (but not all) studies indicate a performance-enhancing effect [251-253]. Fewer studies have employed anaerobic performance-based trials. This evidence is yet to be systematically collated in a way that facilitates the exploration of factors that may influence the ergogenic potential of CHO ingestion. For example, overnight fasting has been shown to reduce liver glycogen stores by up to 80% [254], such that CHO availability may already be suboptimal at the onset of the initial exercise bout. Thus, this methodological approach may augment the influence of CHO supplementation on subsequent athletic performance [255]. Hence, the effect of CHO ingestion on subsequent endurance and anaerobic exercise performance requires elucidation.

While protein (alone) contributes minimally to the energetic demands of exercise, other physiological attributes of this nutrient may facilitate performance enhancements on acute subsequent exercise bouts. For example, when ingested with CHO, protein can potentiate plasma insulin secretion, enhancing muscle glycogen synthase activity and uptake of glucose from circulation [256]. These actions may accelerate muscle glycogen resynthesis post-exercise [164]. Indeed, a previous review [181] concluded that although dietary protein is unlikely to affect glycogen repletion when co-ingested with an "optimal" dose of CHO (i.e. ~1.2 $g \cdot kg^{-1} \cdot h^{-1}$, to maximise glycogen resynthesis), a small quantity of protein (i.e. ~0.2–0.4 $g \cdot kg^{-1} \cdot h^{-1}$) consumed with a "suboptimal" CHO dose (i.e. <1.2 $g \cdot kg^{-1} \cdot h^{-1}$) may be of benefit. (Protein consumption also has the potential to enhance skeletal muscle-damage repair during recovery from exercise [180]). Therefore, in situations where ingesting large quantities of CHO is not feasible (e.g. between exercise sessions), ingesting protein with CHO may provide an opportunity to enhance substrate recovery.

To date, one systematic review [257] has investigated the effect of protein co-ingested with CHO during and/or following an initial bout of activity on subsequent endurance performance. In keeping with the aforementioned evidence, this review concluded that a significant benefit of dietary protein was frequently observed in studies where CHO was delivered "sub-optimally". Conversely, an ergogenic effect was seldom recorded when CHO intake was adequate. However, the practical significance of this finding is unclear as these conclusions were determined on visual inspection of the available evidence and are not supported by statistical procedures. As such, the magnitude of the performance change was not defined. It is also difficult to determine whether a benefit of protein ingestion exists in the absence of such procedures, as several methodological inconsistencies (i.e. the confounding influence of which may be controlled) are evident across experimental investigations. For instance, the additional energy ingested when protein is added to a CHO-containing fluid may explain the performance benefit reported in some studies, and not others (i.e. where "isocarbohydrate" vs. "isoenergetic" beverage treatments are employed) [258]. Hence, the effect of dietary protein intake on subsequent endurance exercise performance requires further clarification.

The aim of the present review was to determine the influence of: (1) CHO co-ingested with water; and (2) protein co-ingested with CHO and water, during and/or following an initial bout of exercise on subsequent endurance and anaerobic performance. In addition, the current investigation sought to clarify the effect of:

a) CHO (co-ingested with water) on performance when individuals are not fasted overnight ahead of experimentation; in other words, does fasting exaggerate the benefit of CHO to performance?;
b) Protein (co-ingested with CHO and water) on performance when CHO intake is "suboptimal" (i.e. <1.2 $g \cdot kg^{-1} \cdot h^{-1}$); and
c) Protein (co-ingested with CHO and water) on performance when the control condition is "isocarbohydrate" rather than "isoenergetic"; in other words, is it the additional energy (i.e. via supplemented protein) that improves performance, or the nutrient itself?).

4.3. Methods

The methodology of this review was devised in accordance with specifications outlined in the *Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols PRISMA-P 2015 Statement* [206] and registered at PROSPERO ahead of the formal study selection process (ID: CRD42016046807).

4.3.1. Literature Search

Potential research studies were identified by searching the online databases SPORTDiscus (via EBSCOhost), PubMed (MEDLINE), Web of Science (via Thomas Reuters)[a] and Scopus from January 1985 to September 2016 using the terms carbohydrate* OR glucose OR fructose OR lactose OR sucrose OR sugar OR glycogen OR "sport* drink" OR "sport* beverage" OR protein OR "amino acid*" each in combination with exercise* OR athletic OR performance OR sport* OR endurance OR sprint OR aerobic OR anaerobic. Records that contained irrelevant terms (obesity, diabetes, rat, mouse, mice, animal, rodent, children, teenagers, adolescents, review, meta-analysis, illness, disease, elderly, older, geriatric, patient and hospital) were excluded from the literature search using the Boolean search operator 'NOT'. (The search was updated in June 2017 to capture recent research studies before proceeding to publication). Two investigators (D.M. and C.I.) independently screened the potential studies to identify relevant texts. Initially, all irrelevant titles were discarded. The remaining articles were systematically screened for eligibility by abstract and full text, respectively. The decision to include or discard potential research studies was made between two investigators (D.M. and C.I.). Any discrepancies were resolved in consultation with a third investigator (B.D.). The reference lists of all included studies were hand searched for missing publications. Full details of the screening process are displayed in Figure 4.1.

[a]Web of Science (via Thomas Reuters) retrieved a comparatively large number of records (68,347 vs. ≤4,789 records via each SPORTDiscus (via EBSCOhost), PubMed (MEDLINE) and Scopus) using search strategy indicated above. To improve efficiency of the study selection process, only those records categorised within the *Sport Sciences* field (3,418 records) were retrieved from Web of Science.

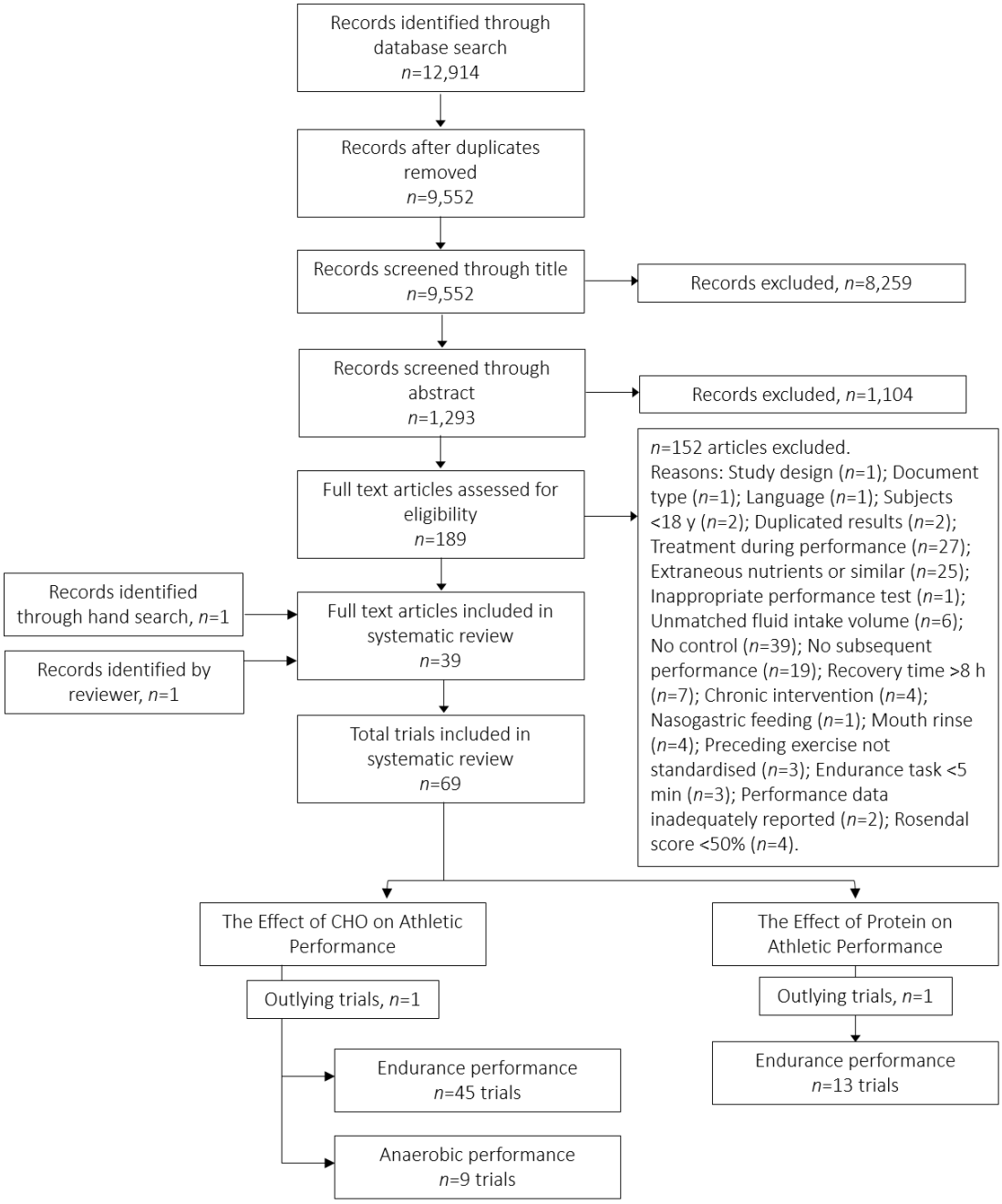

Figure. 4.1. PRISMA Flow Chart (study selection methodology). Where a study contained >1 intervention-arm that was eligible for inclusion (i.e. paired against a suitable control condition), these were treated as separate 'studies' termed *trials*. Note; the updated search did not identify any eligible studies.

4.3.2. Inclusion and Exclusion Criteria

Research studies that fulfilled the following criteria were eligible for inclusion:

1) Controlled trials (random or non-random participant allocation) employing repeated-measures experimental designs;
2) Human studies on adult (≥18 years of age) male and/or female participants devoid of medical conditions and co-morbidities. Studies completed using

subjects with paraplegia due to spinal cord injury were accepted for review (where glucose tolerance was normal);

3) Endurance and/or anaerobic exercise performance (refer to *4.3.4. Primary and Secondary Research Outcomes*) was measured under intervention and control conditions (refer to *4.3.3. Control and Intervention Conditions*);
4) Athletic performance was preceded by an initial bout of exercise (any type), during and/or following which, an experimental condition was imposed. For the purpose of this review, athletic performance was considered "subsequent" to another bout of exercise when: (a) a period of time separated the exercise bouts (i.e. *recovery time*), or (b) there was a change in the demands of the activity (i.e. mode of exercise or intensity, e.g. submaximal exercise followed by a TT performance test). A schematic representation of the experimental protocol is displayed in Figure 4.2;
5) The amount of time separating one exercise bout from another was ≤4 h. This cut-off was instated to reflect time restrictions associated with completing consecutive exercise sessions. No minimum recovery time was set for inclusion;
6) Accessible full text research articles (including complete conference proceedings) written in English. Other documents were discarded.

Studies were excluded from the review if: (1) participants' dietary intake and/or exercise behaviour was experimentally altered ahead of testing (e.g. via a CHO loading regimen or glycogen depletion diet); (2) the preceding bout of exercise was not standardised across experimental conditions (e.g. TTE protocols were employed); (3) an experimental condition was (a) delivered chronically (i.e. a multi-day treatment, e.g. 7 d supplementation period prior to testing); (b) delivered while participants were undertaking the athletic performance test; or (c) not administered orally (e.g. via intravenous or nasogastric routes); (4) extraneous dietary and/or pharmacological constituents (e.g. caffeine), including placebo varieties were also administered during exercise and/or recovery; although additional electrolytes, vitamins and small quantities of fat were accepted; or (5) the performance data were not adequately reported (i.e. Mean±SD was not quantified and could not be calculated). In the event that

data were not adequately reported, and the study was published within the previous 10 years (2006–2016), the corresponding author was contacted via email in an attempt to retrieve missing data. Potential research studies containing at least one eligible comparison between an intervention and control condition were included in the present review; other ineligible study-arms were excluded.

For the purpose of this systematic review, research studies containing multiple intervention-arms that were eligible for inclusion (i.e. each paired against a suitable control) were treated as separate experimental 'studies' termed *trials*. Separate trials derived from a single research study are identifiable by the addition of letters (e.g. a–d) to the citation.

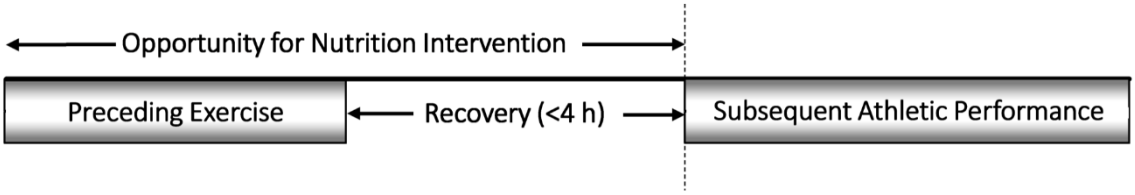

Figure. 4.2. A schematic representation of the experimental protocol employed in studies eligible for inclusion in the present review.

4.3.3. Control and Intervention Conditions

The present systematic review aimed to compare the following experimental conditions, via a two-part investigation: (1) CHO co-ingested with water (CHO+W) vs. water (W); and (2) protein co-ingested with CHO and water (PRO+CHO+W) vs. CHO co-ingested with water (CHO+W). All nutrients consumed during the preceding exercise bout and/or recovery period were considered "co-ingested". The experimental conditions were defined in accordance with Table 4.1. Note, while W was accepted as water intake ≥200 mL, it was also a requirement that the volume was matched (≤5% difference from control) across trials, such that the effect of CHO ingestion could be isolated. Similarly, comparison of PRO+CHO+W vs. CHO+W conditions required one of either total CHO content or total energy content to be matched across trials (≤5% difference from control). Studies administering *whole proteins* were acceptable for review; interventions that contained single amino acids and/or peptides were excluded. Dietary intakes derived from food and/or fluid sources (including "complex" beverages,

such as chocolate milk) were accepted, provided that all of the eligibility criteria were adhered to.

Table 4.1. Experimental conditions

Experimental Condition	Accepted Definition
Part 1: CHO+W vs. W	
W	Total water intake ≥200 mL[a]
CHO+W	Digestible carbohydrate (any type) co-ingested with ≥200 mL water
Part 2: PRO+CHO+W vs. CHO+W	
CHO+W	Digestible CHO[b] (any type) co-ingested with ≥200 mL[a] water
PRO+CHO+W	Whole protein (i.e. single amino acids and/or peptides not accepted) co-ingested with digestible carbohydrate (any type) and ≥200 mL water

CHO+W: CHO that is co-ingested with water; W: Water only; PRO+CHO+W: Protein that is co-ingested with CHO and water. [a]Water intake must be volume-matched (≤5%) to the corresponding intervention condition. [b]Either total CHO intake or total energy intake must be matched (≤5%) to the corresponding intervention condition.

4.3.4. Primary and Secondary Research Outcomes

The primary research outcomes in this investigation were endurance and anaerobic exercise performance. Endurance exercise performance was defined as the $\%_{\Delta}$ MPO on a TT test involving continuous running or cycling exercise for ≥5 min duration. The common metric (i.e. $\%_{\Delta}$ MPO on a TT test), was selected to facilitate interpretation of the intervention effect in the context of competitive performance [259]. Hopkins [260] suggests a ~1% change in endurance power output on a laboratory-based test corresponds to a ~1% change in competitive running performance and ~0.4% change in competitive cycling performance. Effects on performance in TTE tests were converted to effects on performance in TT tests, as described below (see *4.3.4a. Time Trial Performance* and *4.3.4b. Time to Exhaustion Performance*). Similarly, where the $\%_{\Delta}$MPO was not measured directly, it was derived from other performance outcomes. Anaerobic exercise performance was defined as the percent change in peak power output ($\%_{\Delta}$PPO) on anaerobic exercise tests (≤60 s duration) that involved running or cycling exercise (see *4.3.4c. Anaerobic Performance*). GI tolerance was evaluated as a secondary research outcome. Raw scale ratings were extracted and converted to a 0–100 scale [(mean raw score/highest possible score on a given scale) × 100]. Where the

lowest obtainable score was 1 (i.e. rather than *zero*), the raw score was transformed by $x - 1$ and divided by the adjusted maximum score to derive a percentage.

4.3.4a. Time Trial Performance

Time trials included all constant work, distance and duration performance tests. Where TT performance was reported as mean power output (MPO) (Watts) [233,261-268], the change in endurance exercise performance was calculated using the following formula:

$$\%_{\Delta}\,\text{MPO} = \frac{(\text{MPO}_{\text{Intervention}} - \text{MPO}_{\text{Control}})}{\text{MPO}_{\text{Control}}} \times 100$$

Where TT performance was assessed as total work (J) completed on a fixed duration test [269-271], performance scores were divided by test duration (s) to convert to effects on MPO. Conversely, where performance was assessed as time to complete a fixed amount of work [272,273], the target work (J) was divided by the performance score (s) to convert to effects on MPO. (Nb. One study [273] expressed the target work in terms of energy expenditure. These values were multiplied by an energy efficiency of 23.2% [273] before calculating the change in endurance performance). Where TT performance was assessed as time to complete a fixed distance [251,252,274-279], the performance scores (s) were used to determine $\%_{\Delta}$MPO via the speed-power relationship, as described by Hopkins*, et al.* [259]. Briefly, control scores were divided by intervention scores and raised to the power of x, a constant which signifies the coefficient of variation for power output on a given ergometer (Nb. As power output is directly proportional to running speed, x was always equal to 1 on these tests) [280]. Where the Monark [251,252,281,282], VeloTron [261,264] and Schoberer Rad Messtechnik [278] ergometers were used, x was equal to 1.0 [283], 2.0 [284] and 1.6 [285], respectively. The value of x was not known for the Elite cycle trainer, utilised by Cepero*, et al.* [277]. Therefore, $\%_{\Delta}$MPO was derived using the power-speed relationship: $P = 9.65\,S - 86.74$ [286], where S denotes speed ($km \cdot h^{-1}$) and P denotes power (Watts). Where TT performance was measured as distance on a constant duration test [281,282], intervention performance scores (km) were divided by control performance

scores and raised to the power of x (as described above). Where studies evaluated TT performance in terms of MPO [233,261-268], the time taken to complete the task was also recorded. This outcome was used to generate an "imputed $\%_{\Delta}$ MPO" (i.e. using the methods indicated previously) for comparison against the reported value. While the majority of the data were comparable, two studies [261,264] reported a large $\%_{\Delta}$MPO, with minimal effect on the time taken to complete the performance test (i.e. a much smaller imputed $\%_{\Delta}$ MPO, e.g. >2%-points different). This was likely due to the power output data being non-normally distributed across time, such that the mean value did not accurately reflect the result of the performance test. In these situations, the imputed $\%_{\Delta}$MPO was used to perform analyses.

4.3.4b. Time to Exhaustion Performance

Time to exhaustion performance tests included all constant power/load and incremental exercise tests to fatigue. Prior research demonstrates that the percent change in the duration of a constant power/load test is approximately equal to the $\%_{\Delta}$MPO on a TT performance test when it is multiplied by a constant [280]. The constant is calculated as the power/load at which the test was performed (expressed as a percentage of VO_{2max}) divided by 6.4 [280]. Hence, where TTE was assessed as test duration [253,258,287-292], the change in performance was calculated using the following formula [280]:

$$\%_{\Delta}\text{MPO} = \left(\frac{(\text{Mean TTE}_{\text{Intervention}} - \text{Mean TTE}_{\text{Control}})}{\text{Mean TTE}_{\text{Control}}} \times 100\right) \div \left(\frac{\%\text{VO}_{2\ \text{max}}}{6.4}\right)$$

One study [291] expressed performance as a median and range; presumably because the data were non-normally distributed. Effect estimates for this study were therefore calculated using the *median* test duration. Another study [293] assessed TTE as PPO on an incremental test to fatigue. The test commenced at a workload of 180 Watts, and increased by 1 Watt every 2 s, until fatigue. TTE was therefore approximated as mean PPO minus 180 Watts, multiplied by 2 s. Scores were used to derive the change in athletic performance using the following formula [260]:

$$\%_{\Delta}\ \text{MPO} = \left(\frac{(\text{Mean TTE}_{\text{Intervention}} - \text{Mean TTE}_{\text{Control}})}{\text{Mean TTE}_{\text{Control}}} \times 100\right) \times \left(1 - \frac{\%\text{PPO}}{6.4}\right)$$

Where %PPO represents the percentage of peak sustainable power output at which the test was commenced.

4.3.4c. Anaerobic Performance

All anaerobic exercise tests were constant duration TT performance tests. The change in anaerobic performance was calculated where PPO (Watts) was reported, using the following formula:

$$\%_{\Delta}\ \text{PPO} = \frac{(\text{PPO}_{\text{Intervention}} - \text{PPO}_{\text{Control}})}{\text{PPO}_{\text{Control}}} \times 100$$

4.3.5. Methodological Quality Assessment

Included studies were examined for publication bias using the Rosendal Scale [209], where excellent methodological quality is indicated by a Rosendal Score ≥60% [210]. Scoring was determined by dividing the number of 'yes' responses by the total number of applicable items. Studies with a Rosendal score <50% were excluded from this review due to increased risk of experimental bias.

4.3.6. Data Extraction and Synthesis

Data were extracted from relevant publications following the Cochrane Handbook for Systematic Reviews of Interventions *Checklist of Items to Consider in Data Collection or Data Extraction* [211] and entered into a Microsoft Excel spreadsheet. Extracted information included: (1) standardised pre-trial conditions; (2) participant characteristics (i.e. sample description, sample size, age, weight, height, sex, body fat content, VO_{2max} and menstrual phase at performance); (3) characteristics of the preceding exercise bout (i.e. exercise mode, duration, intensity, environmental conditions, sweat loss and recovery time); (4) characteristics of the nutritional intervention (i.e. blinding procedures, nutritional composition of intervention and control treatments (i.e. CHO content, fluid volume, osmolality, temperature, other constituents), time of first intake and time to

consume treatment); (5) characteristics of the subsequent athletic performance (i.e. exercise mode, duration, intensity, environmental conditions, type of performance test, cycle ergometer/treadmill device, incentives and performance), and; (6) subjective ratings of GI discomfort, where these were reported. Where data were presented in graphical form only, high-performance digital calipers (ABSOLUTE Digimatic Caliper 500 Mitutoyo, Kawasaki, Japan) were used to extract numeric values.

4.3.7. Statistical Analyses

All statistical procedures were performed using IBM SPSS Statistical Software, Version 22.0 and Comprehensive Meta-Analysis, Version 3.0. Weighted mean effect estimates and meta-regression coefficients are presented as Mean±SEM. All other data are presented as Mean±SD.

4.3.7a. Weighted Mean Effect

Meta-analyses were performed to determine the influence of: (1) CHO+W vs. W, and; (2) PRO+CHO+W vs. CHO+W on athletic performance. Individual effect sizes were calculated as the $\%_{\Delta}$MPO or the $\%_{\Delta}$PPO (see *4.3.4 Primary and Secondary Research Outcomes*), where a positive effect indicates an increase in power output under the intervention condition. As the current review elected to measure the performance change as a *percentage* of the control score (i.e. rather than a *net* difference), the SD of the performance change could not be determined via standard methods. Instead, *t*-statistics (or *p*-values) derived from paired *t*-tests were used to calculate the SD of the *percent* performance change. Where an exact value was quoted [278,294], the calculation was performed using the following formula [211]:

$$SD_{\Delta} = \frac{|\ \%_{\Delta}\ \text{MPO or PPO}\ |}{t\ \text{statistic}} \times \sqrt{n}$$

Where the SD_{Δ} is the standard deviation of the *percent* performance change and *n* is number of participants. Where $p<x$ ($x \neq 0.05$) was reported [276,287], *p* was taken to equal x and used to derive a *t*-statistic. Where only $p>x$ or $p<0.05$ was reported (and raw performance data could not be retrieved), the missing *t*-statistic was imputed using the

correlation coefficient (R). To do this, the SD_{Δ} of the *net* performance change was first calculated using the formula indicated below [211]:

$$SD_{\Delta} = \sqrt{(SD_{Control}^{2} + SD_{Intervention}^{2}) - (2 \times R \times SD_{Control} \times SD_{Interventions})}$$

Where SD_{Δ} is the standard deviation of the *net* performance change and R is the correlation coefficient. R was approximated as the mean correlation coefficient calculated using t-statistics (or p-values) derived from paired t-tests and/or raw performance data, as indicated by Higgins*, et al.* [211]. Sensitivity analyses were performed to test the robustness of the imputed R value. The imputed SD_{Δ} was then used to derive the required t-statistic, using the following formula:

$$t \text{ statistic} = \frac{\text{Mean Performance Score}_{Intervention} - \text{Mean Performance Score}_{Control}}{(SD_{\Delta} \div \sqrt{n})}$$

The weighted mean treatment effects were subsequently determined using random-effect models, where trials were weighted by the inverse variance for the performance change. Statistical significance was attained if the 95% CIs did not include zero. Heterogeneity was assessed with Cochran's Q and the I^2 index. Low, moderate and high heterogeneity was indicated by an I^2 value of 25, 50 and 75%, respectively [217]. A p-value <0.10 for Cochran's Q was used to indicate significant heterogeneity [211]. Sensitivity analyses were performed to determine risk of bias due to data dependency (i.e. where multiple trials derived from a single publication bias a result). In this case, meta-analyses were completed using data derived from one trial per publication, only. Results are presented in the online supplementary material. The practical significance (i.e. under real-world conditions) of the intervention effect on endurance exercise performance was determined using a spreadsheet by Hopkins [295]. The smallest worthwhile $\%_{\Delta}$MPO was calculated as 1.6% for endurance cyclists and 0.6% for endurance runners. These values were derived by multiplying the CV for a given competitive event (i.e. 1.3% for cycling events (1–40 km) and 1.1% for running events ≤10 km) by 0.5 [280] and transforming the threshold competition time to an equivalent

threshold for cycling/running power output [260]. The effect was interpreted as "unclear" if there was >5% chance of attaining a both a clinically positive and clinically negative influence.

4.3.7b. Meta-Regression Analyses

RML random effects meta-regression analyses were performed to determine the effect of: (1) CHO on performance when individuals are *not fasted* (i.e. ≤4 h post-meal) ahead of experimentation; (2) protein (co-ingested with CHO and water) on performance when CHO intake is "suboptimal" (i.e. <1.2 $g \cdot kg^{-1} \cdot h^{-1}$, as per Beelen*, et al.* [181]), and; (3) protein (co-ingested with CHO and water) on performance when the comparator condition was "isocarbohydrate", rather than "isoenergetic". In order to isolate these effects, it was necessary to control for the potentially cofounding influence of other variables. Simple meta-regression (i.e. one covariate per analysis) was performed to determine the influence of individual covariates on the magnitude of the performance change. If a significant relationship was identified (i.e. $p<0.05$), each of the covariates were re-examined, this time using multiple meta-regression (i.e. >1 covariate per analysis) to control for the influential factor. All covariates are defined in Table 4.2. At least 10 data points were required for a variable to qualify for meta-regression analysis. Categorical variables were dummy-transformed with m - 1, where m is the number of levels of the original variable. Regression analyses were examined for influential cases and outliers (i.e. studentized residuals, Cook's distance, and centered leverage values), normality of residuals (Shapiro-Wilk Test) and multicollinearity (VIF). Statistical significance was accepted as $p<0.05$.

Table 4.2. Covariates investigated

Covariate	Accepted Definition
Study Design	
Study Blinding	Single-blinded protocols vs. double-blinded protocols. Studies that did not employ a blinded protocol were omitted from the analysis of this variable [274,289,290] as there were insufficient data to construct a third 'non-blinded' category.
Time Since Last Meal	"Fed" subjects were tested in a post-prandial state (≤4 h post-meal, as defined by Pochmuller*, et al.* [255]) vs. "Fasted" subjects ≥10 h post-meal). When subjects were 4–10 h post-prandial, studies were omitted from the analysis of this variable [269,270].
Participant Population	
$VO_{2\ max}$	Studies that reported VO_{2max} in units of $mL \cdot min^{-1}$ were divided by the mean BM of the subject group to convert to $VO_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$), with the exception of Temesi*, et al.* [271] where standardisation against BM was not considered appropriate due to effects of paraplegia on BM.
Intervention Characteristics	
Time from First Intake to Performance	The length of time (h) between the first intervention exposure and commencement of the athletic performance task.
Total Fluid Intake	The total volume of fluid (L) consumed during the preceding exercise bout and/or subsequent recovery period under the intervention. Studies that administered an unspecified (but controlled) quantity of water alongside the experimental treatment [261,278,291,292] were omitted from the analysis of this variable.
Total CHO Intake	The Total CHO Intake (g) during the preceding exercise bout and/or subsequent recovery period under the intervention. Values that were reported relative to BM (kg) were multiplied by the mean BM of the subject group to approximate intake.
Relative CHO Intake	The Relative CHO Intake ($g \cdot kg^{-1}$) was determined by dividing the Total CHO Intake by the mean BM of the subject group. Ferguson-Stegall*, et al.* [264] was excluded as values could not be reliably calculated.
Rate of CHO Delivery	The Rate of CHO Delivery ($g \cdot kg^{-1} \cdot h^{-1}$) was determined by dividing the Relative CHO Intake by the Time From 1st Intake to Performance. Ferguson-Stegall*, et al.* [264] was excluded as values could not be reliably calculated.
Total Protein Intake	The Total Protein Intake (g) during the preceding exercise bout and/or subsequent recovery period under the intervention. Values that were reported relative to BM (kg) were multiplied by the mean BM of the subject group to approximate intake.
Relative Protein Intake	The Relative Protein Intake ($g \cdot kg^{-1}$) was determined by dividing the Total Protein Intake by the mean BM of the subject group. Ferguson-Stegall*, et al.* [264] was excluded as values could not be reliably calculated.
Energy Difference Between Beverages	The energy content (kJ) of the intervention minus the energy content of the control. Where the energy content of a treatment was not reported, it was calculated from the macronutrient composition, assuming an energy density of 16.7, 17.0 and 37.0 $kJ \cdot g^{-1}$ of CHO, protein and fat, respectively [18].
Performance Characteristics	
Performance Test	TTE vs. TT Performance Tests, defined as per section *4.3.4a. Time Trial Performance* and *4.3.4b. Time to Exhaustion Performance*.
Duration of the Performance Test	The length of time (min) between commencing and concluding the athletic performance task under the control condition. Temesi*, et al.* [271] was excluded as duration on an arm crank test may not be comparable to duration on a running or cycling test.
Total Exercise Time	Total Exercise Time represents the Duration of the Performance Test plus the length (min) of the preceding exercise bout. Temesi*, et al.* [271] was excluded as duration on an arm crank test may not be comparable to duration on a running or cycling test.
Exercise Mode	Running vs. cycling. The arm-crank test used in one study [271] was unable to be included in the analysis of this variable.

4.4. Results

4.4.1. Overview of Included Studies and Study Quality

The literature search identified 43 eligible investigations. However, one of these studies [296] was removed from the review because the performance data could not be converted to the common metric for endurance exercise performance (%$_{\Delta}$MPO on a TT test). Four studies [297-300] scored <50% on the Rosendal scale during the methodological quality assessment and were subsequently ineligible for inclusion. A further two trials were omitted from the analyses as outlying data (+17.95%$_{\Delta}$MPO [281]; +16.22%$_{\Delta}$ MPO [264]) with studentized residuals ≥3.3; excluding these trials did not influence the result of the CHO+W (%$_{\Delta}$MPO=4.246, 95% CI's: 3.413, 5.080, p<0.001) [281] or PRO+CHO+W (%$_{\Delta}$MPO=0.848, 95% CI's: -0.393, 2.089, p=0.180) [264] meta-analyses. Overall, 67 repeated-measures trials (n=745 participants, 90.4% male) derived from 37 original publications were reviewed. The included studies yielded a Rosendal Score of 63±9% (Mean±SD). The highest Rosendal Score of 81% was calculated for Betts*, et al.* [258]. Complete results of the quality assessment are published in the online supplementary material. A summary of included investigations is indicated in Table 4.3.

Table 4.3. Summary of experimental trials included in the current review

	CHO+W vs. W (Endurance Performance) 45 Trials; *n*=486 (92.9% male)		CHO+W vs. W (Anaerobic Performance) 9 Trials; *n*=134 (73.1% male)		PRO+CHO+W vs. CHO+W (Endurance Performance) 13 Trials; *n*=125 males	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
Study Characteristics						
Sample Size	11.1 ± 3.4	6–20	14.9 ± 12.0	8–36	9.6 ± 3.1	6–15
Double-Blinded Design	*n* = 32	-	*n* = 6	-	*n* = 12	-
Single-Blinded Design	*n* = 10	-	*n* = 2	-	*n* = 1	-
Subjects "Fasted" (≥10 h post-prandial)	*n* = 25	-	*n* = 6	-	*n* = 12	-
Subjects "Fed" (2 – 4 h post-prandial)	*n* = 11	-	*n* = 3	-	*n* = 1	-
Subject Characteristics						
Age (y)	29 ± 4	23–35	24 ± 3	22–30	26 ± 7	21–39
BM (kg)	73.4 ± 4.4	62.2–80.0	69.7 ± 4.7	63.4–78.6	72.3 ± 6.5	61.1–83.5
$VO_{2\,max}$ (mL· kg BM^{-1}·min^{-1})	56.4 ± 6.1	42.8–69.8	56.1 ± 4.5	47.1–61.7	60.8 ± 3.9	51.4–65.6
Intervention Characteristics						
Total Fluid Volume (L)	1.6 ± 0.7	0.2–3.6	0.5 ± 0.3	0.3–1.1	1.2 ± 0.5	0.7– 2.6
Time From 1st Intake to Performance (min)	124 ± 73	40–375	53 ± 9	36–68	168 ± 61	75–240
CHO Concentration (%)	9.4 ± 7.5	1.5–40.0	12.6 ± 7.1	6–20	7.5 ± 1.8	4.8–10.0
Protein Concentration (%)	-	-	-	-	2.0 ± 0.7	0.9–3.3
Total CHO Intake (g)	102 ± 50	30–247	51 ± 8	36–68	115 ± 61	50–232
Rate of CHO Delivery (g· kg^{-1}· h^{-1})	0.8 ± 0.6	0.2–1.3	0.8 ± 0.1	0.8–0.9	0.6 ± 0.3	0.2–1.05
Total Protein Intake (g)	-	-	-	-	35 ± 26	10–87
Relative Protein Intake (g· kg^{-1})	-	-	-	-	0.5 ± 0.4	1.2–0.1
Endurance Exercise Test						
TT Performance Test	*n* = 34	-	*n* = 9	-	*n* = 5	-
TTE Performance Test	*n* = 11	-	*n* = 0	-	*n* = 8	-
Performance Test Duration	24.8 ± 15.4 min	8.4–86.1 min	-	30–40 s	38.3 ± 28.8 min	7.2–100 min
Environmental Temperature (°C)	21 ± 4	10–32	NS	NS	NS	NS
Mode of Exercise Cycling	*n* = 38	-	*n* = 8	-	*n* = 8	-
Mode of Exercise Running	*n* = 7	-	*n* = 1	-	*n* = 5	-

BM: Body mass; M: Males; *n*: Number; NS: Not specified (or rarely specified). Values are presented as Mean±SD or a proportion (*n*) of the total number of trials for which the given characteristic is known. Note: Percentage body fat mass, peak sustainable power output and sweat loss were reported in too few studies for the data to accurately reflect the reviewed sample and were therefore omitted from the current summary.

4.4.2. The Effect of CHO (Co-Ingested with Water) on Athletic Performance

4.4.2a. Endurance Exercise Performance

Forty-five trials (*n*=486 participants; 92.9% male) derived from 25 publications investigated the effect of CHO+W on endurance performance (Table 4.4). The mean correlation coefficient (*R*=0.715) was imputed using raw performance data from 12 trials[a] and two *p*-values [276,287]. The weighted mean treatment effect suggests that CHO+W significantly improves endurance exercise performance (%$_{\Delta}$MPO=3.974, 95% CI's: 3.209, 4.739, $p<0.001$) when it is preceded by an initial bout of activity (Figure 4.3). The magnitude and significance of the effect was stable during sensitivity analyses where trials were sequentially removed (%$_{\Delta}$MPO range: 3.792–4.094; CIs did not include zero). Findings were also comparable across different levels of correlation (see online supplementary material). The magnitude of this effect is such that, >99% of the time, CHO+W will *almost certainly* produce a clinically positive effect on endurance performance, assuming a +1.6%$_{\Delta}$ in competitive cycling performance or a +0.6%$_{\Delta}$ in competitive running performance is required to covey a meaningful performance-enhancement. Moderate heterogeneity was present across trials (I^2=43.899, p=0.001).

Simple meta-regression identified a significant effect of Performance Test (i.e. "TTE" *n*=11 vs. "TT" *n*=33) (p=0.003, R^2=0.71) on %$_{\Delta}$MPO. Hence, the influence of this variable was controlled when modelling the effect of the remaining covariates on the change in endurance exercise performance. These analyses revealed a significant effect of Time Since Last Meal (i.e. "Fed" *n*=10 vs. "Fasted" *n*=25) (p=0.012), where Performance Test was controlled ($p<0.001$; R^2=1.00) (Figure 4.4). (One trial [261]$_{(a)}$ yielded comparatively large Cook's Distance values in the aforementioned analyses (Cook's d=0.50, all other trials ≤0.06; Cook's d=1.4, all other trials ≤0.13, respectively) and was omitted due to potential confounding effects). These data suggest that the effect of CHO+W to enhance endurance exercise performance may be attenuated in individuals who have consumed food 2–4 h prior to testing (%$_{\Delta}$MPO=0.605, if TTE; %$_{\Delta}$MPO=3.562, if TT) in comparison to individuals who are fasted overnight ahead of experimentation (%$_{\Delta}$MPO=3.112, if TTE; %$_{\Delta}$MPO=6.070, if TT). No other covariates significantly affected the magnitude of the performance change (p's >0.05) (Table 4.5).

[a] [264,266,271,272,274,281,289,290]

Table 4.4. Characteristics of studies investigating the effect of CHO co-ingested with water on endurance exercise performance

Citation	Participants	$VO_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Weight (kg)	Study Design	Time Since Last Meal (h)	Preceding Exercise	Recovery Time (min)	Beverage Administration	Mean Beverage Volume (mL)	Intervention Beverage CHO Content (%)	CHO Intake (g)	CHO Type(s) (%)	Time from First Intake to Performance (min)	Athletic Performance	Duration (min)	$\%_{\Delta}$ MPO
Murray et al. (1989a – c) [251], U.S.	12 (7 M)	42.8 ± 6.2	69.2 ± 14.5	DB	4	Cycle; 60 min; 65% $VO_{2\ max}$	0	2.5 $mL \cdot kg^{-1}$ pre-, & ea. 20 min of P-EX	692	(a) 6.0 (b) 8.0 (c) 10.0	41.5 54.3 69.2	S (6.0) S (8.0) S (10.0)	70	Cycle, TT (500 revs); 24°C	13.6	+4.53 +2.41 +0.37
Murray et al. (1991a – c) [252], U.S.	10 (8 M)	48.3 ± 2.6	72.9 ± 3.5	DB	2 – 3	Cycle; 120 min; 65-75% $VO_{2\ max}$	0	2.0 $mL \cdot kg\ LBM^{-1}$ ea. 15 min of P-EX	880	(a) 6.0 (b) 12.0 (c) 18.0	52.8 106 158	G (6.0) G+GP G+GP	105	Cycle, TT (4.8 km); 10°C	16.1	+6.09 +4.55 +6.47
Burgess et al. (1991) [253], U.S.	9 M Trained cyclists	59.9 ± 5.4	74.1 ± 5.4	SB	O/N	Cycle; 165 min; 70% $VO_{2\ max}$	0	3.5 $mL \cdot kg^{-1}$ ea. 20 min of P-EX	1,900	1.8	34.0	NS	140	Cycle, TTE (80% $VO_{2\ max}$); 22°C	16.1	-0.95
Millard-Stafford et al. (1992) [276], U.S.	8 M Trained long-distance runners	69.8 ± 3.7	70.5 ± 7.2	DB	O/N	Run; 35 km; "moderate" pace	0	400 mL pre-, & 250 mL ea. 5 km of P-EX	1,985	7.0	139	F (2.0)+GP (5.0)	180	Run, TT (5 km); 28°C	24.4	+11.42
Cole et al. (1993a – c) [269], U.S.	10 M Trained cyclists	59.6 ± 1.3	77.3 ± 1.9	SB	6 – 10	Cycle; 105 min; 70% $VO_{2\ max}$	0	~175 mL ea. 15 min of P-EX	1,506	(a) 6.0 (b) 8.3 (c) 8.3	90.0 125 125	G+S [a]G (3.6)+F (4.7) [a]G(2.8)+F(3.5)+GP(2.0)	90	Cycle, TT (15 min); 23°C	15.0	+2.42 +2.19 +2.10
Below et al. (1995a – b)[233], U.S.	8 M Endurance trained	62.9 ± 3.2	70.6 ± 8.5	DB	O/N	Cycle; 50 min; 5% above LT	0	40% pre- & 20% at 15, 25 & 34 min of P-EX	(a) 1,330 (b) 200	6.0 40	79.0	GP (6.0) GP (40.0)	50	Cycle, TT[b]; 31°C	10.9	+5.84 +7.49
El-Sayed et al. (1995) [282], U.K.	9 M Competitive cyclists	60.7 ± 6.6	69.9 ± 22.1	DB	4	Cycle; 60 min; 70% $VO_{2\ max}$	0	3.0 $mL \cdot kg^{-1}$ pre-, & ea. 20 min of P-EX	839	7.5	62.9	G (7.5)	75	Cycle, TT (10 min); 22°C	10.0	+8.49
McConell et al. (1996a – b) [270], Australia	9 M Trained cyclists/ triathletes	68.9 ± 5.6	71.7 ± 4.0	DB	6 – 12	Cycle; 120 min; 70% $VO_{2\ max}$	0	(a) 250 mL pre-, & ea. 15 min of P-EX (b) 250 mL at 90, 105 &120 min P-EX	2,250 750 (+1.5 L H_2O)	7.0 21.0	158 158	NS	120 45	Cycle, TT (15 min); 21°C	15.0	+10.74 +4.55
Casey et al. (2000a – b) [292], U.K.	10 M Well trained	52.7	76.1 ± 5.7	DB	O/N	Cycle; 83 ± 25 min; 70% $VO_{2\ max}$	240	1.0 g $CHO \cdot kg^{-1}$ at the onset of REC, only	410 (+H_2O intake NS)	18.5	76.1	(a) G (18.5) (b) S (18.5)	240	Cycle, TTE (70% $VO_{2\ max}$)	35	+1.31 +2.87
Wong et al. (2000) [287], U.K.	9 M Endurance trained	59.5 ± 4.5	71.0 ± .8.1	DB	O/N	Run; 90 min; 70% $VO_{2\ max}$	240	725 mL at 30 min REC & equal dose ea. 30 min of REC	3,582	6.9	247	NS	210	Run, TTE (70% $VO_{2\ max}$); 21°C	45.0	+4.94
Ivy et al. (2003a) [288], U.S.	9 M Trained cyclists	61.3 ± 7.2	69.6 ± 7.5	DB	O/N	Cycle; 190 min; 45-75% $VO_{2\ max}$	0	200 mL pre- & ea. 20 min of P-EX	2,000	7.8	155	NS	180	Cycle, TTE (85% $VO_{2\ max}$); 20°C	12.7	+4.15
Abbiss et al. (2008a – b) [261], Australia	10 M Endurance trained cyclists	61.7 ± 5.0	77.9 ± 6.6	DB	<4	Cycle; 90 min; 62% $VO_{2\ max}$	0	0.50 g $CHO \cdot kg^{-1}$ pre-, & 0.25 ea. 15 min of P-EX	600 (+H_2O intake NS)	25.0	150	S (25.0)	90	(a) Cycle, TT (16.1 km); 18°C (b) Cycle, TT (16.1 km); 32°C	25.4 27.5	0.00 +6.88

[a] The high fructose corn syrup used in this study was presumed to contain 55% fructose (with the remaining CHO as free-glucose) [301].

[b] Target work (J) = work rate at VO_2 at 10% above LT x 10 min 1 $60 s \cdot min^{-1}$ (value unpublished)

Table 4.4. *(continued)*

Citation	Participants	$VO_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Weight (kg)	Study Design	Time Since Last Meal (h)	Preceding Exercise	Recovery Time (min)	Beverage Administration	Mean Beverage Volume (mL)	Intervention Beverage CHO Content (%)	CHO Intake (g)	CHO Type(s) (%)	Time from First Intake to Performance (min)	Athletic Performance	Duration (min)	$\%_{\Delta}$ MPO
Osterberg et al. (2008) [262], U.S.	13 M Trained cyclists/ triathletes	56.0 ± 6.9	73.4 ± 9.0	DB	O/N	Cycle; 120 min; 5% below LT	0	250 mL ea. 15 min of P-EX	2,000	6.0	120	G+F+S (2.0 ea.)	105	Cycle, TT[a]; 23°C	39.7	+6.10
Cox et al. (2010) [272], Australia	16 M, Endurance trained cyclists/triathletes	61.7 ± 5.0	75.0 ± 6.7	NB	2	Cycle; 100 min; 70% $VO_{2\ max}$	~5	5 $mL \cdot kg^{-1}$ ea. 20 min of P-EX	1,875	10.0	188	G (10.0)	85	Cycle, TT[d]; 21°C	31.9	+5.87
Smith (2010a – c) [263], U.S.	12 M Recreational cyclists/triathletes	55.3 ± 3.6	77.6 ± 6.9	SB	O/N	Cycle; 120 min; 77% $VO_{2\ max}$	0	250 mL pre-, & ea. 15 min of P-EX	2,000	(a) 1.5 (b) 3.0 (c) 6.0	30.0 60.0 120	G (1.5) G (3.0) G (6.0)	105	Cycle, TT (20 km); 23°C	36.4	+7.14 +8.10 +10.48
Temesi et al. (2010) [271], Australia	6 (5 M) Tetraplegics/ paraplegics	62.2 ± 19.7	22.2 ± 7.8	DB	O/N	Arm-cycle; 60 min; 65% $VO_{2\ max}$	~5	125 mL pre-, & 0, 15, 30 min of P-EX	500	Variable (0.5g CHO·kg^{-1})	31.0	GP	75	Cycle, TT (20 km); 21°C	15	+2.73
Alghannam (2011) [289], U.K.	6 M Amateur soccer players	51.4 ± 5.0	71 ± 5	SB	O/N	Run; 45 min[b]	0	During P-EX	515 (+460 mL H_2O)	6.9	70.8	GP (6.9)	105	Run, TTE (80% $VO_{2\ max}$); 21°C	11	+3.99
Ferguson-Stegall et al. (2011) [264], U.S.	10 (5 M) Trained cyclists/ triathletes	52.6 ± 6.5	67.8 ± 7.3	DB	O/N	Cycle; 90 min; 75% $VO_{2\ max}$ and 5 × 60 s @ 90% $VO_{2\ max}$	240	50% at 0 and 120 min of REC	1,200 (+1.25 L H_2O)	15.2	181	G (15.2)	240	Cycle, TT (40 km); 21°C	86.1	+2.11
Lee et al. (2011a – b) [290], Singapore	12 M Physically active	53.9 ± 8.8	65.2 ± 6.6	(a) DB (b) NB	O/N	Cycle; 75 min; 65% $VO_{2\ max}$	300	Pre-, ea. 15 min of P-EX & at 15, 30, 45 & 60 min REC (dose NS)	2,325	6.8	158	G (4.8)+S (2.0)	375	Cycle, TTE (65% $VO_{2\ max}$); 32°C	32.0	+2.35 +3.36
Robson-Anseley et al. [275] (2011)	9 M Trained runners	58 ± 4	75.5 ± 7.4	DB	O/N	Run; 2 h; 60% $VO_{2\ max}$	0	2.0 $mL \cdot kg^{-1}$ pre-, & ea. 20 min of P-EX	1,057	8.0	85	NS	120	Run, TT (5 km); 20°C	24.0	+8.70
Bonetti & Hopkins et al. (2012a – c) [293], N.Z.	16 (Sex NS) Trained cyclists/ triathletes	52.4 ± 6.1	82.0 ± 8.8	DB	NS	Cycle; 120 min; 55-60% PPO	~10	250 mL ea. 15 min of P-EX	2,000	(a) 3.9 (b) 2.8 (c) 7.6	78 56 152	NS NS NS	130	Cycle, incremental TTE; 20°C	6.1	+4.15 +1.30 +3.63
McGawley et al. (2012) [279], Sweden	10 (6 M) Amateur triathletes	62.8 ± 9.3	66.8 ± 9.2	SB	NS	Swim 1500 m & Cycle 40 km (Intensity standardised)	0	25% ea. 10 km of P-EX (Cycle)	808	14.4	115	GP (9.6)+F (4.8)	40	Run, TT (10 km); 16°C	40.4	+4.39
Too et al. (2012a – b) [274], U.S.	11 M Competitive runners	58.2 ± 4.8	72.4 ± 11.1	NB	O/N	Run; 80 min; 75% $VO_{2\ max}$	0	0.50 g CHO·kg^{-1} pre-, & 0.2 ea. 20 min of P-EX	(a) Raisins (+1.23 L H_2O) (b) "Chews" (+1.23 L H2O)	N/A	94	NS	80	Run, TT (5 km); 22°C	21.6	+4.85 +4.35

[a] Target work =7 kJ·kg^{-1} (value unpublished)

[b] The soccer-specific exercise protocol consisting of various exercise intensities that are often observed during competitive soccer matches (e.g. walking, jogging and sprinting) [302]

Table 4.4. *(continued)*

Citation	Participants	$VO_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Weight (kg)	Study Design	Time Since Last Meal (h)	Preceding Exercise	Recovery Time (min)	Beverage Administration	Mean Beverage Volume (mL)	Intervention Beverage CHO Content (%)	CHO Intake (g)	CHO Type(s) (%)	Time from First Intake to Performance (min)	Athletic Performance	Duration (min)	$\%_{\Delta}$ MPO
Heesch et al. (2013a – c) [265], U.S.	8 M Recreational cyclists	56.8 ± 5.2	80.0 ± 6.3	DB	O/N	Cycle; 120 min; 62% $VO_{2\ max}$	0	(a) 250 mL pre-, & ea. 15 min of P-EX	2,000	3.0	60	GP (3.0)	120	Cycle, TT (10 km); 22°C	18.1	+5.49
								(b) 250 mL pre-, & ea. 15 min of 1st h P-EX	1,000 (+1.0 L H_2O)	6.0		GP (6.0)	120			+5.06
								(c) 250 mL ea. 15 min of 2nd h P-EX	1,000 (+1.0 L H_2O)	6.0		GP (6.0)	60			+7.17
Newell et al. (2015a – c) [266], U.K	20 M Trained cyclists/ triathletes	62 ± 9	74.6 ± 7.9	DB	O/N	Cycle; 120 min; 59% $VO_{2\ max}$	~5	240 mL pre-, & ~220 mL ea. 15 min of P-EX	2,000	(a) 2.0	40.0	NS	125	Cycle, TT[a]; 19°C	37.0	+5.70
										(b) 3.9	78.0	NS				+8.00
										(c) 6.4	128	NS				+9.00
Greer et al. (2011) [281], U.S.	9 M Untrained	36.3 ± 2.2	84.2 ± 17.0	SB	4	Cycle; 90 min; 55% $VO_{2\ max}$	0	50% pre-, & 50% 60 min into P-EX	837	6.1	51.1	NS	65	Cycle, TT (15 min); 22°C	15	+17.95

$\%_{\Delta}$ MPO: Percent change in mean power output; CHO: Carbohydrate; DB: Double-blinded; ea.: Each; F: Fructose; G: Glucose; GP: Glucose polymers; LBM: Lean body mass; M: Male; NB: Non-blinded; NS: Not specified; O/N: Fasted overnight (≥10 h); P-EX: Preceding exercise; REC: Recovery time; S: Sucrose; SB: Single-blinded; TT: Time trial performance test; TTE: Time to exhaustion performance test. Shaded trials were excluded from the meta-analysis.

[a] Target work = 0.7 × PPO (W) × 1800 (value unpublished)

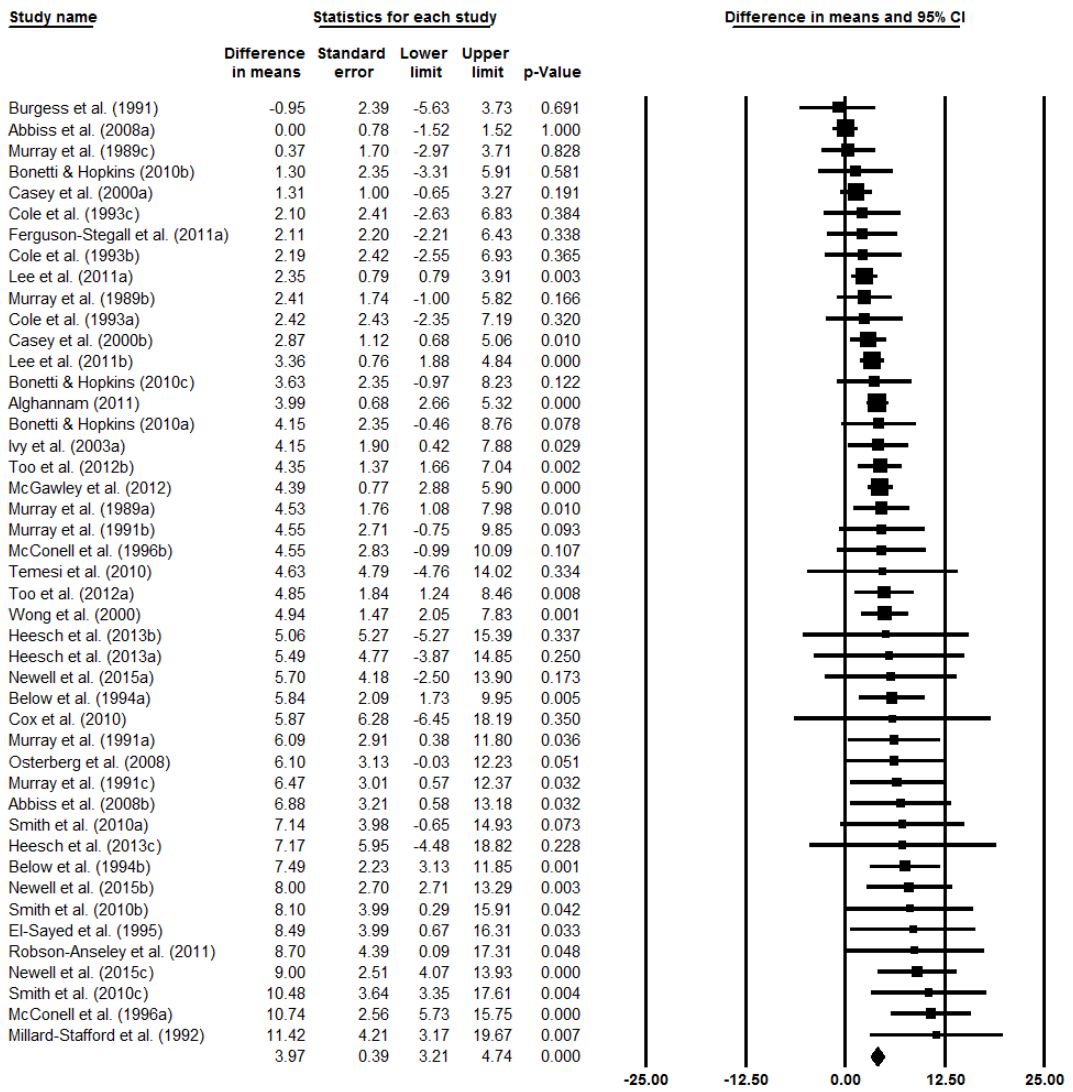

Figure 4.3. Forest plot displaying the effect of CHO+W vs. W on %$_{\Delta}$ MPO. Size of the squares are proportional to the weight of the study. A positive effect estimate indicates greater power output with CHO+W than W.

Table 4.5. Summary of covariates analysed (via RML multiple meta-regression analyses) for the CHO+W treatment. The influence of Performance Test and Time Since Last Meal was controlled in each model.

Effect Estimate	Mean Difference (%$_{\Delta}$ MPO)	
Covariate	Coefficient (95% CI)	*p* value
Study Blinding (SB vs. DB)	1.128 (-0.371, 2.626)	0.134
$VO_{2\,max}$	0.126 (-0.025, 0.276)	0.100
Time from First Intake to Performance	-0.004 (-0.010, 0.003)	0.231
Total Fluid Intake	0.001 (-0.001, 0.001)	0.885
Total CHO Intake	-0.637 (-2.627, 1.353)	0.518
Relative CHO Intake	0.031 (-0.779, 0.840)	0.939
Rate of CHO Delivery	-0.637 (-2.627, 1.353)	0.518
Duration of Performance Test	-0.027 (-0.061, 0.008)	0.127
Total Exercise Duration	-0.002 (-0.018, 0.014)	0.787
Exercise Mode (Run vs. Cycle)	0.821 (-0.512, 2.153)	0.218

DB: Double blinded studies; SD: Single blinded studies.

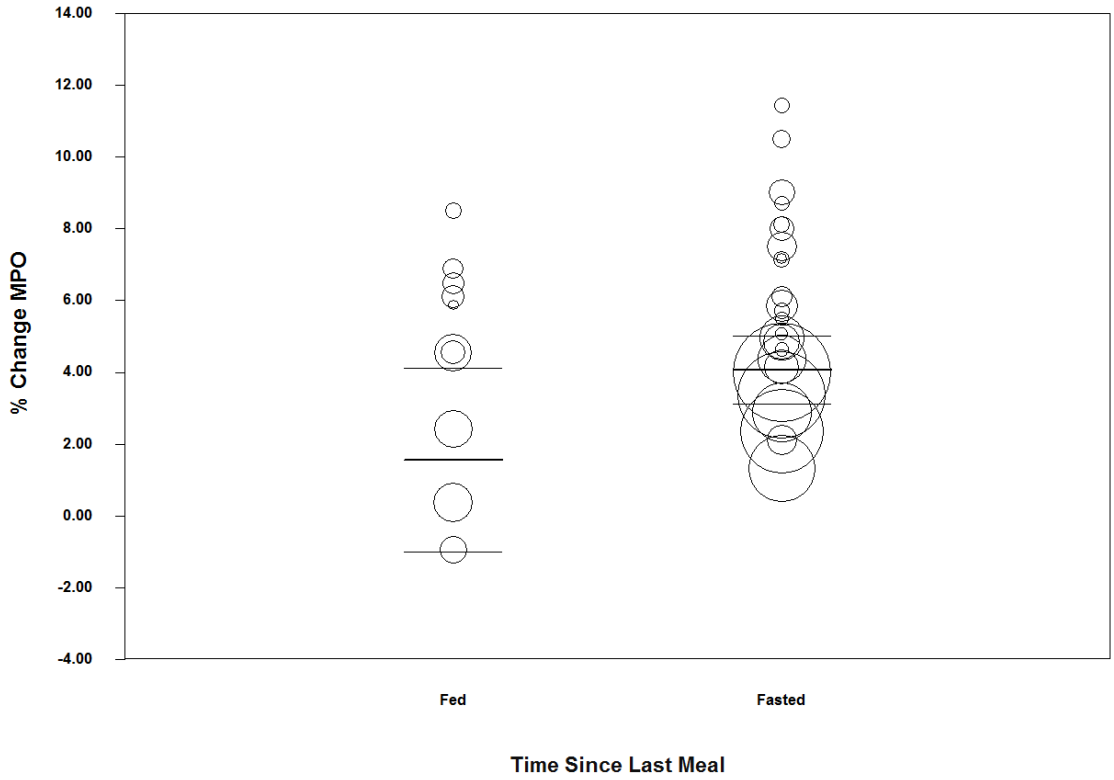

Figure 4.4. Correlation between Time Since Last Meal (Fed vs. Fasted) and the $\%_{\Delta}$ MPO (95% CI's), controlling for Performance Test (TT vs. TTE). Circle diameter corresponds to the weight of each trial (*n* =35).

4.4.2b. Anaerobic Exercise Performance

Nine trials (*n*=134 participants; 73.1% male) derived from 5 publications investigated the effect of CHO+W on anaerobic exercise performance (Table 4.6). The mean correlation coefficient (*R*=0.905) was imputed using raw performance data from one trial [303] and one *p*-value [294]. The weighted mean treatment effect (Figure 4.5) suggests that CHO+W improves anaerobic performance ($\%_{\Delta}$PPO=2.548, 95% CI's: 1.114, 3.982, $p<0.001$) when it is preceded by an initial bout of exercise. Low heterogeneity was present across trials ($I^2=0.000$, $p=0.679$). The magnitude and significance of the effect was stable during sensitivity analyses ($\%_{\Delta}$PPO range: 2.026–2.845, CIs did not include zero). Findings were also comparable across different levels of correlation (see online supplementary material).

Table 4.6. Characteristics of studies that investigated the effect of CHO co-ingested with fluid on anaerobic exercise performance

Citation	Participants	$VO_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Weight (kg)	Study Design	Time Since Last Meal (h)	Preceding Exercise	Recovery Time (min)	Beverage Administration	Mean Beverage Volume (mL)	Intervention Beverage CHO Content (%)	CHO Intake (g)	CHO Type(s) (%)	Time From First Intake to Performance (min)	Athletic Performance	$\%_{\Delta}$ PPO
Ball et al. (1995) [294], U.S.	8 M Competitive cyclists	61.7 ± 5.2	78.6 ± 8.2	SB	O/N	Cycle; 50 min; 80% $VO_{2\ max}$	0	2 $mL \cdot kg^{-1}$ at 10, 20, 30 & 40 min P-EX	629	8.0	50.3	NS	40	30 s Wingate Test	+6.21
Sugiura et al. (1998a – d) [304], Japan	8 M Competitive cyclists/triathlete	56.1 ± 3.8	66.9 ± 4.5	DB	O/N	(a, b) Cycle; 2 × 45 min blocks (with 15 min REC); 75% $VO_{2\ max}$	0	250 mL during 15 min REC	250	20.0	50.0	(a) GP (20.0)	60	40 s Wingate Test	+3.81
												(b) F (20.0)			+1.79
						(c, d) Cycle; 2 × 45 min blocks (with 15 min REC); 65-100% $VO_{2\ max}$						(c) GP (20.0)			+2.78
												(d) F (20.0)			+2.03
Jarvis et al. (1999) [305], U.S.	10 (0 M) Trained cyclists	47.1 ± 3.8	63.4 ± 7.3	DB	O/N	Cycle; 50 min; 80% $VO_{2\ max}$	0	2.0 $mL \cdot kg^{-1}$ at 0, 20, 30 & 40 min P-EX	507	7.0	35.5	GP (7.0)	40	40 s Wingate Test	+3.40
Clarke et al. (2005) [303], U.K.	12 M University soccer players	59.4 ± 6.0	74.5 ± 6.0	DB	3 – 4	Run, 45 min[a]	15	7 $mL \cdot kg^{-1}$ before P-EX & 7 $mL \cdot kg^{-1}$ during 15 min REC	1,065	6.4	67.7	NS	60	Run, 9 × 3.3 s sprints (30 s total)	-0.67
O'Neil et al. (2013a – b) [306], U.S.	36 (23 M)	NS	71.4 ± 12.1	(a) SB	2 – 4	Cycle; 5 × 10 min blocks (with 2 min REC); 60-65% HR_{max}	0	25% at 0, 20, 40 & 60 min P-EX	847	6.0	50.8	NS	50	30 s Wingate Test	+2.36
				(b) NB											+1.25

$\%_{\Delta}$ PPO: Percent change in peak power output; CHO: Carbohydrate; DB: Double-blinded; F: Fructose; G: Glucose; GP: Glucose polymers; HR_{max}: Age predicted maximum heart rate; M: Males; NB: Non-blinded; NS: Not specified; O/N: Fasted overnight (≥10 h); P-EX: Preceding exercise; REC: Recovery time; SB: Single-blinded. Shaded trials were excluded from the meta-analysis.

[a] The soccer-specific exercise protocol consisting of various exercise intensities that are often observed during competitive soccer matches (e.g. walking, jogging and sprinting) [302]

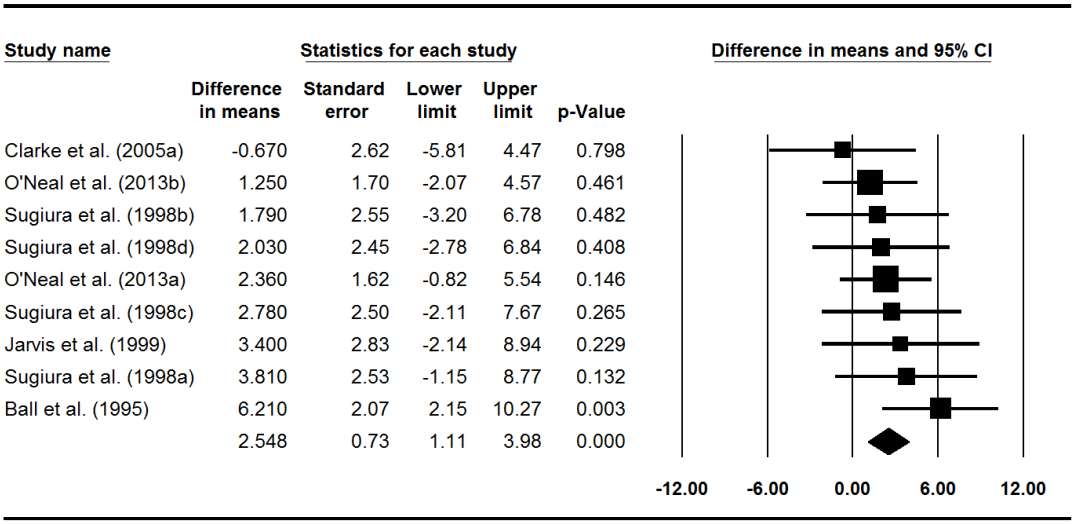

Figure 4.5. Forest plot displaying the effect of CHO+W vs. W on %ΔPPO. Size of the squares are proportional to the weight of the study. A positive effect estimate indicates greater power output with CHO+W than W.

4.4.3. The Effect of Protein (Co-Ingested with CHO and Water) on Endurance Exercise Performance

Thirteen trials (*n*=125 male participants) derived from 9 publications investigated the effect of PRO+CHO+W on subsequent endurance exercise performance (Tables 4.7 & 4.8). The mean correlation coefficient (*R*=0.752) was imputed using raw performance data from 4 trials [264,273,289] and one *p*-value derived from a paired *t*-test [278]. The weighted mean treatment effect indicates no difference in endurance exercise performance between PRO+CHO+W and CHO+W (%ΔMPO=0.547, 95% CI: -0.523, 1.616, *p*=0.316) (Figure 4.6), despite the CHO dose being "suboptimal" (<1.2 g· kg BM^{-1}· h^{-1}) on all trials. The magnitude and significance of the effect was stable during sensitivity analyses (%ΔMPO range: 0.188–0.866, all 95% CIs included zero). Findings are also comparable across different levels of correlation (see online supplementary material). The magnitude of this effect is such that, 97% of the time, PRO+CHO+W will *very likely* produce a clinically trivial effect on cycling performance; and 51% of the time will *possibly* produce a clinically trivial effect on running performance, assuming a +1.6%Δ in competitive cycling performance or a +0.6%Δ in competitive running performance is required to covey a meaningful performance-enhancement. Moderate to high heterogeneity was observed across trials (%Δ MPO: I^2 = 72.92, *p*<0.001).

Initially, none of the proposed moderators were able to account for the between-trial variability observed (all simple meta-regression analyses, *p*>0.10). However, on

removing the study that received the lowest Rosendal score (53%) (and the only investigation that did not employ a double-blinded design) [289] a significant effect of the Energy Difference Between Beverages was observed (p=0.015, R^2=1.00) (Figure 4.7). (Nb. One trial [258]$_{(b)}$ yielded a very large Cook's Distance (Cook's d=8.12, all other trials ≤0.25) and was omitted from this analysis due to potential confounding effects). These data suggest that the %$_{\Delta}$MPO may be greater in trials that administered an intervention beverage that contained more energy than the control beverage (i.e. those that matched beverage CHO content). While it important to acknowledge that the two trials omitted from this analysis observed a large benefit of protein ingestion using isoenergetic beverages, a trend for a significant effect of this covariate on the %$_{\Delta}$MPO (p=0.098, R^2=1.00) remained detectable when the outlying study [264] was reintroduced to the analysis. The remaining covariates were investigated using simple meta-regression analyses, given that the small cohort of trials (n=11) was not appropriate for multiple meta-regression. These covariates did not significantly affect the magnitude of the performance change (all p's >0.05) (Table 4.9).

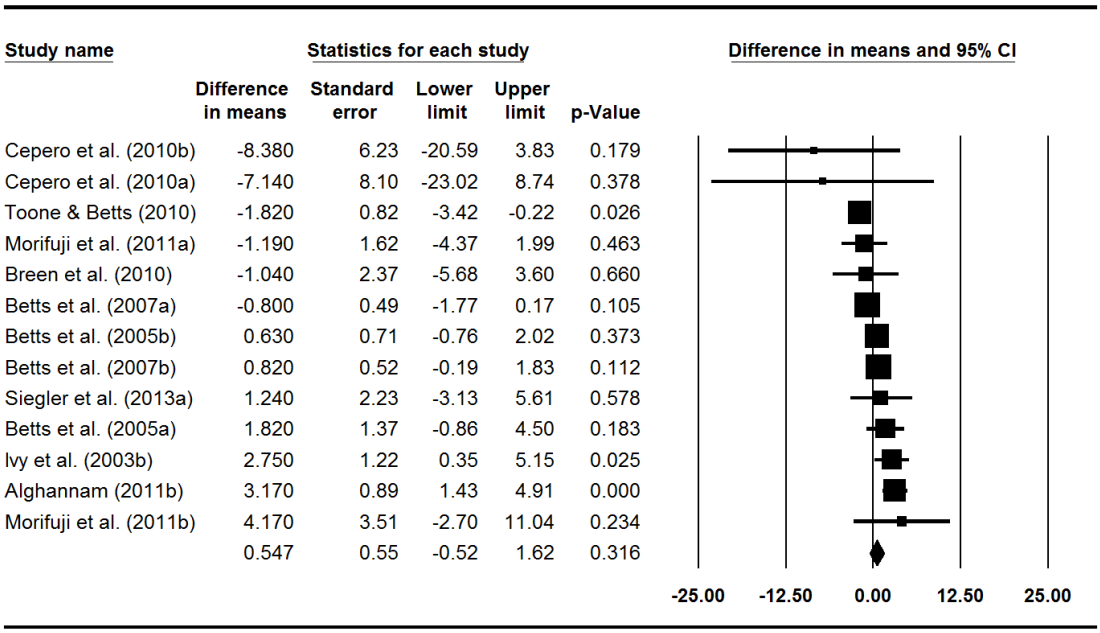

Figure 4.6. Forest plot displaying the effect of PRO+CHO+W vs. CHO+W on %$_{\Delta}$ MPO. Size of the squares are proportional to the weight of the study. A positive effect estimate indicates greater power output with PRO+CHO+W than CHO+W.

Table 4.7. Characteristics of studies that investigated the effect of protein co-ingested with CHO and fluid on endurance exercise performance.

Citation	Participants	$VO_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Weight (kg)	Study Design	Time Since Last Meal (h)	Preceding Exercise	Recovery Time (h)	Beverage Administration	Time from First Intake to Performance (min)	Athletic Performance	Duration (min)	%Δ MPO
Ivy et al. (2003b) [288], US	9 M Trained cyclists	61.3 ± 7.2	69.6 ± 7.5	DB	O/N	Cycle; 180 min; 45/75% $VO_{2\ max}$	0	200 mL pre-, & ea. 20 min of P-EX	180	Cycle, TTE (85% $VO_{2\ max}$); 20°C	19.7	+2.75
Betts et al. (2005a – b) [291], U.K.	(a) 9 M Recreationally active	59.7 ± 11.4	79.6 ± 11.2	DB	O/N	Run, 90 min; 70% $VO_{2\ max}$	4	12.5% ea. 30 min of REC	240	Run, TTE (85% $VO_{2\ max}$); 23°C	23.4	+1.82
	(b) 7 M Recreationally active	55 ± 11	83.5 ± 11.8								25.0	+0.63
Betts et al. (2007a – b) [258], U.K.	6 M Recreationally active	61.4 ± 7.3	72.6 ± 8.4	DB	O/N	Run, 90 min; 70% $VO_{2\ max}$	4	12.5% ea. 30 min of REC	240	Run, TTE (70% $VO_{2\ max}$); 21°C	(a) 99.9 (b) 83.7	(a) -0.80 (b) +0.82
Breen et al. (2010) [267], U.K	12 M Trained cyclists	62.7 ± 6.3	70.5 ± 5.0	DB	NS	Cycle, 120 min; 50% PPO	0	270 mL ea. 15 min of P-EX	105	Cycle, TT (880 ± 94 kJ)	60.2	-1.04
Cepero et al. (2010a – b) [277], Spain	15 M Cyclists	65.6 ± 10.3	74.4 ± 7.2	DB	O/N	Cycle, 60 min; 75% $VO_{2\ max}$	2	1 L during 2 h REC	120	Cycle, TT (20 km)	29.5	(a) -7.14 (b) -8.38
Toone & Betts (2010) [278]	12 M Highly trained cyclists	64.3 ± 6.4	72.5 ± 5.2	DB	O/N	Cycle, 45 min; 60-90% $VO_{2\ max}$	0	7.0 $mL \cdot kg^{-1}$ pre-, & 2.5 ea. 15 min of P-EX	195	Cycle, TT (6 km)	7.2	-1.82
Alghannam et al. (2011b) [289]	6 M Amateur footballers	51.4 ± 5.0	71 ± 5	SB	O/N	Run, 75 min[a]	0	During P-EX	75	Run, TTE (80% $VO_{2\ max}$)	16.5	+3.17
Morifuji et al. (2011a – b) [273]	8 M Trained	60.1 ± 8.8	61.1 ± 5.6	DB	2	Cycle, 70 min; 68/88% $VO_{2\ max}$	2	350 mL at 0, 30, 60, 90 & 120 min of REC	120	Cycle, TT (365 ± 40 kJ); 21°C	36.8	(a) -1.19 (b) +4.17
Siegler et al. (2013) [268]	12 M	52.5 ± 5.2	76.0 ± 8.3	DB	O/N	Cycle, 90 min; 50% $VO_{2\ max}$	0	180 mL ea. 15 min of P-EX	90	Cycle, TT (5 km); 21°C	7.6	+1.24
Ferguson-Stegall et al. (2011b) [264]	10 (5M) Trained cyclists/triathletes	52.6 ± 6.5	67.8 ± 7.3	DB	O/N	Cycle, 100 min; 70% $VO_{2\ max}$	4	50% at 0 and 120 min of REC	240	Cycle, TT (40 km)	86.1	+16.22

%Δ MPO: Percent change in mean power output; DB: Double-blinded; ea.: Each; M: Male subjects; NS: Not specified; O/N: Fasted overnight (≥10 h); P-EX: Preceding exercise; REC: Recovery; TT: Time trial performance test; TTE: Time to exhaustion performance test. Shaded trials were excluded from the meta-analysis.

[a] A soccer-specific exercise protocol consisting of various exercise intensities that are often observed during competitive soccer matches (e.g. walking, jogging and sprinting) Clarke, *et al.* [302]

Table 4.8. Characteristics of beverages used in studies that investigated the effect of protein co-ingested with CHO and fluid on endurance exercise performance

		Control Beverage				Intervention Beverage									
Citation	Mean Beverage Volume (mL)	CHO Content (%)	CHO Intake (g)	CHO Intake ($g \cdot kg \cdot h^{-1}$)	CHO Type(s) (%)	CHO Content (%)	CHO Intake (g)	CHO Intake ($g \cdot kg \cdot h^{-1}$)	CHO Type(s) (%)	PRO Content (%)	PRO Intake (g)	PRO Intake ($g \cdot kg^{-1}$)	PRO Type(s) (%)	Mean Energy Intake (kJ)	Energy Difference from Control Beverage (kJ)
Ivy et al. (2003b) [288], US	2,000	7.8	157	0.75	NS	7.8	157	0.75	NS	1.9	38.8	0.56	NS	3,282	+660
Betts et al. (2005a – b) [291], U.K.	(a) 1,031 ($+H_2O$ intake NS)	9.3	95.9	0.30	G (6.2) + F (3.1)	9.3	95.9	0.30	G (6.2) + F (3.1)	1.5	15.5	0.19	WP(H)	1,867	+264
	(b) 722 ($+H_2O$ intake NS)	9.3	67.1	0.20		9.3	67.1	0.20		1.5	10.8	0.13		1,303	+184
Betts et al. (2007a – b) [258], U.K.	581 (+1.3 L H_2O)	(a) 13.3	320	1.10	S (13.0)	10.0	232	0.80	S (10.0)	3.3	87.0	1.20	WP	5,342	0
		(b) 10.0	232	0.80	S (10.0)	10.0	232	0.80	S (10.0)	3.3	87.0	1.20		5,342	+1,459
Breen et al. (2010) [267], U.K	2160 (+430 mL H_2O)	6.0	130	1.05	GP (6.0)	6.0	130	1.05	GP (6.0)	1.8	39.0	0.55	Protein (H)	2,834	+663
Cepero et al. (2010a – b) [277], Spain	1,000	9.0	90.0	0.60	NS	7.0	70.0	0.47	NS	2.0	20.0	0.27	(a) WP(H)	1,505	0
													(b) CP(H)		0
Toone & Betts (2010) [278]	1,053 ($+H_2O$ intake NS)	9.0	94.8	0.40	S (9.0)	6.8	71.6	0.30	S (6.8)	2.2	23.0	0.32	WP(I)	1,586	0
Alghannam et al. (2011b) [289]	515 (+460 mL H_2O)	6.9	70.8	0.80	GP (6.9)	4.8	49.6	0.56	GP (4.8)	2.1	21.2	0.30	WP	1,189	0
Morifuji et al. (2011a – b) [273]	1,750 (+300 mL H_2O)	5.0	87.5	0.72	GP (5.0)	5.0	87.5	0.72	GP (5.0)	(a) 0.9	15.0	0.25	WP(H)	1,725	+254
										(b) 2.3	40.0	0.65		2,150	+680
Siegler et al. (2013) [268]	1,260	8.3	105	0.92	GP (8.3)	6.3	79.4	0.70	GP (6.3)	1.6	20.2	0.27	WP(I)	1,680	-74
Ferguson-Stegall et al. (2011b) [264]	1,200	15.2	181	0.67	NS	11.5	138	0.51	NS	3.7	44.0	0.65	Milk-Protein	3,965	0

(H): Hydrolysate; (I): Isolate; CHO: Carbohydrate; CP: Casein Protein; F: Fructose; G: Glucose; GP: Glucose polymers; NS: Not specified; S: Sucrose; WP: Whey Protein. Shaded trials were excluded from the meta-analysis.

Table 4.9. Summary of covariates analysed (via RML simple meta-regression) for PRO+CHO+W (Excluding Alghannam [289]).

Effect Estimate	Mean Difference (%ΔMPO)	
Covariate	**Coefficient (95% CI's)**	***p* value**
$VO_{2\ max}$	-0.223 (-0.569, 0.122)	0.181
Time from First Intake to Performance	0.004 (-0.020, 0.028)	0.717
Total CHO Intake	0.002 (-0.016, 0.020)	0.778
Relative CHO Intake	0.173 (-0.010, 1.445)	0.769
Rate of CHO Delivery	0.414 (-4.295, 5.122)	0.859
Total Protein Intake	-0.001 (-0.040, 0.040)	0.994
Relative Protein Intake	0.024 (-2.824, 2.873)	0.985
Performance Test (TT vs. TTE)	-1.761 (-3.972, 0.450)	0.106
Duration of Performance Test	-0.006 (-0.042, 0.031)	0.738
Total Exercise Duration	0.012 (-0.011, 0.035)	0.270
Exercise Mode (Run vs. Cycle)	0.566 (-1.886, 3.018)	0.618

DB: Double blinded studies; SD: Single blinded studies. Analysis of Study Blinding (SB vs. DB), Time Since Last Meal (Fed vs. Fasted) and Total Fluid Intake could not be completed due to insufficient trials.

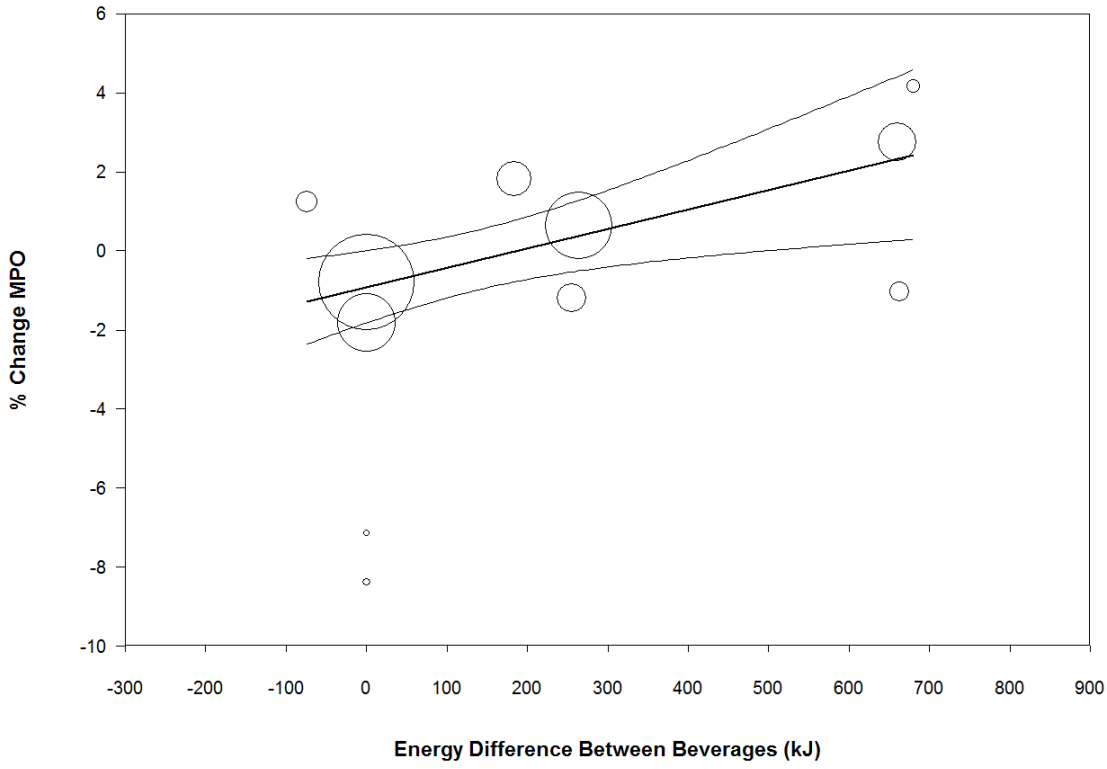

Figure 4.7. Correlation between Energy Difference Between Beverages (kJ) and %Δ MPO (95% CI's). Circle diameter corresponds to the weight of each trial (*n*=11).

4.4.4. Subjective Gastrointestinal Tolerance

Twelve trials derived from 6 publications measured GI tolerance following dietary intervention [251,252,274,279,287,289]. These data are summarised in (see online

supplementary material). The median CHO intake (i.e. at the time symptomology was assessed) was ~50 g (range: 10.4–247 g) whereas fluid intake was 522 mL (range: 174–3582 mL) (excluding baseline values); only one trial [289] assessed GI tolerance following protein ingestion (21.2 g). The majority of trials observed negligible to mild GI distress (e.g. scores 0–25%); no treatment elicited a score >50%. That said, one trial [258]$_{(a)}$ (which did not present GI symptomology data graphically or numerically) commented that two participants experienced such severe GI distress on the CHO+W treatment the performance test had to be terminated. This trial delivered the largest quantity of CHO in the present review (320 g). Only Wong*, et al.* [287] assessed GI tolerance *during* the athletic performance test. The collective data do not appear to indicate a trend for increased GI discomfort on intervention vs. control trials.

4.5. Discussion

The present systematic review and meta-analysis summarises evidence for the effect of: (1) CHO co-ingested with water; and (2) protein co-ingested with CHO (and water), during and/or following an initial bout of exercise on subsequent athletic performance. Results indicate a beneficial effect of CHO on subsequent endurance exercise performance. While the magnitude of the improvement was significantly diminished when participants were "Fed" (i.e. a meal ≤4 h prior to initial bout) as opposed to "Fasted" (i.e. at the onset of the initial exercise bout), a positive effect of CHO was still observed under the "Fed" condition. No further benefit was derived with the addition of protein to a CHO-containing beverage. Indeed, the performance-enhancing effect of protein demonstrated in some studies appeared to be a consequence of the additional energy it delivered (i.e. compared to an isocarbohydrate control), rather than an effect of the nutrient per se. A significant improvement in anaerobic exercise performance was also observed with CHO ingestion. Collectively, findings from the present investigation indicate that athletes with limited time for nutritional intake between consecutive exercise sessions should prioritise CHO ingestion (with fluid) to enhance subsequent athletic performance.

4.5.1. The Effect of CHO (Co-Ingested with Water) on Athletic Performance

The weighted mean effect estimate indicates that CHO co-ingested with water during and/or following an initial bout of activity improves subsequent endurance exercise performance, compared to water only. More specifically, CHO administration (102 ± 50 g; 0.8 ± 0.6 $g \cdot kg^{-1} \cdot h^{-1}$) increased MPO on a TT test by ~4.0%, such that >99% of the time, the magnitude of the performance-enhancement (i.e. during competitive endurance cycling or running) is almost certain to be meaningful. While the precise mechanisms underpinning these effects were not assessed in this review, accelerated muscle glycogen resynthesis [160], sparing of endogenous substrate stores [307], maintenance of blood glucose concentration and CHO oxidation rates in the latter stages of exercise [308], and activation of central mechanisms [175] may be contributing factors. It is important to acknowledge that the inferences in this investigation are based on calculations of the smallest change required to enhance performance in a competitive endurance event (i.e. a *single* maximum effort) [260]. A performance test that is conducted after an initial exercise bout (and a period of recovery) may demonstrate greater test-retest variability; such that the magnitude of improvement required to enhance performance may be increased. However, the research candidate is not aware of calculated CVs that would facilitate this assessment. In any case, the smallest worthwhile change would need to increase considerably to alter the outcome of the present analysis.

Except for one trial [253], all individual effect-estimates indicated a beneficial effect of CHO ingestion on endurance exercise performance. However, the magnitude of improvement was heterogeneous (I^2=43.9). Meta-regression analyses determined that differences in Time Since Last Meal ("Fed" vs. "Fasted") and Performance Test (TT vs. TTE) could explain a large proportion of this heterogeneity (R^2=1.00). In regard to the influence of Time Since Last Meal, results suggest that the CHO-mediated performance-effect may be exaggerated in "Fasted", compared to "Fed", individuals. This may be due to a larger contrast in substrate availability under W vs. CHO+W treatments, resulting from lower glycogen concentrations post-exercise, and subsequently, accelerated glycogen resynthesis on exposure to CHO [160]. In most circumstances, athletes are recommended to avoid commencing exercise in a fasted state [2]. The current data indicate greater variability in the effect of CHO within the "Fed" sub-group (Figure 4.4).

This may be partly due to the smaller number of "Fed" trials analysed. However, it could also reflect differences in the nutritional composition of the pre-exercise diet. Indeed, where the CHO content of the pre-exercise diet was specified, it ranged between 1.0–2.1 $g \cdot kg^{-1}$ [252,261,272]. This, along with other food-related factors (e.g. GIx, other macronutrients and dietary constituents, and timing of intake) [2], could potentially influence the response to CHO. A detailed description of participants' pre-exercise diet was not always indicated in the manuscripts reviewed; hence, it was not possible to explore the influence of these factors on subsequent performance further. Despite the observed variability, a significant benefit of CHO ingestion was still detectable in the presence of a pre-exercise meal.

The current results also suggest that the CHO-mediated performance-effect may be accentuated on TT compared to TTE performance tests. This observation is consistent with evidence from Vandenbogaerde*, et al.* [309], who detected a small difference in the magnitude of the effect of CHO supplementation across different performance tests in a meta-analytic investigation.

The weighted treatment effect indicates that CHO (53 ± 9 g; 0.8 ± 0.1 $g \cdot kg^{-1} \cdot h^{-1}$) co-ingested with water during and/or following an initial bout of exercise significantly increases PPO on a subsequent anaerobic performance test, compared to water only. Endogenous CHO availability is not usually a limiting factor in anaerobic exercise performance. Furthermore, pre-exercise muscle glycogen concentrations do not generally influence PPO on short-duration performance tests [310,311]. One factor that might explain the observed effect of CHO is enhanced central drive and/or motivation due to the presence of CHO in the oral cavity (i.e. oral CHO receptor-mediated effects) [312]. Indeed, CHO mouth-rinsing (i.e. repeating CHO exposures during exercise), has been shown to enhance exercise performance [175]. In the reviewed studies, the time between the final CHO exposure and the onset of performance was typically ≥10 min (up to 45 min [304]). At present, it is unclear how long CHO receptor-mediated effects persist. In addition, given that the CHO in the current studies was ingested, gut-mediated responses (not just via the oral cavity), may be involved in influencing performance results [175]. The capacity for nutrient-sensitive receptors within the GI tract to modulate exercise performance is not well understood [175].

4.5.2. The Effect of Protein (Co-Ingested with CHO and Water) on Endurance Exercise Performance

Protein (35 ± 26 g; 0.5 ± 0.4 g·kg^{-1}) co-ingested with CHO (115 ± 61 g; 0.6 ± 0.3 g·kg^{-1}·h^{-1}) (and water) during and/or following an initial bout of activity did not influence subsequent endurance exercise performance compared to control conditions (i.e. CHO+W). Indeed, the present analyses indicated only a ~0.5% increase in MPO on a TT test, such that 97% of the time, the effect of PRO+CHO+W on real-world endurance cycling performance is *very likely* to be trivial (i.e. no practical benefit or harm). Similarly, PRO+CHO+W will *possibly* produce a trivial effect on real-world endurance running, 51% of the time. Again, it is important to acknowledge that these inferences are based on the smallest change required to enhance performance in a *single* maximum effort; which may not reflect the performance variability observed under the conditions of a subsequent exercise task.

While prior research suggests that protein is unlikely to influence muscle glycogen resynthesis when co-ingested with an "optimal" dose of CHO (i.e. ≥1.2 g·kg^{-1}·h^{-1}, to maximise muscle glycogen repletion), protein consumed with a "suboptimal" CHO dose (i.e. <1.2 g· kg^{-1}· h^{-1}) may accelerate this process [181]. (Protein ingestion also has the potential to influence skeletal muscle-damage repair during recovery from endurance exercise [180]; however, the amount of protein synthesis that occurs within ≤4 h is probably small). No studies in the current review administered protein with an "optimal" CHO dose. Rather, the rate of CHO delivery ranged between 0.2–1.05 g·kg^{-1}·h^{-1}. Even with a "suboptimal" CHO intake, endurance performance was unaffected by PRO+CHO+W. Furthermore, subsequent regression analyses failed to indicate a significant effect of Relative CHO Intake (g·kg^{-1}) on %$_{\Delta}$ MPO, suggesting that the performance effect of dietary protein may be unrelated to CHO availability. These data are inconsistent with findings from a previous review [257] which reported that protein ingestion could improve subsequent endurance performance, provided CHO delivery was inadequate. However, this investigation defined "optimal" and "suboptimal" based on the rate of *nutrient* delivery (i.e. ≥1.0 g CHO/PRO· kg^{-1}· h^{-1} was "optimal"), as opposed to the rate of CHO delivery. Furthermore, the conclusions of the review were determined via visual inspection of the available data and were unsupported by statistical methods. One possible explanation for the lack of effect of dietary protein is that the

difference in muscle glycogen concentrations under the PRO+CHO+W vs. CHO+W conditions ≤4 h post-treatment is too small to convey a practical benefit.

The magnitude and direction of the individual effect-estimates in the PRO+CHO+W analysis was heterogeneous (I^2=72.92). Initially, none of the proposed moderator variables were able to account for the inconsistencies observed. However, a significant effect of the Energy Difference Between Control and Intervention Beverages (R^2=1.00) did become apparent on removing one study. This study [289] received the lowest Rosendal score (53%) and was the only investigation in the analysis that did not employ a double-blind experimental design. Clearly, blinding of investigators is an important consideration in experimental trials. This may be particularly true of *performance-based trials* where conscious or unconscious actions of an investigator (e.g. differences in verbal or non-verbal encouragement) have the potential to impact performance [313]. During Part 1 of this investigation (W vs. CHO+W), regression analyses were performed to evaluate the influence of blinding on the performance result observed. However, this was not possible in the current analysis where only one study failed to employ a double-blind experimental design. Therefore, we determined that the most conservative approach was to conduct the analyses while both including, and excluding, this investigation. The significant influence of the Energy Difference Between Beverages (detected where Alghannam [289] was omitted) suggests that the magnitude of the performance effect may be related to the quantity of additional energy administered under the PRO+CHO+W condition, such that the benefit of protein demonstrated in some studies appears to be a consequence of the energy delivered in this nutrient, rather than an isolated effect of protein itself. This observation is consistent with experimental data by Betts*, et al.* [258], who demonstrated a benefit of protein ingestion in comparison to an "isocarbohydrate" control (+1400 kJ); where no effect was observed against an "isoenergetic" control.

4.5.3. Gastrointestinal Tolerance

A subgroup of 12 trials assessed GI symptomology following dietary intervention. Collectively, these data indicate similar mild levels of GI distress following either CHO+W or W ingestion (only one trial [289] assessed GI discomfort following PRO+CHO+W). Thus, ingestion of CHO with fluid provides a performance benefit without exacerbating GI

intolerance. However, there are several limitations to the current evidence. First, the quantity of CHO and fluid ingested at the time of performance assessment was relatively low (~50 g and 500 mL, respectively). Current guidelines [2] recommend individuals ingest fluid in volumes equal to 1.25–1.50 L· kg BM lost^{-1} and consume 1.0–1.2 g CHO ·kg^{-1}·h^{-1} (for 4 h) to restore fluid losses and optimise glycogen resynthesis, where the length of time separating one bout of exercise from another is <8 h. Thus, nutrients ingested as per the guidelines may elicit different GI responses. Second, only Wong, *et al.* [287] assessed GI tolerance during the athletic performance test. GI symptomology may be exacerbated during exercise [314]; therefore, ratings obtained at rest or during submaximal intensity exercise may not provide a true indication of tolerance. Nevertheless, it appears that CHO ingested with fluid in amounts likely to benefit athletic performance does not augment GI distress any more than water alone. However, the extent to which CHO, protein and fluid are tolerated when ingested between consecutive exercise sessions in amounts corresponding with current nutrition recommendations requires further consideration.

4.5.4. Limitations

This review does contain several limitations. First, only studies with accessible full text articles written in English were included. Second, it is likely that differences in the preceding exercise bout affected the level of glycogen depletion incurred across trials. While these differences may moderate the effect of dietary intervention on the magnitude of the performance change, it was not possible to reliably estimate the severity of substrate depletion based on a description of the exercise task, and subsequently control for this influence. Third, the practical relevance of the effect of CHO ingestion on endurance exercise performance in a "Fed" state could not be calculated while simultaneously controlling for the influence of Performance Test (TT vs. TTE); the practical relevance of the effect of CHO ingestion on %$_{\Delta}$PPO is also unknown, as the significance of this outcome in a real-life context is yet to be fully characterised. Finally, while pre-loaded exercise protocols were accepted in this review, these may not precisely reflect the demands of consecutive exercise sessions, due to the limited amount of time separating the pre-load task from the performance test.

4.6. Conclusion

Results of the present review suggest that individuals who have limited opportunity for nutritional recovery between exercise sessions (e.g. ≤4 h) should prioritise CHO ingestion (with fluid) during and/or following the initial exercise bout to enhance performance on subsequent tasks involving endurance and anaerobic activity. Protein ingestion is unlikely to benefit or harm subsequent endurance performance and should be consumed as recommended to facilitate muscle protein synthesis [2].

Chapter 5: Summary of Thesis Part I

Thesis Part I investigated how common beverage constituents; namely, water, CHO and protein, affect subsequent athletic performance when consumed between exercise sessions with limited recovery time (i.e. ≤4 h).

Initially, a systematic and meta-analytic literature review (Study 1; presented in Chapter 3) was conducted to explore the effect of fluid consumption during or following dehydration on subsequent (i.e. ≤4 h recovery) athletic performance. Briefly, the investigation indicated that fluid (water) intake improved performance on a subsequent bout of continuous exercise and that the magnitude of this improvement tended to increase as both the environmental temperature and length of exercise increased. Research investigating the effect of fluid intake on intermittent exercise, resistance exercise, sport-specific technical skills and cognition was relatively limited, and a narrative synthesis of the available data failed to indicate a clear improvement, suggesting that further investigation is required in these areas. With Study 1 identifying a benefit of fluid intake, Study 2 was conducted to determine whether the addition of other nutrients; specifically, CHO and protein, to a post-exercise beverage could enhance subsequent athletic performance further. Unlike Study 1, Study 2 primarily focused on endurance performance, since these tasks indicated a clear benefit of fluid intake. Overall, the investigation revealed that fluid co-ingested with CHO during and/or following an initial bout of exercise improved subsequent endurance performance compared to fluid ingested alone. The administration of additional CHO also improved subsequent anaerobic exercise performance. Conversely, protein added to a CHO-containing post-exercise beverage did not influence enhance subsequent endurance performance further (though, fewer studies were found to have examined the influence of this nutrient). One limitation that should be acknowledged is that the aforementioned investigations included a combination of studies – some of which administered fluid or nutrients during, and others following, the initial bout of exercise or the dehydration protocol. These approaches could potentially elicit different physiological responses (e.g. effects on T_c or substrate utilisation) and should be considered when generalising the results to one context or the other. That said, given the strength and magnitude of the fluid- and CHO-mediated performance benefits (i.e. on endurance tests), it is unlikely that these situations would indicate substantially different effects.

Collectively, the research presented in Thesis Part I suggests that athletes who have limited opportunity for nutritional recovery between consecutive bouts of dehydrating exercise (e.g. ≤4 h) should prioritise consumption of CHO and water to enhance subsequent endurance performance. Protein consumption seems unlikely to benefit or harm athletic performance and should therefore occur recommended to facilitate muscle damage repair. Given their ability to deliver fluid and CHO simultaneously, CHO-containing beverages (e.g. CHO-electrolyte sports beverages and milk/milk-based formulations), may be ideal beverages to consume when undertaking consecutive endurance exercise sessions and attempting to optimise performance on the subsequent activity. However, studies employing ecologically valid experimental protocols in which participants are free to eat and drink voluntarily (i.e. *ad libitum*), are needed to determine how different beverages impact short-term post-exercise recovery (i.e. the restoration of fluid and substrate losses) and subsequent endurance performance in a free-living environment.

THESIS PART II

“Whole Beverages”

The Effect of Different Post-Exercise Beverages on *Ad Libitum* Fluid Recovery, Nutrient Provision and Subsequent Athletic Performance

Chapter 6: Literature Review II

6.1. Preface

6.2. Post-Exercise Fluid Recovery: Beverage Composition, Volume and Ingestion Rate

6.3. Other Determinants of Post-Exercise Fluid Recovery

6.4. Different Post-Exercise Beverages: Effects on Nutrient Provision and Subsequent Athletic Performance

6.5. Chapter Summary

6.1. Preface

This chapter summarises research investigating the effect of different post-exercise beverages on short-term (i.e. ≤4 h) post-exercise recovery and subsequent athletic performance; studies employing longer (though still relatively short) recovery periods (i.e. up to 6 h) are, however, considered where appropriate. While Thesis Part I considered the manner in which individual beverage constituents (i.e. fluid and nutrients) influence short-term post-exercise recovery and subsequent athletic performance, Part II examines how different post-exercise beverages affect these processes when consumed in a more ecologically valid context.

The review initially describes factors that affect post-exercise fluid recovery, including beverage volume, composition and rate of ingestion. It then summarises findings from studies investigating the ability of different beverages to rehydrate post-exercise (i.e. "rehydration potential") and outlines the major limitations of the research completed to date. In particular, the fact that studies typically "prescribe" drinking (i.e. administer fixed quantities of fluid) and deny participants access to food. Subsequently, results from the small number of investigations that have allowed participants to consume fluid *ad libitum* and/or with food are reviewed in detail. Finally, the review considers how different post-exercise beverages affect nutrient provision and subsequent athletic performance when consumed with food.

Peer-reviewed research articles were identified by searching the online databases PubMed (MEDLINE), Web of Science (via Thomas Reuters) Google Scholar and Scopus using a combination of terms, including fluid, beverage, rehydration, fluid recovery, fluid balance, fluid restoration, energy, nutrients, post-exercise, endurance, aerobic and

athletic performance. Cross-matching of citation reference lists and forward citation searches were also completed to ensure all relevant articles were captured.

6.2. Post-Exercise Fluid Recovery: Beverage Volume, Composition and Ingestion Rate

This section of the review describes factors that affect post-exercise fluid recovery, including beverage volume, composition and rate of ingestion. Most of the studies presented here follow a similar format; whereby, after completing a controlled bout of exercise to induce hypohydration, participants are administered a "test beverage" and then instructed to collect their urine output and measure their BM at regular intervals throughout an observation period (i.e. ~1–6 h) to determine fluid balance. A review of the literature preceding February 2019 identified 53 original research studies (*n*=526, 88% male) investigating the restoration of fluid balance post-exercise in healthy adults (i.e. ≥18 y) using a repeated-measures design. Each of the studies described in this section of the review prescribed drinking (i.e. administered a fixed quantity of fluid) and prohibited the consumption of food in the post-exercise period. A summary of these investigations is indicated in Table 6.1.

6.2.1 Fluid Recovery and Beverage Volume

Beverage volume is an important determinant of post-exercise rehydration. Shirreffs*, et al.* [142] administered hypohydrated participants (i.e. –2.1% BM) either 0.5, 1.0, 1.5 or 2.0 L·kg BM lost^{-1} in the hour following exercise; reporting that the volume of fluid ingested needed to *exceed* the amount lost to restore euhydration. Simply replacing fluid losses (i.e. 1.0 L·kg BM lost^{-1}) was insufficient, since – even in a hypohydrated state – the consumption of fluid (i.e. particularly, large quantities of dilute beverages, see *6.2.2. Fluid Recovery and Beverage Composition*) reduced plasma Na^+ concentration and P_{OSM}, stimulating fluid-induced diuresis [142]. Importantly, the same study demonstrated that these large quantities of fluid were only effective (i.e. able to completely restore euhydration) when the beverage consumed had a high Na^+ concentration (i.e. 61 mmol·L^{-1}) [142]. A lower Na^+ beverage (i.e. 23 mmol·L^{-1}) could not restore euhydration, even when it was consumed in very large amounts (i.e. ≥1.5 L·kg BM lost^{-1}).

Table 6.1. Studies investigating fluid retention of different beverages after exercise-induced dehydration using a repeated-measures experimental design. Each of these studies *prescribed* drinking (i.e. administered a fixed quantity of fluid at a pre-determined rate) and prohibited the consumption of food during the post-exercise (i.e. rehydration) period.

Citation	Subjects	BM loss (%)	Fluid Intake (% of BM loss); Drink Time	Time Post-Meal	Post-Drink Observation Period	Fluid (Product name, if known)	Beverage Nutrient Composition (Energy and macronutrients per 100 g)							Fluid Retention (%)	Net TBW Balance (L)
							Energy (kJ)	Protein (g)	Fat (g)	CHO (g)	Na^+ ($mmol \cdot L^{-1}$)	K^+ ($mmol \cdot L^{-1}$)	Osmolality		
Nielson, *et al*. [315](1986), Denmark	6 M	3.1	~110%[a]; 2 h	Fasted	0	CHO + Electrolyte Drink	42	0	0	2.5	116	0	229	79	- 0.4
						CHO + Electrolyte Drink	157	0	0	9.4	35	17	465	88	- 0.1
						CHO + Electrolyte Drink	42	0	0	2.5	346	0	387	92	- 0.1
						CHO + Electrolyte Drink	42	0	0	2.5	116	138	323	84	- 0.3
Gonzalez-Alonso, *et al*. [316] (1992), USA	10 (8 M)	2.5	100%; 2 h	2–3 h	0	Water	0	0	0	0	0	0	10	64 ± 16	- 0.7
						Cola Drink (Diet Coca-Cola®)	0	0	0	0	4	0	59	54 ± 16	- 0.9
						CHO + Electrolyte Drink (Gatorade®)	100	0	0	6.0	20	3	395	69 ± 16	- 0.6
Gonzalez-Alonso, *et al*. [316] (1992), USA	19 (16 M)	2.5	100%; 2 h	2–3 h	0	Water	0	0	0	0	0	0	10	65 ± 13	- 0.6
						CHO + Electrolyte Drink (Gatorade®)	100	0	0	6.0	20	3	395	73 ± 13	- 0.5
Lambert, *et al*. [317] (1992), USA[b]	8 M	4.1	100%; 195 min	NS	45 min	CHO + Electrolyte Drink (Carbonated)	167	0	0	10.0	11	4	648	66	-1.1 ± 0.3
						CHO + Electrolyte Drink	167	0	0	10.0	11	4	627	65	-1.2 ± 0.3
						Electrolyte Drink (Carbonated)	0	0	0	0	7	5	57	57	-1.4 ± 0.3
						Electrolyte Drink	0	0	0	0	7	5	36	61	-1.3 ± 0.3
Maughan, *et al*. [318] (1994), UK	8 M	2.1	100%; 0.5 h	Fasted	6 h	CHO Drink	27	0	0	1.6	0	0	123	65	- 0.6
						Electrolyte Drink	0	0	0	0	60	0	141	85	- 0.3
						Electrolyte Drink	0	0	0	0	0	25	76	82	- 0.3
						CHO + Electrolyte Drink	27	0	0	1.6	60	25	281	85	- 0.3
Mitchell, *et al*. [143] (1994), USA	9 M	2.5	100%; 3 h	NS	0	Electrolyte Drink	0	0	0	0	15	8	35	55[c]	- 0.7
			150%; 3 h			Electrolyte Drink	0	0	0	0	15	8	35	55[c]	- 0.3
Maughan & Leiper [319] (1995), UK	6 M	1.9	150%; 0.5 h	Fasted	5.5 h	Water	0	0	0	0	2	0	NS	33	- 0.7 ± 0.3
						Electrolyte Drink	0	0	0	0	26	0	NS	49	- 0.4 ± 0.2
						Electrolyte Drink	0	0	0	0	52	0	NS	67	- 0.0 ± 0.2
						Electrolyte Drink	0	0	0	0	100	0	NS	71	+ 0.1 ± 0.2
Shirreffs, *et al*. [142] (1996), UK	6 M	2.1	50%; 1 h	6 h	6 h	Electrolyte Drink	0	0	0	0	23	0	NS	78 ± 6	- 0.9
			100%; 1 h			Electrolyte Drink	0	0	0	0	23	0	NS	69 ± 14	- 0.5
			150%; 1 h			Electrolyte Drink	0	0	0	0	23	0	NS	66 ± 15	- 0.1
			200%; 1 h			Electrolyte Drink	0	0	0	0	23	0	NS	51 ± 13	- 0.1

[a] Fixed volume of 2.7 L ≈ 110% of BM loss

[b] Before, immediately after dehydration, and at 1, 2, 3 and 4 h the subjects performed 5 min cycle exercise at 70% VO2 max (40°C and 40% RH). Therefore, net TBW balance (mL) and fluid retention (%) are a product of both urinary and (ongoing) sweat water loss due to physical activity.

[c] Fluid retention (%) is adjusted to account for the volume of fluid remaining in the stomach at the end of the observation period (98.9 vs. 86.0%, for the 1.00 and 1.50 L· kg BM $lost^{-1}$ conditions, respectively).

Table 6.1. *(continued)*

Citation	Subjects	BM loss (%)	Fluid Intake (% of BM loss); Drink Time	Time Post-Meal	Post-Drink Observation Period	Fluid (Product name, if known)	Beverage Nutrient Composition (Energy and macronutrients per 100 g)							Fluid Retention (%)	Net TBW Balance (L)
							Energy (kJ)	Protein (g)	Fat (g)	CHO (g)	Na^+ ($mmol \cdot L^{-1}$)	K^+ ($mmol \cdot L^{-1}$)	Osmolality		
Shirreffs, *et al*. [142] (1996), UK	6 M	2.1	50%; 1 h	6 h	6 h	Electrolyte Drink	0	0	0	0	61	0	NS	81 ± 3	- 1.0
			100%; 1 h			Electrolyte Drink	0	0	0	0	61	0	NS	83 ± 7	- 0.3
			150%; 1 h			Electrolyte Drink	0	0	0	0	61	0	NS	72 ± 12	+ 0.1
			200%; 1 h			Electrolyte Drink	0	0	0	0	61	0	NS	65 ± 11	+ 0.4
Shirreffs and Maughan [320] (1997), UK	6 M	2.0	150%; 1 h	6 h	6 h	Beer, 0% alc.	140	0.2	0.1	8.0	2	10	NS	59 ± 16	- 0.2
						Beer, 1% alc.	169	0.2	0.1	8.0	2	10	NS	53 ± 11	- 0.3
						Beer, 2% alc.	198	0.2	0.1	8.0	2	10	NS	50 ± 16	- 0.4
						Beer, 4% alc.	256	0.2	0.1	8.0	2	10	NS	41 ± 17	- 0.6
Shirreffs and Maughan [321] (1998), UK	6 M	1.9	150%; 1 h	Fasted	6 h	Water	0	0	0	0	0	1	19	40	- 0.6 ± 0.3
						Electrolyte Drink	0	0	0	0	25	1	64	46	- 0.4 ± 0.4
						Electrolyte Drink	0	0	0	0	50	1	107	53	- 0.3 ± 0.4
						Electrolyte Drink	0	0	0	0	100	1	199	68	+ 0.0 ± 0.3
Wong, *et al*. (1998) [322], China	7 (2 M)	2.5	100%; 4 h	Fasted	0	CHO + Electrolyte Drink (Lucozade-Sport)	115	0	0	6.9	24	3	300	54	- 0.7
Bilzon, *et al*. [323] (2000), UK	13 M	1.7	140%; ~195 min	1.5 h	~45 min	Water	0	0	0	0	NS	NS	NS	71	+ 0.0 ± 0.2
						CHO + Electrolyte Drink (Lucozade-Sport)	115	0	0	6.9	24	3	300	74	+ 0.0 ± 0.1
Mitchell, *et al*. [324] (2000), USA	10 M	2.9	100%; 3 h	NS	0	Electrolyte Drink	0	0	0	0	25	NS	NS	71	- 0.7
			100%; 3 h			Electrolyte Drink	0	0	0	0	50	NS	NS	76	- 0.6
			150%; 3 h			Electrolyte Drink	0	0	0	0	35	NS	NS	68	+ 0.0
			150%; 3 h			Electrolyte Drink	0	0	0	0	50	NS	NS	69	+ 0.1
Wong, *et al*. [287] (2000), China	9 M	3.0	89-200[a]%; 3 h	Fasted	1 h	Water	0	0	0	0	NS	NS	NS	73	+ 0.4 ± 0.7
						CHO + Electrolyte Drink (Lucozade-Sport)	115	0	0	6.9	24	3	300	71	+ 0.4 ± 0.6
Wong, *et al*. [325] (2000), China	9 M	2.5	150%; 3 h	Fasted	1 h	CHO + Electrolyte Drink (Lucozade-Sport)	115	0	0	6.9	24	3	300	64 ± 21	+ 0.0 ± 0.6
Kovacs, *et al*. [326] (2002), Netherlands	8 M	3.0	120%; 3 h	2 h	3 h	CHO + Electrolyte Drink	127	0	0	7.6	31	5	NS	82 ± 14	- 0.4 ± 0.3
			120%; 5 h		1 h	CHO + Electrolyte Drink	127	0	0	7.6	31	5	NS	79 ± 17	- 0.5 ± 0.4
Saat, *et al*. [327] (2002), Malaysia	8 M	2.8	120%; 2 h	Fasted	0	Water	0	0	0	0	0	0	0	73 ± 14	- 0.2
						CHO + Electrolyte Drink (Isomax™)	54	0	0	3.2	19	4	404	75 ± 14	- 0.2
						Coconut Water	42	0	0	2.5	5	52	405	80 ± 11	- 0.1
Singh, *et al*. [297], (2002), Malaysia	13 M	3.2	120%; 2 h	~2–3 h	0	Water	0	0	0	0	NS	NS	NS	60 ± 18	- 0.6
						CHO + Electrolyte Drink (Isostar®)	127	0	0	7.6	30	5	303	70 ± 11	- 0.4
Seifert, *et al*. [328] (2006), USA	13 (8 M)	2.4	100%; 3 h	NS	0	Water	0	0	0	0	NS	NS	2	53 ± 16	- 1.1
						CHO, Electrolyte + Protein Drink (Accelerade™)	126	1.5	0	6.0	23	7	305	88 ± 5	- 0.5
						CHO + Electrolyte Drink (Gatorade®)	100	0	0	6.0	20	3	280	75 ± 15	- 0.9

[a] Some subjects were unable to consume all of the prescribed fluid (a volume equal to 200% of BM loss) due to GI discomfort

Table 6.1. *(continued)*

Citation	Subjects	BM loss (%)	Fluid Intake (% of BM loss); Drink Time	Time Post-Meal	Post-Drink Observation Period	Fluid (Product name, if known)	Beverage Nutrient Composition (Energy and macronutrients per 100 g)							Fluid Retention (%)	Net TBW Balance (L)
							Energy (kJ)	Protein (g)	Fat (g)	CHO (g)	Na^+ ($mmol \cdot L^{-1}$)	K^+ ($mmol \cdot L^{-1}$)	Osmolality		
Ismail, *et al*. [329] (2007), Malaysia	10 M	3.1	120%; 1.5 h	1 h	30 min	Water	0	0	0	0	NS	NS	NS	59 ± 5.7	- 0.6
						CHO + Electrolyte Drink	62	0	0	3.7	20	4	321	68 ± 5.1	- 0.4
						Coconut Water	67	1.0	0	3.0	9	50	384	65 ± 5.3	- 0.4
						Coconut Water (Sodium Enriched)	67	1.0	0	3.0	20	50	411	69 ± 4.4	- 0.3
Shirreffs, *et al*. [330] (2007), UK	8 (4 M)	1.9	150%; 1 h	Fasted	4 h	Water	0	0	0	0	0	0	16	42	- 0.5 ± 0.3
						Water	0	0	0	0	1	0	15	49	- 0.4 ± 0.4
						"Apfelshorle"[a]	112	0	0	6.7	8	30	404	51	- 0.4 ± 0.3
						CHO + Electrolyte Drink (Gatorade®)	100	0	0	6.0	23	6	353	60	- 0.2 ± 0.4
Shirreffs, *et al*. [331] (2007), UK	11 (5 M)	1.8	150%; 1 h	Fasted	4 h	Water	0	0	0	0	0	0	0	36 ± 10	- 0.6 ± 0.1
						CHO + Electrolyte Drink (Powerade®)	102	0	0	6.0	23	2	283	38 ± 16	- 0.6 ± 0.3
						Cow's Milk (Low Fat)	148	3.6	0.3	5.0	38	45	299	69 ± 10	+ 0.0 ± 0.2
						Cow's Milk (Low Fat; Sodium Enriched)	148	3.6	0.3	5.0	58	45	345	72 ± 4	+ 0.1 ± 0.1
Merson, *et al*. [332] (2008), UK	8 M	2.0	150%; 1 h	Fasted	4 h	Water	0	0	0	0	2	0	27	39 ± 14	- 0.7
						Electrolyte Drink	0	0	0	0	30	0	83	50 ± 13	- 0.4
						Electrolyte Drink	0	0	0	0	40	0	102	60 ± 14	- 0.2
						Electrolyte Drink	0	0	0	0	50	0	120	64 ± 11	- 0.1
Watson, *et al*. [333] (2008), U.K	7 M	2.0	150%; 1 h	Fasted	3 h	CHO + Electrolyte Drink (Powerade®)	100	0	0	6.0	23	2	280	62	- 0.1 ± 0.4
						Cow's Milk (Fat Free)	142	3.3	0.1	5.0	32	42	278	75	+ 0.2 ± 0.2
Evans, *et al*. [334] (2009), UK	6 M	1.9	150%; 1 h	Fasted	6 h	Electrolyte Drink	0	0	0	0	32	1	79	27 ± 13	- 0.8
						CHO + Electrolyte Drink	33	0	0	2.0	32	1	193	40 ± 14	- 0.6
						CHO + Electrolyte Drink	167	0	0	10.0	32	1	667	46 ± 9	- 0.4
Hobson, *et al*. [335] (2010), UK	12 M	1.9	~70%[b]; 30 min	4 h	4 h	Beer, 0% alc. (Bavaria™)	105	0.2	0	5.8	2	NS	NS	74	-1.7 ± 0.4
						Beer, 4% alc. (Bavaria™)	105	0.2	0	5.8	2	NS	NS	58	-1.9 ± 0.3
Osterberg, *et al*. [336] (2010), USA	15 M	2.6	100%; 1 h	3 h	3 h	Water	0	0	0	0	0	0	15	66 ± 14	- 0.7
						Electrolyte Drink	0	0	0	0	18	3	49	72 ± 10	- 0.6
						CHO + Electrolyte Drink	50	0	0	3.0	18	3	187	75 ± 8	- 0.5
						CHO + Electrolyte Drink	100	0	0	6.0	18	3	338	75 ± 16	- 0.5
						CHO + Electrolyte Drink	200	0	0	12.0	18	3	691	82 ± 9	- 0.4
James, *et al*. [337] (2011), UK	8 M	1.9	150%; 1 h	Fasted	4 h	CHO + Electrolyte Drink	115	0.0	0.0	6.5	7	5	247	43 ± 15	- 0.5 ± 0.3
						CHO, Electrolyte + Protein Drink	115	2.5	0.0	4.0	7	4	229	55 ± 12	- 0.3 ± 0.3

[a]Apfelschorle is a carbonated apple drink comprising of apple juice (60%) and carbonated mineral water (40%).

[b] Fixed volume of 1.0 L ≈ 70% of BM loss

Table 6.1. *(continued)*

Citation	Subjects	BM loss (%)	Fluid Intake (% of BM loss); Drink Time	Time Post-Meal	Post-Drink Observation Period	Fluid (Product name, if known)	Beverage Nutrient Composition (Energy and macronutrients per 100 g)							Fluid Retention (%)	Net TBW Balance (L)
							Energy (kJ)	Protein (g)	Fat (g)	CHO (g)	Na^+ ($mmol \cdot L^{-1}$)	K^+ ($mmol \cdot L^{-1}$)	Osmolality		
Wong & Chen [338] (2011), China	13 M	2.0	150%; 2.5 h	Fasted	1	Water	0	0	0	0	NS	NS	NS	30 ± 14	- 0.8
						Lemon Tea (Vita Light™)	202	0	0	12.0	5	NS	NS	36 ± 15	- 0.7
						CHO + Electrolyte Drink (Pocari Sweat®)	109	0	0	6.6	21	NS	NS	52 ± 18	- 0.4
James, *et al.* [339] (2012), UK	12 M	1.9	150%; 1 h	Fasted	4	CHO + Electrolyte Drink	109	0	0	6.5	20	5	NS	50 ± 18	- 0.4 ± 0.4
						CHO, Electrolyte + Protein Drink	109	1.5	0	5.0	20	5	NS	49 ± 13	- 0.4 ± 0.3
Kalman, *et al.* [340] (2012), USA	12 M	2.1	125%; 1 h	1 h	1	Water	0	0	0	0	NS	NS	NS	35 ± 24	- 0.9
						Coconut Water (VitaCo®)[a]	78	0	0	4.6	4	50	NS	39 ± 38	- 0.7
						CHO + Electrolyte Drink	92	0	0	~5.5	NS	NS	NS	40 ± 25	- 0.8
Kamjio, *et al*, [341] (2012), Japan	7 M	2.3	100%[b]; 0.5 h	~30 min	2.5	Electrolyte Drink	0	0	0	0	21	5	77	67	- 0.1
						CHO + Electrolyte Drink	55	0	0	3.3	21	5	277	68	- 0.1
						CHO + Electrolyte Drink	109	0	0	6.5	21	5	350	74	+ 0.0
Desbrow, *et al.* [342] (2013), Australia	7 M	2.0	150%; 1 h	~30 min	4	Beer, 2.3% alc.	110	0.3	0	2.3	25	NS	NS	30	-1.2 ± 0.4
						Beer 2.3% alc. (Sodium Enriched)	110	0.3	0	2.3	50	NS	NS	37	-1.0 ± 0.4
						Beer, 4.8% alc.	177	0.3	0	2.0	25	NS	NS	14	-1.6 ± 0.3
						Beer, 4.8% alc. (Sodium Enriched)	177	0.3	0	2.0	50	NS	NS	14	-1.6 ± 0.3
James, *et al.* [343] (2013), UK	8 M	1.8	150%; 1 h	Fasted	4	CHO + Electrolyte Drink	109	0	0	6.0	20	5	280	46 ± 9	- 0.5 ± 0.2
						CHO, Electrolyte + Protein Drink	109	2.0	0	4.0	20	5	264	59 ± 12	- 0.2 ± 0.3
						CHO, Electrolyte + Protein Drink	109	4.0	0	2.0	20	5	252	64 ± 6	- 0.1 ± 0.1
Clayton, *et al.* [344] (2014), UK	8 M	1.8	150%; 1 h	Fasted	3	CHO + Electrolyte Drink	40	0	0	2.0	29	<1	193	52 ± 10	- 0.3
						CHO + Electrolyte Drink	176	0	0	10.0	29	<1	656	64 ± 11	- 0.1
Tai, *et al.* [345] (2014), USA	20 (10 M)	2.4	150%; 1 h	Fasted	3	Water	0	0	0	0	0	4	NS	42 ± 14	- 0.6
						CHO + Electrolyte Drink (Gatorade®)	111	0	0	6.7	18	4	NS	41 ± 18	- 0.6
						Electrolyte + Protein Drink (Amino1™)	5	1.0	0	0	5	7	NS	44 ± 17	- 0.5
Desbrow, *et al.* [147] (2014), Australia	15 M	2.0	150%; 1 h	Fasted	4	CHO + Electrolyte Drink (Powerade®)	129	0	0	7.3	12	NS	NS	17 ± 17	-1.4 ± 0.3
						Cow's Milk (Full Fat)	286	3.6	3.8	4.9	18	NS	NS	40 ± 25	- 0.9 ± 0.5
						Soy Milk (So Good™)	273	3.2	3.5	5.1	20	NS	NS	47 ± 20	- 0.8 ± 0.4
						Milk-Based Formulation (Sustagen Sport®)	417	6.5	0.2	17.6	29	NS	NS	65 ± 15	- 0.5 ± 0.4
Hobson and James [346] (2014), UK	16 (13 M)	1.9	150%; 1 h	Fasted	4	CHO + Electrolyte Drink	106	0	0	6.2	26	<1	312	51 ± 12	- 0.3
						CHO, Electrolyte + Protein Drink	141	2.0	0	6.2	26	<1	329	55 ± 15	- 0.3
Flores-Salamanca, *et al.* [347] (2014), Costa Rica	11 M	2.1	100%; 1 h	1 h	2	Water	0	0	0	0	0	0	NS	48 ± 21	- 0.8
						Beer, 4.6% alc.	193	0.2	0	3.4	3	2	NS	26 ± 15	-1.2
						Beer, 0.5% alc.	62	0.3	0	3.4	3	2	NS	53 ± 19	- 0.7

[a] Product nutritional information taken from manufacturer's website (www.vitacoco.com/products/pure-coconut-water/original)

[b] An additional ~550 mL 0.9% saline was infused intravenously over ~4.5 h during rehydration and recovery (30 min at 5 $mL \cdot min^{-1}$ and 4 h at 1.67 $mL \cdot min^{-1}$). This volume has been accounted for in the calculation of net TBW balance (mL) and fluid retention (%).

Table 6.1. *(continued)*

Citation	Subjects	BM loss (%)	Fluid Intake (% of BM loss); Drink Time	Time Post-Meal	Post-Drink Observation Period	Fluid (Product name, if known)	Beverage Nutrient Composition (Energy and macronutrients per 100 g)							Fluid Retention (%)	Net TBW Balance (L)
							Energy (kJ)	Protein (g)	Fat (g)	CHO (g)	Na^+ ($mmol \cdot L^{-1}$)	K^+ ($mmol \cdot L^{-1}$)	Osmolality		
James, *et al.* [348] (2014), UK	10 (7 M)	2.0	150%; 1 h	Fasted	4	Water	0	0	0	0	0	0	2	40 ± 14	- 0.6 ± 0.3
						Protein Drink	34	2.0	0	0	0	0	14	37 ± 14	- 0.7 ± 0.3
Perez-Idarraga, *et al.* [349] (2014), Columbia	12 (10 M)	2.1	120%; 1 h	1 h	3	Water	0	0	0	0	0	0	2	51	- 0.6
						CHO + Electrolyte Drink	67	0	0	4.0	18	4	264	68	- 0.4
						Coconut Water	92	1.0	0	4.5	5	71	444	63	- 0.4
						CHO + Electrolyte Drink	67	0	0	4.0	18	50	312	66	- 0.2
Stasiule, *et al.* [350] (2014), Lithuania	9 (0 M)	2.8	150%; 2 h	NS	2	Water	0	0	0	0	3	<1	NS	42	- 0.7
						Water	0	0	0	0	<1	<1	NS	39	- 0.8
Wong, *et al.* [351] (2014), China	9 M	2.0	150%; 3 h	Fasted	1	Water	0	0	0	0	NS	NS	NS	49	- 0.4
						CHO + Electrolyte Drink (Pocari Sweat®)	109	0	0	6.6	21	NS	NS	60	- 0.2
						Lemon Tea (Vita Light™)	168	0	0	10.0	5	NS	NS	59	- 0.2
Wong, *et al.* [351] (2014), China	10 (0 M)	1.4	150%; 3 h	Fasted	1	Water	0	0	0	0	NS	NS	NS	31	- 0.4
						CHO + Electrolyte Drink (Pocari Sweat®)	109	0	0	6.6	21	NS	NS	40	- 0.3
						Lemon Tea (Vita Light™)	168	0	0	10.0	5	NS	NS	25	- 0.5
Desbrow, *et al* [352] (2015), Australia	12 M	2.0	150%; 1 h	~30 min	4	Beer, 2.3% alc. (Sodium Enriched)	110	0.3	0	2.3	25	NS	NS	10	-1.3 ± 0.2
						Beer, 2.3% alc. (Sodium Enriched)	110	0.3	0	2.3	50	NS	NS	24	- 1.0 ± 0.2
						Beer, 3.5% alc.	140	0.3	0	2.0	0	NS	NS	-2	-1.6 ± 0.3
						Beer, 3.5% alc. (Sodium Enriched)	140	0.3	0	2.0	25	NS	NS	4	-1.4 ± 0.3
Li, *et al.* [353] (2015), China	10 M	2.4	150%; 2.5 h	Fasted	1.5	CHO + Electrolyte Drink	110	0	0	6.6	14	3	NS	47 ± 17	- 0.5
						CHO, Electrolyte + Protein Drink	110	2.2	0	4.4	14	3	NS	55 ± 9	- 0.3
						CHO, Electrolyte + Protein Drink	110	2.2	0	4.4	14	3	NS	46 ± 17	- 0.5
Seery, *et al.* [354] (2016), Ireland	7 M	1.8	150%; 2 h	1 h	3	Water	0	0	0	0	1	NS	11	47 ± 15	- 0.5 ± 0.4
						CHO + Electrolyte Drink (Powerade®)	70	0	0	3.9	22	NS	299	52 ± 16	- 0.4 ± 0.5
						Cow's Milk (Fat Free)	145	3.3	0.1	5.0	18	NS	280	71 ± 4	+ 0.1 ± 0.1
Wijnen, *et al.* [355] (2016), Netherlands	11 M	1.0	100%; 45 min	~30 min	5	Water	0	NS	0	0	1	NS	NS	34 ± 25	- 0.5
						CHO + Electrolyte Drink	125	NS	0	7.0	30	NS	NS	42 ± 23	- 0.4
						Beer, 0% alc. (Heineken™)	104	NS	0	6.0	1	NS	NS	36 ± 30	- 0.5
						Beer, 2% alc. (Heineken™)	125	NS	0	4.0	1	NS	NS	36 ± 31	- 0.5
						Beer, 5% alc. (Heineken™)	167	NS	0	3.0	1	NS	NS	21 ± 24	- 0.6
Li, *et al.* [356] (2018), China	10 M	2.2	150%; 2.5 h	Fasted	1.5	CHO + Electrolyte Drink	110	0	0	6.6	21	3	NS	38 ± 17	- 0.6
						CHO + Electrolyte Drink	55	0	0	3.3	21	3	NS	36 ± 14	- 0.7
						CHO, Electrolyte + Protein Drink	110	3.3	0	3.3	21	3	NS	55 ± 13	- 0.2
						CHO, Electrolyte + Protein Drink	110	2.2	0	4.4	21	3	NS	51 ± 19	- 0.3
						CHO, Electrolyte + Protein Drink	110	1.5	0	5.1	21	3	NS	43 ± 13	- 0.5

Table 6.1. *(continued)*

Citation	Subjects	BM loss (%)	Fluid Intake (% of BM loss); Drink Time	Time Post-Meal	Post-Drink Observation Period	Fluid (Product name, if known)	Beverage Nutrient Composition (Energy and macronutrients per 100 g)							Fluid Retention (%)	Net TBW Balance (L)
							Energy (kJ)	Protein (g)	Fat (g)	CHO (g)	Na^+ ($mmol \cdot L^{-1}$)	K^+ ($mmol \cdot L^{-1}$)	Osmolality		
Evans, *et al*. [357] (2018), UK	8 M	1.9%	150%; 1 h	Fasted	3	CHO Drink	28	0	0	1.6	0	NS	NS	32 ± 15	- 0.7
						Protein Drink	34	1.6	<1	<1	<1	NS	NS	37 ± 13	- 0.6
Sayer *et al.* [358] (2018) Australia	12 M	1.9%	100%; 30 min	~30 min	2.5	Water	0	0	0	0	NS	NS	NS	57 ± 16	- 0.6
						Cow's Milk (Low Fat)	210	4.0	1.4	5.3	21	NS	NS	83 ± 6	- 0.3
			100%; 1.5 h		1.5	Water	0	0	0	0	NS	NS	NS	60 ± 20	- 0.6
						Cow's Milk (Low Fat)	210	4.0	1.4	5.3	21	NS	NS	85 ± 7	- 0.2
Rodriguez-Sanchez *et al.* [359] (2018), UK	12 (9 M)	2.0%	100%; 15 min	~30 min	2.75	Water	0	0	0	0	NS	NS	NS	50 ± 11	- 0.8
			100%; 45 min		2.25	Water	0	0	0	0	NS	NS	NS	52 ± 22	- 0.7
			100%; 1.5 h		1.5	Water	0	0	0	0	NS	NS	NS	57 ± 13	- 0.6
			100%; 15/45 min[a]		2.75/2.25	Water	0	0	0	0	NS	NS	NS	53	- 0.7

BM: Body mass; M: Male Subjects; NS: Not Specified. Beverage osmolality in $mOsm \cdot kg^{-1}$. Time Post-Meal: Length of time between the last meal and the commencement of the dehydration protocol.

[a] Half of the participants ingested fluid in 15 min and half in 45 min during this trial.

6.2.2. Fluid Recovery and Beverage Composition

Water is a potent stimulator of fluid-induced diuresis, and thus, a relatively inefficient rehydration beverage [144]. Indeed, except for the study by Wong*, et al.* [287], which administered a particularly large quantity of fluid (i.e. ~1.70 L·kg BM lost^{-1}), none of the studies indicated in Table 6.1 saw participants return to a euhydrated state when exclusively consuming water as the rehydration beverage. In contrast, beverages containing other nutrients (e.g. CHO, protein and electrolytes), have demonstrated a capacity to enhance retention of ingested fluids, either by impacting P_{OSM} directly or by reducing the rate of fluid absorption into the vascular space [360]. This section of the review will discuss how different nutritional constituents and common post-exercise beverages affect rehydration.

6.2.2a. The Role of Electrolytes, Carbohydrate (CHO) and Protein in Post-Exercise Rehydration

The first dose-response studies investigating the effect of beverage Na^+ concentration on post-exercise rehydration were conducted by Maughan*, et al.* [319] and Shirreffs*, et al.* [321]. In these studies [319,321], hypohydrated participants consumed fluid containing either ~0, ~25, ~50 or 100 mmol·L^{-1} of Na^+ post-exercise. Differences in cumulative urine output were apparent across the beverage treatments ≤2 h after drinking ceased, with both studies identifying a significant inverse relationship between the total quantity of urine produced and the Na^+ concentration of the rehydration beverage [319,321]. In total, ~70% of the high Na^+ (100 mmol·L^{-1}) beverage was retained at the conclusion of the observation period, whereas ~35, ~45 and ~60% of the ingested 0, ~25 and ~50 mmol Na^+·L^{-1} beverages were retained, respectively [319,321]. A more recent investigation [332] comparing fluid retention of lower Na^+ beverages (i.e. ~0, ~30, ~40 vs. 50 mmol·L^{-1}) identified a similar relationship, suggesting that even small increases in the Na^+ concentration of a beverage have the potential to aid rehydration. That said, in 2 out of the 3 aforementioned studies [321,332], a Na^+ concentration of 50 mmol·L^{-1} (i.e. ~2-fold greater than most CHO-electrolyte sports beverages, which typically contain ~20–25 mmol Na^+·L^{-1}) was insufficient to facilitate the restoration of fluid balance.

While the addition of K^+ to a rehydration beverage has been proposed to increase intracellular rehydration, studies investigating post-exercise rehydration have produced

inconsistent results. Initially, Maughan*, et al.* [318] observed significantly greater fluid retention of an ingested KCl solution (i.e. 25 mmol·L^{-1}) than a 1.6% CHO (electrolyte-free) beverage; in fact, the KCl solution was as effective as a high-Na^+ solution (i.e. 60 mmol·L^{-1}). The study did not detect any additional improvement when both electrolytes were combined in a single beverage (i.e. 25 mmol K^+·L^{-1} and 60 mmol Na^+·L^{-1}); though, this might have been because urine outputs were already very low (i.e. leaving little room for further improvement) [318]. In contrast, Shirreffs*, et al.* [330] found no difference in fluid retention of a commercial carbonated apple drink (Apfelshorle) containing 30 mmol·L^{-1} of K^+ and a standard CHO-electrolyte sports beverage. These contrasting findings might be explained by the use of KCl in the study by Maughan*, et al.* [318], but not in the study of Shirreffs*, et al.* [330]. Indeed, Cl^- concentrations in the extracellular fluid are second only to Na^+ and the addition of $Cl^-$to drinks, as with Na^+, might enhance retention of extracellular fluid. While few other studies have investigated the impact of beverage K^+ concentration on post-exercise fluid recovery, several [327,329,340,349] have shown that the naturally high-K^+ fluid, *coconut water* (i.e. ~50 mmol·L-1), is a not significantly more effective as a rehydration agent than water.

Some evidence suggests that the addition of CHO to an electrolyte-containing beverage (i.e. ~20–30 mmol Na^+·L^{-1}) enhances post-exercise rehydration [334,336,341]. Indeed, studies have observed greater fluid retention of ingested 6.5% [341], 10.0% [334] and 12.0% [336] CHO solutions than CHO-free control beverages. While the ability of Na^+ to enhance post-exercise rehydration has been attributed to its direct effect on P_{OSM} [361], Clayton*, et al.* [344] reported that CHO is likely to improve fluid retention by reducing gastric emptying rate, in turn, reducing the rate of water flux into the vascular space and attenuating haemodilution. Indeed, large differences in the total amount of fluid remaining inside the stomach 3 h after the consumption of a “high” 10.0% vs. “low” 2.0% CHO solution (i.e. 42 vs. <1%) have been reported [344]. Nonetheless, it is important to recognise that improvements in post-exercise rehydration are not usually observed when beverages with lower CHO concentrations (i.e. ≤6%) are administered [334,336,341], since these do not typically reduce gastric emptying rates sufficiently to attenuate haemodilution [362,363]. Thus, it appears that the addition of CHO to an electrolyte-containing beverage has potential to enhance post-exercise rehydration, but only if the CHO concentration is sufficiently high [360].

Several studies have investigated the effect of protein consumption on post-exercise rehydration [328,337,339,343,346,353,356]. The earliest of these [328] reported enhanced fluid retention of a 6.0% CHO + 1.5% whey protein beverage compared to a 6.0% CHO (only) control. However, as this investigation employed an isocarbohydrate (i.e. rather than isoenergetic) control condition, the observed effect could have been due to the additional nutrition delivered and not the 'protein' per se [360]. Subsequent studies utilising isoenergetic CHO controls have generated mixed results. For instance, James*, et al.* [337] & [343] repeatedly observed improved rehydration with a combined CHO and milk protein beverage (i.e. compared to an isoenergetic CHO control), but found no difference in fluid retention of a 5.0% CHO + 1.5% whey protein and a 6.5% CHO beverage [339]. The authors attributed their findings to differences in the gastric-emptying properties of milk and whey proteins [339]. Indeed, on exposure to gastric acid, it seems the casein fraction of milk protein clots, and therefore, empties from the stomach more slowly than the soluble whey protein fraction [364]. This could, in turn, attenuate haemodilution. However, the only study to have compared the rehydration effects of whey and casein protein directly [353] reported *better* fluid retention of the 4.4% CHO + 2.2% whey protein beverage than a 4.4% CHO + 2.2% casein protein solution; in fact, retention of the casein beverage did not differ from an isoenergetic CHO control. The precise reason for these contrasting results is not clear. Though, glutamine and alanine (more prevalent in whey than casein protein), have been demonstrated to promote intestinal sodium and water absorption may offer one explanation [353]. Thus, while, additional research is required to understand the effect of protein consumption on post-exercise rehydration, it seems that, in general, the addition of this nutrient to a recovery beverage is unlikely to impair rehydration and at the very least may have a small effect to enhance fluid recovery [360].

6.2.2b. The Rehydration Potential of Common Post-Exercise Beverages

The fluid retention rates reported in Table 6.1 are summarised graphically by beverage type in Figure 6.1. The beverages presented are: Water, CHO-electrolyte

sports beverages[a], milk/milk-based formulations[b] and alcoholic beer; "high" and "low" electrolyte beverages[c] are presented for comparisons only, as these are not common post-exercise beverages. Each of the studies graphed administered ≥1.0 L·kg BM lost^{-1} and allowed ≥1 h rest post-fluid consumption. Collectively, the evidence suggests that ~45±10%, ~55±16%, ~67±15% and 30±18% of water, CHO-electrolyte sports beverage, milk/milk-based formulation and alcoholic beer ingested post-exercise is typically retained, respectively. Before considering these data further, it is important to acknowledge the large amount of variation in reported fluid retention rates across individual studies. This variation is likely due to differences in the research methodology employed (e.g. the degree of exercise-induced fluid loss; time of last meal; the beverage volume, composition and rate of ingestion; and, period of observation) and should be considered when interpreting these findings.

[a] These are "CHO + Electrolyte Drinks" (see Table 6.1) that have Na^+ and K^+ concentrations <35 and <25 mmol·L^{-1}, respectively; in total, *n*=3 CHO + Electrolyte Drinks did not meet these criteria.

[b] These include cow's milk, non-dairy alternatives (e.g. soy milk) and other milk-based products (e.g. Sustagen Sport®).

[c] These are "Electrolyte Drinks" (see Table 6.1) that have a "high" Na^+ or K^+ concentration (i.e. ≥35 or ≥25 mmol·L^{-1}, respectively), or "low" Na^+ and K^+ concentration (i.e. <35 and <25 mmol·L^{-1}, respectively).

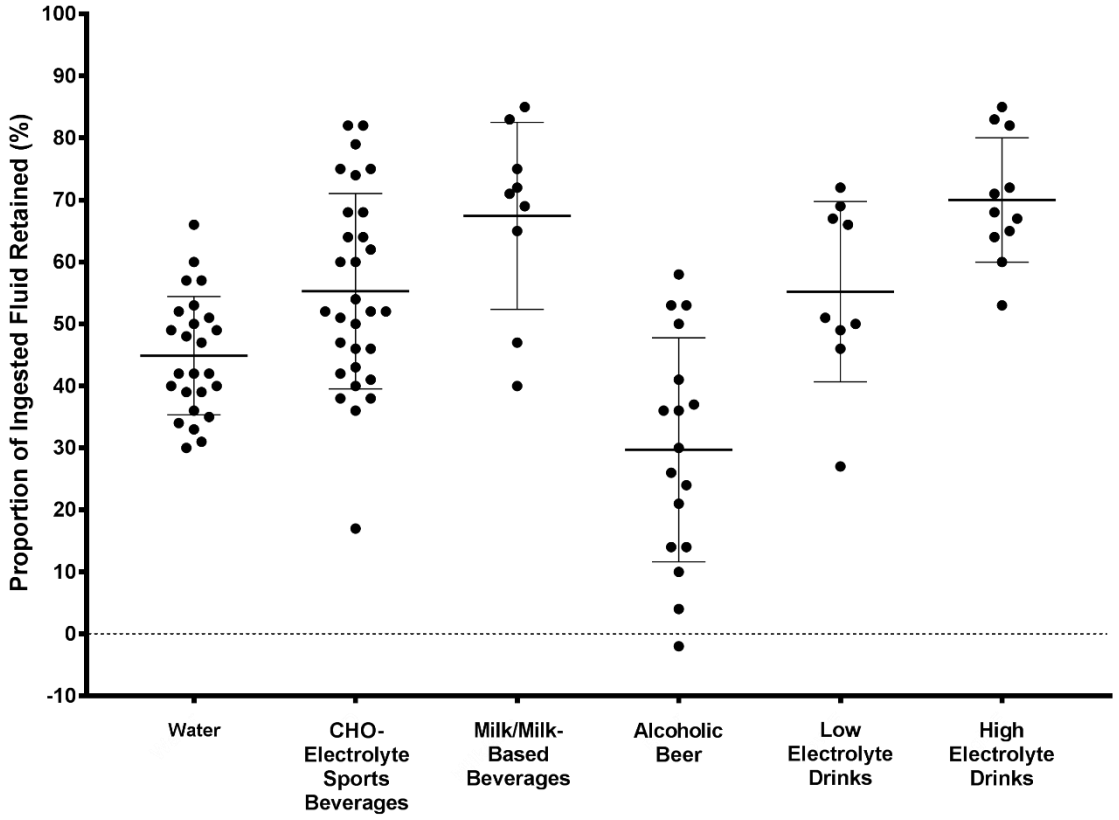

Figure 6.1. A graphical summary of the fluid retention rates reported in Table 6.1. The studies presented in this figure administered a volume of fluid ≥100% of exercise-induced BM loss and allowed ≥1 h rest post-fluid consumption. Beverages that were infrequently investigated have been omitted. Values are Mean±SD.

The data summarised in Figure 6.1 suggest that CHO-electrolyte sports beverages (designed for consumption *during* exercise) are only slightly more effective than water at promoting post-exercise rehydration (i.e. ~45±10 vs. 55±16%). Indeed, experimental studies sometimes[a] and sometimes do not[b] detect significant differences in the rehydration potential of these fluids. The fact that sports beverages are poorly retained is likely due to their low Na^+, K^+ and CHO content; indeed, those graphed contained ~6.2±2.2% CHO and ~22±6 mmol $Na^+ \cdot L^{-1}$. Higher concentrations of CHO and electrolytes are typically required to maximise fluid retention of post-exercise beverages [360]. While a relatively small number of studies have assessed the rehydration potential of milk/milk-based formulations, including fat-free/low-fat/full-cream cow's milk[c], soy milk [147] and a milk-based formulation [147], results suggest that these products are well-retained post-exercise (i.e. ~67±16%). In fact, experimental studies consistently

[a] [297,316,328-330,336,338,349,351]

[b] [287,316,323,327,331,340,345,354,355]

[c] [147,331,333,354,358]

report greater fluid retention of milk/milk-based formulations than water and CHO-electrolyte sports beverages, likely because their higher energy-density (i.e. ~150–400 $kJ \cdot dL^{-1}$) decreases the rate of gastric emptying (i.e. and subsequently, haemodilution) [147,331,333,354,358]. In contrast, alcoholic beer is, generally, an inefficient post-exercise rehydration beverage (i.e. ~30±18%); though reported fluid retention rates do vary widely across investigations. It is likely that some of this variation can be attributed to differences in the alcohol content of the beer (i.e. ~0.5–5.0%), as studies have demonstrated better fluid retention of light beer (i.e. ≤2.0% alc.) than mid- and heavy-strength beer, which, because of its higher alcohol content, typically elicits a detectable diuretic effect [320,342,347,352,355].

6.2.3. Fluid Recovery and Beverage Ingestion Rate

The rate at which fluid is consumed has also been suggested to influence post-exercise rehydration, as a high ingestion rate could, theoretically, exacerbate haemodilution (i.e. the fluid-induced reduction in plasma Na^+ concentration and P_{OSM}) and increase urine production [360]. However, the effect of "drinking rate" on post-exercise rehydration has not been widely researched [326,358,359,365]. Initially, Kovacs*, et al.* [326] (Table 6.3) failed to detect a difference in fluid retention of a CHO-electrolyte sports beverage consumed over 3 vs. 5 h (i.e. ~79±6 vs. 82±5%). However, Jones*, et al.* [365] (Table 6.3) later observed greater fluid retention of water ingested over 4 vs. 1 h (i.e. ~75±12 vs. 55±18%). Increased fluid retention of a CHO-electrolyte sports beverage consumed 30 vs. 90 min (~56 vs. 61%) was similarly reported by Archer*, et al.* [366]. Differences in the fluid ingestion rates and test beverages employed could possibly explain this inconsistency. Recently, two companion studies [358,359] attempted to clarify the impact of: (1) drinking rate (i.e. 30 vs. 90 min) on fluid retention of different post-exercise beverages (i.e. water vs. low-fat milk) [358]; and (2) faster drinking rates (i.e. that better reflect 'typical' *ad libitum* drinking behaviour) on post-exercise fluid retention (i.e. 15, 45 vs. 90 min) [359]. Study 'A' [358] found no effect of drinking rate on fluid retention of either beverage; Study 'B' [359] observed greater retention of fluid consumed over 90 than 15 min (i.e. ~57±13 vs. 50±11%), but noted that the magnitude of the effect was within the range of normal day-to-day variation. Thus, the effect of drinking rate on rehydration is likely to be subtle and is, perhaps, a less

important determinant of post-exercise fluid recovery than beverage volume and composition.

6.3. Other Determinants of Post-Exercise Fluid Recovery

Clearly, considerable research has investigated post-exercise rehydration, in particular the impact of beverage type and composition on fluid retention. Findings from the studies described thus far suggest that beverages with "complex" nutritional profiles (e.g. milk/milk-based formulations) are more effective rehydration agents than those with "simple" nutritional profiles (e.g. water, CHO-electrolyte sports beverages) (Figure 6.1). However, it is important to recognise the limitations of this research; specifically, that these studies prescribe drinking (i.e. administer fixed quantities of fluid) and prohibit eating during the post-exercise period. In reality, individuals usually control the volume of fluid they consume and often have access to food in the initial hours after exercise. This section of the review will summarise research investigating the ability of different beverages to rehydrate when consumed *ad libitum* and/or with food post-exercise.

Table 6.2. Studies investigating fluid recovery with different beverages consumed *ad libitum* after exercise-induced dehydration using a repeated-measures experimental design. These studies did not permit the consumption of food during the post-exercise (i.e. rehydration) period.

Citation	Subjects	BM loss (%)	Drink Time (h)	Time Post-Meal	Post-Drink Observation Period	Fluid (Product name, if known)	Beverage Nutrient Composition (Energy and macronutrients per 100 g)							Fluid Intake (L·kg BM Lost^{-1})	Fluid Retention (%)	Net TBW Balance (L)
							Energy (kJ)	Protein (g)	Fat (g)	CHO (g)	Na^+ (mmol·L^{-1})	K^+ (mmol·L^{-1})	Osmolality			
Nose, *et al*. [144] (1988), USA	6 M	2.3	5	NS	0	Water	0	0	0	0	0	NS	NS	0.7	75	- 0.7
						Water (Na^+ via capsule)	0	0	0	0	80	NS	NS	0.8	86	- 0.5
Maughan, *et al*. [367] (1994), UK	8 M	2.1	2	Fasted	4	Water (Carbonated)	0	0	0	0	NS	NS	NS	1.8 L	Values NR and cannot be calculated.	- 0.5 ± 0.4
						CHO + Electrolyte Drink	150	0	0	1.6	60	24	NS	1.8 L		- 0.1 ± 0.7
						CHO + Electrolyte Drink	NS	0	0	NS	25	4	NS	2.5 L		- 0.1 ± 0.4
						Orange Juice/Lemonade Mix	NS	0	0	NS	0	2	NS	2.5 L		+ 0.0 ± 0.3
Wemple, *et al*. [368] (1997), USA	6 (NS)	3.0	3	Fasted	0	Water (AS)	0	0	0	0.0	0	NS	NS	1.2 ± 0.2	74	- 0.2
						CHO + Electrolyte Drink	100	0	0	6.0	25	NS	NS	1.6 ± 0.3	80	+ 0.6
						CHO + Electrolyte Drink	100	0	0	6.0	50	NS	NS	1.3 ± 0.2	79	+ 0.1
Passe, *et al*. [369] (1997), USA	52 (30 M)	NS	<15 min	NS	0	CHO + Electrolyte Drink	100	0	0	6.0	20	3	NS	0.2 ± <0.1 L	Parameters not assessed. Fluid intake, only.	
						CHO + Electrolyte Drink (Carbonated x 1.1)	100	0	0	6.0	20	3	NS	0.2 ± <0.1 L		
						CHO + Electrolyte Drink (Carbonated x 2.3)	100	0	0	6.0	20	3	NS	0.1 ± <0.1 L		
						CHO + Electrolyte Drink (Carbonated x 3.0)	100	0	0	6.0	20	3	NS	0.1 ± <0.1 L		
Wilmore, *et al*. [370] (1998), Australia	15 M	1.1	1.5	NS	0	Water	0	0	0	0	NS	NS	NS	0.8	Parameters not assessed. Fluid intake, only.	
						CHO + Electrolyte Drink	100	0	0	6.0	20	3	NS	1.3		
						CHO + Electrolyte Drink	134	0	0	8.0	10	5	NS	1.2		
Wong, *et al*. [322] (1998), China	7 (2 M)	2.5	4	Fasted	0	CHO + Electrolyte Drink (Lucozade-Sport)	115	0.0	0.0	6.9	24	3	300	1.0	43	- 0.9
Evans, *et al*. [371] (2009), UK	9 (6 M)	2.0	2	Fasted	3	Electrolyte Drink (AS)	0	0.0	0.0	0.0	31	1	74	1.5	48 ± 20	- 0.4
						CHO + Electrolyte Drink (AS)	33	0.0	0.0	2.0	31	1	188	1.7	49 ± 13	- 0.3
						CHO + Electrolyte Drink (AS)	167	0.0	0.0	10.0	31	1	654	1.4	57 ± 15	- 0.3
Park, *et al*. [372], (2012) Korea	8 M	1.9	1.5	Fasted	0	Water (10°C)	0	0.0	0.0	0.0	NS	NS	NS	0.9	Values NR and cannot be calculated.	
						Water (26°C)	0	0.0	0.0	0.0	NS	NS	NS	0.7		
						CHO + Electrolyte Drink (Gatorade®) (10°C)	111	0.0	0.0	6.7	18	4	NS	1.1		
						CHO + Electrolyte Drink (Gatorade®) (26°C)	111	0.0	0.0	6.7	18	4	NS	1.0		
Mears, *et al*. (2014)	10 M	1.2	2	Fasted	0	Water	0	0	0	0	NS	NS	NS	0.5	Values cannot be calculated.	
		1.0				Water	0	0	0	0	NS	NS	NS	0.5		
Baguley, *et al*. [373] (2016), Australia	7 M	1.9	2	3	1	CHO + Electrolyte Drink (Powerade®)	129	0.0	0.0	7.3	12	NS	NS	1.6	54 ± 20	- 0.5 ± 0.5
						CHO + Electrolyte Drink (Powerade®)	129	0.0	0.0	7.3	12	NS	NS	1.9	44 ± 12	- 0.4 ± 0.4
						Milk-Based Formulation (Sustagen Sport®)	417	6.5	0.2	17.6	29	NS	NS	1.0	87 ± 8	- 0.6 ± 0.7
						Milk-Based Formulation (Sustagen Sport®)	417	6.5	0.2	17.6	29	NS	NS	1.0	85 ± 7	- 0.5 ± 0.6
Desbrow, *et al*. [374] (2018), Australia	54 (41 M)	1.5	0.5–1	NS	0	CHO + Electrolyte Drink (Gatorade®)	100	0	0	6.0	23	6	NS	0.9	Parameters not assessed. Fluid intake, only.	
						Beer, 0.9% alc. (Hahn Ultra®)	57	0	0	1.8	2	NS	NS	0.7		
Desbrow, *et al*. [374] (2018), Australia	78 (38 M)	1.5	0.5–1	NS	0	Water	0	0	0	0	NS	NS	NS	0.7	Parameters not assessed. Fluid intake, only.	
						CHO + Electrolyte Drink (Gatorade®)	100	0	0	6.0	23	6	NS	0.8		

AS: Artificially Sweetened; BM: Body mass; M: Male Subjects; NS: Not Specified. Beverage osmolality in mOsm·kg^{-1}. Time Post-Meal: Length of time between the last meal and the commencement of the dehydration protocol.

Table 6.3. Studies investigating fluid recovery with different beverages consumed *ad libitum* or in prescribed quantities after exercise-induced dehydration using a repeated-measures experimental design. These studies permitted the consumption of food during the post-exercise (i.e. rehydration) period.

Citation	Subjects	BM loss (%)	Fluid Intake (% of BM loss); Drink Time	Time Post-Meal	Post-Drink Observation Period	Fluid (Product name, if known)	Food (Product name, if known)	Eating protocol	Fluid Intake (L·kg BM Lost^{-1})	Fluid Retention (%)	Net TBW Balance (L)
Studies Measuring the Effect of Food Intake on Post-Exercise Rehydration											
Maughan, *et al*. [375] (1996), UK	8 (5 M)	2.1	Prescribed 1 h; 150%	Fasted	6	CHO + Electrolyte Drink	Nil	n/a		53	- 0.3
						CHO + Electrolyte Drink	Nil	n/a	n/a	52	- 0.4
						Water	Beef, Rice and Beans	Standard meal at the onset of drinking		67	- 0.0
Ray, *et al*. [376] (1998), USA	30 (15 M)	2.5	Prescribed 2 h; 100%	Fasted	0	Water	Water	2 x 175 mL at 0 and 20 min after the onset of drinking	n/a	87	- 0.4
						Water	CHO + Electrolyte Drink			83	- 0.4
						Water	Chicken Broth			91	- 0.2
						Water	Chicken Noodle Soup			86	- 0.2
Pryor, *et al*. [377] (2015), U.S.	8 M	Participants in this study completed 3 weeks of unsupervised training, consuming a different post-exercise treatment each week. Thus, acute hydration measures were not taken, and 'trial' conditions were not standardised.				CHO + Electrolyte Drink (Powerade®)	Nil	n/a	n/a	Parameters not assessed. No differences in long-term indicators of hydration status.	
						Nil	Beef Jerky	Standard snack immediately post-exercise			
						CHO + Electrolyte Drink (Powerade®)	Beef Jerky				
Studies in which Food was Administered During Rehydration (i.e. Effects on Rehydration were not Assessed)											
Brouns, *et al*. [378] (1998), Netherlands	8 (NS)	3.2	*Ad libitum*; 2 h	2 h	2	Water	Ham or cheese sandwich	Standard meal 3 h after the onset of drinking	1.0	59	- 1.0
						Cola Drink (Coca-Cola®)			1.2	57	- 0.7
						CHO + Electrolyte Drink (Isostar®)			1.3	58	- 0.6
Kovacs, *et al*. [326] (2002), Netherlands	8 M	3.0	Prescribed 3 h; 120%	2 h	3	CHO + Electrolyte Drink	Ham or cheese sandwich	Standard meal 3 h after the onset of drinking	n/a	79 ± 6	- 0.5
			Prescribed 5 h; 120%		1	CHO + Electrolyte Drink				82 ± 5	- 0.4
Jones, *et al*. [365] (2010), USA	8 M	2.0	Prescribed 1 h; 100%	Fasted	6	Water	Breakfast bar, banana, peanut butter and jelly sandwich, and potato chips	Standardised meal post-exercise and 4 h the onset of drinking	n/a	55 ± 18	- 0.7
			Prescribed 4 h; 100%		3	Water				75 ± 12	- 0.4
Evans, *et al*. [379] (2017), UK	8 (5 M)	1.5	Prescribed 1 h; 150%	Fasted	3	Water	Pasta, tomato pasta sauce, olive oil and cheese	Standard meal 15 min after the onset of drinking	n/a	50 ± 20	- 0.5
						Electrolyte Drink				70 ± 21	+ 0.0
Campagnolo, *et al*. [8] (2017), Australia	10 M	2.5	*Ad libitum*; 4 h	Fasted	0	Water	Assorted Snack Foods	2 x 15 min *ad libitum* eating occasions at 1 and 2 h after the onset of drinking	1.2	72 ± 8	+ 0.0 ± 0.3
						Water			1.2	73 ± 11	+ 0.0 ± 0.3
						CHO + Electrolyte Drink (Powerade®)			1.5	72 ± 17	+ 0.0 ± 0.2
						Milk-Based Formulation (Sustagen Sport®)			1.0	74 ± 10	- 0.1 ± 0.2

BM: Body mass; M: Male Subjects; n/a: Not applicable. Time Post-Meal: Length of time between the last meal and the onset of the dehydration protocol; Fluid Intake: Voluntary fluid consumption in studies employing *ad libitum* rehydration protocols.

Table 6.4. Characteristics of food and beverage items used in studies presented in Table 6.3.

		Beverage Nutrient Composition (Energy and macronutrients per 100 g)								Total Nutrient Content (i.e. intake *per serve*)				
Citation	Fluid (Product name, if known)	Energy (kJ)	Protein (g)	Fat (g)	CHO (%g	Na^+ ($mmol \cdot L^{-1}$)	K^+ ($mmol \cdot L^{-1}$)	Osmolality	Food (Product name, if known)	Energy (kJ)	Protein (g)	Fat (g)	CHO (g)	Na^+ (mmol)
Maughan, *et al*. [375] (1996), UK	CHO + Electrolyte Drink	NS	NS	NS	NS	21	3	NS	Nil	-	-	-	-	-
	CHO + Electrolyte Drink	NS	NS	NS	NS	21	3	NS	Nil	-	-	-	-	-
	Water	0	0	0	0	1	<1	NS	Beef, Rice and Beans	4170	47	31	132	492
Ray, *et al*. [376] (1998), USA	Water	0	0	0	0	0	0	25	Water	0	0	0	0	0
	Water								CHO + Electrolyte Drink	317	0	0	19	5
	Water								Chicken Broth	85	5.0	<1	<1	33
	Water								Chicken Noodle Soup	776	9.0	4.2	28	100
Pryor, *et al*. [377] (2015), U.S.	CHO + Electrolyte Drink (Powerade®)	97	0	0	5.8	18	NS	NS	Nil	-	-	-	-	-
	Nil	-	-	-	-	-	-	-	Beef Jerky	420	18	1.0	4.0	32
	CHO + Electrolyte Drink (Powerade®)	97	0	0	5.8	18	NS	NS	Beef Jerky	420	18	1.0	4.0	32
Brouns, *et al*. [378] (1998), Netherlands	Water	0	0	0	0	0	0	4	Ham or cheese sandwich	757/890	8.5/11.0	2.3/4.9	31.6/31.2	20/20
	Cola Drink (Coca-Cola®)	175	0	0	10.5	2	0	554						
	CHO + Electrolyte Drink (Isostar®)	127	0	0	7.6	31	5	305						
Kovacs, *et al*. [326] (2002), Netherlands	CHO + Electrolyte Drink	127	0	0	7.6	31	5	NS	Ham/cheese sandwich	757/890	8.5/11.0	2.3/4.9	31.6/31.2	20/20
	CHO + Electrolyte Drink	127	0	0	7.6	31	5	NS						
Jones, *et al*. [365] (2010), USA	Water	0	0	0	0	NS	NS	NS	Breakfast bar, banana, peanut butter and jelly sandwich, and potato chips	NS	NS	NS	NS	36
	Water	0	0	0	0	NS	NS	NS						
Evans, *et al*. [379] (2017), UK	Water	0	0	0	0	NS	NS	NS	Pasta, tomato pasta sauce, olive oil and cheese	3767 ± 273	32 ± 5	33 ± 5	118 ± 17	31 ± 5
	Electrolyte Drink	0	0	0	0	50	NS	NS						
Campagnolo, *et al*. [8] (2017), Australia	Water	0	0	0	0	NS	NS	NS	Assorted Snack Foods	7826 ± 888	67 ± 16	80 ± 21	197 ± 52	70 ± 18
	Water	0	0	0	0	NS	NS	NS		7578 ± 1112	63 ± 16	75 ± 23	195 ± 70	62 ± 13
	CHO + Electrolyte Drink (Powerade®)	129	0	0	7.3	12	NS	NS		6783 ± 1834	56 ± 15	64 ± 20	186 ± 69	56 ± 22
	Milk-Based Formulation (Sustagen Sport®)	417	6.5	<1	17.6	29	NS	NS		3171 ± 1063	24 ± 12	28 ± 13	107 ± 54	26 ± 15

NS: Not Specified. Beverage osmolality in $mOsm \cdot kg^{-1}$.

6.3.1. *Ad libitum* Post-Exercise Fluid Recovery

A review of the literature preceding February 2019 identified 14 original investigations examining *ad libitum* post-exercise fluid consumption behaviour; 8 of these measured fluid restoration, specifically (i.e. beverage retention, TBW balance). Most of the studies identified (*n*=12) prohibited eating during the post-exercise (i.e. rehydration) period and are summarised in Table 6.2; two studies [8,378] that did administer food are displayed in Table 6.3 (Nb. The other studies included in Table 6.3 prescribed drinking and are not discussed here). Reported rates of *ad libitum* fluid consumption for water[a], CHO-electrolyte sports beverages[b] and milk/milk-based formulations are indicated graphically in Figure 6.2. Collectively, results suggest that individuals may consume different beverages in different volumes post-exercise, likely influencing their effectiveness as rehydration agents. Factors that appear to affect *ad libitum* fluid consumption behaviour of different post-exercise beverages include thirst, beverage palatability and GI tolerance.

[a] This is "Water" (see Tables 6.2 & 6.3) that was not administered with Na^+ or artificial sweetener/flavouring.

[b] These are "CHO + Electrolyte Drinks" (see Tables 6.2 & 6.3) that have Na^+ and K^+ concentrations <35 and <25 $mmol \cdot L^{-1}$, respectively; all CHO + Electrolyte Drinks met these criteria.

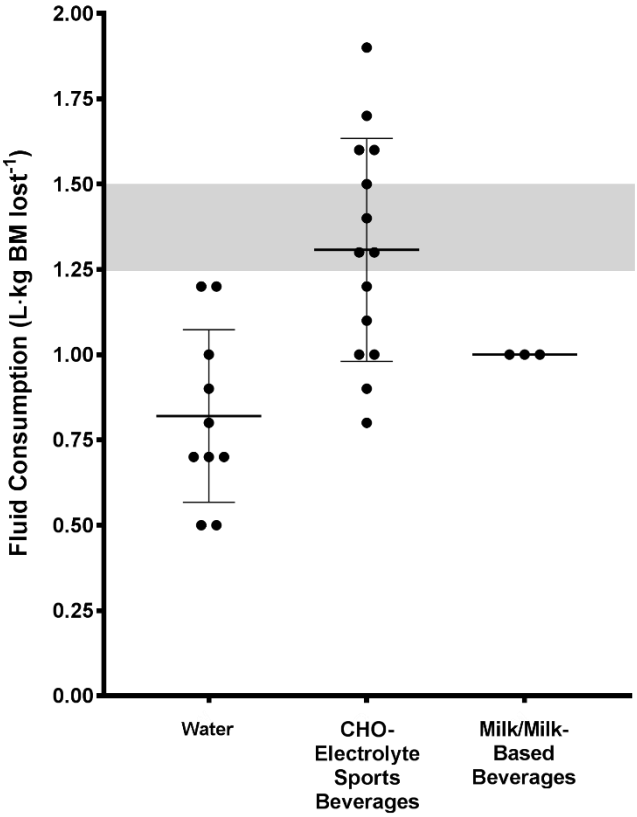

Figure 6.2. A graphical summary of the *ad libitum* fluid consumption rates reported in Tables 6.2 & 6.3. Shaded area represents the amount of fluid individuals are recommended to consume to promote rapid and complete recovery of TBW content (1.25–1.50 L·kg BM lost^{-1}) [1,2]. Values are Mean±SD.

6.3.1a. Thirst, Beverage Palatability and GI Tolerance

Early research by Nose*, et al.* [144] demonstrated the importance of Na^+ in maintaining thirst and promoting *ad libitum* consumption of post-exercise beverages. Participants in this study rehydrated *ad libitum* using water and capsules containing either placebo or ~8 mmol Na^+ (per 100 mL of fluid) [144]. While the additional Na^+ did not affect fluid consumption in the first hour of recovery, late-stage (i.e. 60–180 min) and total fluid consumption were increased as a result of supplementation, suggesting that ingested water attenuates the osmotic drive for drinking (i.e. thirst sensation) to a greater extent in the absence of Na^+ [144]. Interestingly, no study included in Tables 6.2 or 6.3 observed participants voluntarily consume a quantity of water consistent with current recommendations (i.e. 1.25–1.50 L·kg BM lost^{-1} [1,2]); in fact, the only studies that saw participants replace their fluid losses (i.e. consume ≥1.0 L·kg BM lost^{-1}) administered food (and as a consequence Na^+) during the post-exercise period [8,378]. Subsequent investigations [367,368] have attempted to determine if smaller amounts of Na^+ incorporated directly into post-exercise beverages promote drinking in a similar

manner. Maughan*, et al.* [367] observed no difference in *ad libitum* fluid consumption of an electrolyte-free orange juice/lemonade beverage and CHO-electrolyte beverage (i.e. 24 mmol $Na^{+}\cdot L^{-1}$); however, the fluids differed in both flavour and composition. In contrast, Wemple*, et al.* [368] found that participants voluntarily consumed more of a CHO beverage containing 25 mmol $Na^{+}\cdot L^{-1}$ (compared to an electrolyte-free version), suggesting that small amounts of Na^{+} might be sufficient to promote post-exercise rehydration. Importantly, both investigations [367,368] also observed a significant reduction in *ad libitum* fluid consumption when higher Na^{+} beverages (i.e. ~25 vs. 50 $mmol\cdot L^{-1}$) were administered [368]. Thus, it appears that high Na^{+} concentrations reduce beverage palatability to such an extent that voluntary fluid consumption is reduced, even if the osmotic drive for drinking is maintained [367,368].

Several factors, aside from Na^{+} content, also appear to influence beverage palatability and *ad libitum* post-exercise fluid consumption. For instance, studies have reported greater *ad libitum* fluid consumption of beverages that are flavoured, sweetened and/or served at cool temperatures (i.e. 0–22°C) [370,380]; whereas those that are carbonated or warmer in temperature (i.e. >22°C) are typically ingested in smaller volumes [369,380]). This, along with their moderate Na^{+} content (i.e. ~20–25 mmol $Na^{+}\cdot L^{-1}$), very likely explains why CHO-electrolyte sports beverages are voluntarily ingested in large amounts (Figure 6.2). Indeed, all of the studies comparing *ad libitum* post-exercise fluid consumption of CHO-electrolyte sports beverages and water (Tables 7.2 & 7.3) report increased intakes of sports beverages [8,367,368,370,372,374,378]. Thus, CHO-electrolyte sports beverages may provide a greater opportunity for effective rehydration than water in a free-living environment, despite indicating only marginally greater capacity for fluid retention (Figure 6.1). Conversely, there is some evidence to suggest that milk/milk-based formulations are not considered to be palatable. Indeed, Baguley*, et al.* [373] reported that Sustagen Sport® was perceived as "unpleasant" to consume. While the specific sensory characteristics that contributed to this perception are not clear, a "heavy mouthfeel" and viscous consistency have previously been associated with reduced acceptance of post-exercise beverages [7].

Finally, there is some (albeit limited) evidence to suggest that GI factors (e.g. bloating and/or stomach distention) affect *ad libitum* fluid consumption behaviour. For instance, an early study by Phillips*, et al.* [381] demonstrated that a reduction in thirst

and an increase in perceived stomach fullness acted to terminate drinking under 'normal', daily (i.e. non-exercise) conditions. While the role that GI factors play in moderating post-exercise drinking behaviour is not well understood, these factors do seem to be important – particularly, when beverages with high calorie loads, and therefore, slower rates of gastric emptying are involved [146]. Indeed, both Baguley*, et al.* [373] and Campagnolo*, et al.* [8] found that the milk-based formulation 'Sustagen Sport®' elicited strong feelings of bloatedness and that participants ceased drinking this beverage even while they were still thirsty. Thus, the GI effects that milk/milk-based beverages provide, and their low palatability might explain why these products are not usually ingested in large quantities post-exercise (Figure 6.2). In fact, the available data suggest that while milk/milk-based beverages are well retained, hypohydrated individuals are unlikely to consume them in sufficiently large volumes to restore euhydration in a free-living environment. Of course, further research utilising other milk/milk-based beverages that are less energy-dense than Sustagen Sport® (i.e. ~400 $kJ \cdot dL^{-1}$) is required to clarify these effects.

One important limitation of this research area as a whole is that participants in these studies are aware they are hypohydrated, and therefore, motivated to drink. In many applied contexts, athletes may not know they are dehydrated, particularly, if they have consumed some fluid during exercise (i.e. attenuating thirst). As such, it is important to bear in mind that the studies presented may overestimate the volume of fluid a hypohydrated athlete might consume *ad libitum* post-exercise.

6.3.1b. Other Contextual Factors

Several other factors might also impact *ad libitum* post-exercise drinking behaviour in a free-living environment. For instance, cognitive factors (e.g. learning, prior experience), cultural factors, the social environment, the beverage container size and/or shape, exposure to advertising material, nutrition knowledge and/or beliefs, and/or beverage availability [7]. However, the role that these factors play in modifying drinking behaviour has not been well researched. Hence, further studies employing protocols that better reflect post-exercise conditions and practices is warranted.

6.3.2. Food and Post-Exercise Fluid Recovery

Studies investigating the ability of different beverages to rehydrate when consumed either *ad libitum* or in prescribed quantities with food post-exercise are summarised in Tables 6.3 & 6.4. Three of these studies investigated the effect of co-ingested food on fluid retention of different post-exercise beverages [375-377]. The earliest of these [375] administered a fixed volume (i.e. 1.5 L·kg BM lost^{-1}) of either CHO-electrolyte sports beverage or water in the post-exercise period; a standard meal containing rice, beans and beef was consumed alongside the water. While water is generally considered an inefficient rehydration agent [360], Maughan*, et al.* [375] observed significantly greater fluid retention of this beverage co-ingested with food than the CHO-electrolyte sports beverage alone. In fact, while participants left the laboratory in negative TBW balance (i.e. –0.3 L) on the sports beverage treatment, the water essentially restored euhydration [375]. Ray*, et al.* [376] also reported greater post-exercise fluid retention of water (i.e. 1.0 L·kg BM lost^{-1}) co-ingested with (i.e. vs. without) a standard meal of chicken noodle soup. Though, as the soup had an extremely high Na^+ concentration (i.e. ~334 mmol·L^{-1}), this result is not entirely unexpected. In contrast, Pryor*, et al.* [377] failed to detect a significant benefit of co-ingesting a CHO-electrolyte sports beverage with (i.e. vs. without) beef jerky post-exercise. However, acute changes in hydration status were not measured in this study (i.e. BM, urine volume and U_{SG} measurements 24 h post-exercise, only). Furthermore, as much of the experiment occurred in a free-living environment, differences in participants diet and/or physical activity behaviours up to 24 h post-exercise could have confounded the results. Thus, the available evidence suggests that co-ingested food improves fluid retention of beverages with simple nutritional profiles during the post-exercise period [375,376]. Still, most of the studies conducted have prescribed a small number of food items that may not reflect the participants' usual dietary behaviour.

To date, only Campagnolo*, et al.* [8] has investigated the ability of different beverages to rehydrate when both the fluid and a variety of foods are consumed *ad libitum* post-exercise (Table 6.3). This study gave 10 endurance-trained male participants *ad libitum* access to water, a CHO-electrolyte sports beverage or a milk-based formulation (i.e. Sustagen Sport®) during a 4 h post-exercise recovery period; individuals were also given two 15 min opportunities to access food *ad libitum* (e.g. muesli bars,

fresh/dried fruit, bread and condiments) in the first 2 h of recovery. Overall, the study demonstrated that the different beverages were *equally* effective at replenishing fluid losses, with participants' BM returning to near pre-exercise levels (Figure 6.3). The observed effect was attributed to the fact that water and the CHO-electrolyte sports beverage were voluntarily ingested in larger volumes than the milk-based formulation and that the co-ingested food improved retention of these "simple" fluids. Thus, rehydration may not be influenced by beverage type or composition when fluid is consumed voluntarily and with food post-exercise [8]. Given the preliminary nature of these findings, additional studies employing protocols that better reflect real-life post-exercise conditions are still required to improve our understanding of the interaction between fluid, food and nutrients in rehydration during the initial hours after exercise. In particular, future studies should explore different participant populations (e.g. females) and exercise contexts (e.g. between consecutive bouts of activity) – as these could potentially elicit different eating and/or drinking behaviours, thereby influencing rehydration (see *6.4.2. Dietary Behaviour: The Influence of Sex and Exercise Context*).

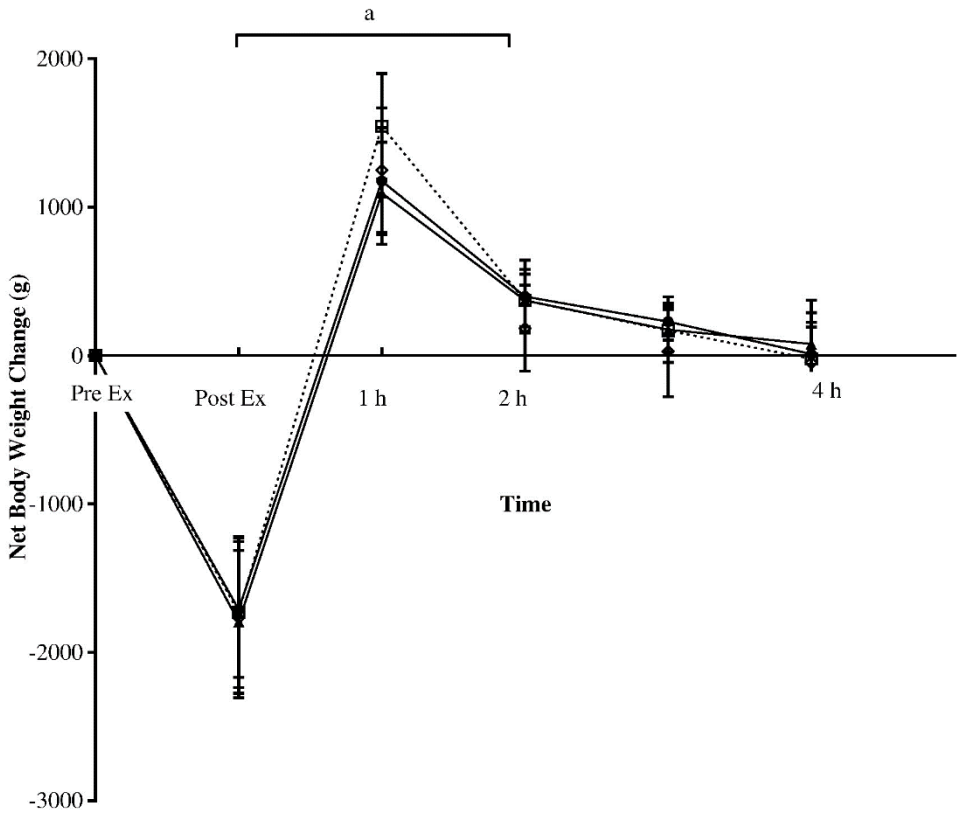

Figure 6.3. Net BM responses throughout experimental trials. Values are Mean±SD for Water #1 (●); Water #2 (▲); CHO-electrolyte sports beverage (□); Milk-based formulation (◊). *a*, indicates a significant difference in net BM change compared to previous hour. Pre-Ex: Pre-exercise; Post-Ex: Post-Exercise. Figure reproduced from Campagnolo*, et al.* [8]

6.4. Different Post-Exercise Beverages: Effects on Nutrient Provision and Subsequent Athletic Performance

This review has, so far, examined the utility of different beverages as post-exercise rehydration agents. However, in addition to fluid, many popular beverages also contain nutrients (e.g. CHO and/or protein), such that they have the potential to influence energy and nutrient provision in the initial hours after exercise. Such differences could potentially affect muscle glycogen resynthesis and therefore subsequent athletic performance. This section of the review summarises research investigating the effect of different post-exercise beverages consumed with food on nutrient provision, exercise recovery (e.g. glycogen resynthesis) and subsequent athletic performance. The chronic effects of consuming nutritive beverages post-exercise are also briefly discussed.

6.4.1. Post-Exercise Beverage and Nutrient Provision

To the research candidate's knowledge, no studies have investigated the effect of different post-exercise beverages consumed with food on substrate repletion; however, a small number have examined nutrient provision (Table 6.5). Participants in these investigations are typically administered a fixed volume of fluid post-exercise and later (i.e. ~1–2 h) given *ad libitum* access to food. (Nb. A caloric beverage will increase nutrient provision compared to a non-nutritive control unless individuals reduce their solid food intake sufficiently to offset the additional energy it delivers). Brown*, et al.* [382] and Clayton*, et al.* [383] found that participants reduced their *ad libitum* intake of energy from food when consuming milk or a dairy-based beverage post-exercise compared to water. In both cases, the magnitude of this reduction was sufficient to offset the additional energy delivered in the caloric beverage, such that the total amount of energy ingested (i.e. from food and beverage) was comparable across trials. Participants did not, however, compensate for the energy delivered in the CHO-containing beverages [382,383]. Still, these studies administered relatively small quantities of fluid (i.e. ~500 mL) that were not intended to restore fluid balance.

The aforementioned study by Campagnolo*, et al.* [8] (i.e. in which male participants consumed different rehydration beverages with food *ad libitum* post-exercise) also assessed nutrient provision. In this study, individuals ingested larger volumes of fluid that, in turn, delivered a greater quantity of nutrition (Table 6.5). While this investigation also found that participants reduced their intake of energy from food when consuming a milk-based post-exercise beverage, the magnitude of the reduction was too small to offset the additional energy derived from fluid. As such, both the CHO-electrolyte sports beverage and the milk-based formulation increased total (i.e. from food and beverage) energy (i.e. ~30%) and CHO (i.e. ~95%) consumption compared to water; the milk-based formulation also increased protein consumption relative to the other beverages (i.e. ~115%) [8]. This study [8] suggests that, when food is available, the type of beverage consumed is more likely to influence nutrient provision than fluid recovery. Hence, an athlete's nutritional needs should be considered when recommending a suitable post-exercise beverage. Once again, however, additional research is required to clarify these effects in different participant populations (i.e. females) and exercise contexts (e.g. between consecutive bouts of activity) that could

potentially elicit different dietary behaviours. Whether or not the nutrient differences associated with access to the different beverages influence exercise recovery and subsequent athletic performance also remains unknown.

Table 6.5. Studies investigating the effect of different post-exercise beverages on dietary behaviour (i.e. *ad libitum* energy intake from food).

Citation	Subjects	Exercise Task	Post-Exercise Beverage	Beverage Volume (L)	*Ad Libitum* Meal	Test Meal Energy Intake (MJ)	Total Energy Intake (MJ)
Clayton *et al*. [383] (2014), UK	12 M	Cycle; 45 min; 60–85% $VO_{2\ max}$	Placebo (0 MJ)	0.5	Pasta, tomato sauce, olive oil and cheese 60 min post-beverage	6.4 ± 0.5	6.4 ± 0.5
			Protein Drink (0.5 MJ)			5.8 ± 1.0[b]	6.4 ± 1.0
			CHO Drink (0.5 MJ)			6.1 ± 1.0	6.6 ± 0.9
Rumbold *et al*. [384]) (2014), UK	9 (0 M)	Cycle; 30 min; 65% $VO_{2\ max}$	Skim Cow's Milk (0.9 MJ)	0.6	Pasta, tomato sauce, olive oil and cheese 60 min post-beverage	2.4 ± 0.7[a]	3.3
			Orange-Flavoured Drink (0.9 MJ)			3.2 ± 0.8	4.1
Brown *et al*. [382] (2016), UK	13 (0 M)	Cycle; 60 min; 65% $VO_{2\ max}$	Water (0 kJ)	0.5	Pasta, tomato sauce, olive oil and cheese 120 min post-beverage	5.5 ± 0.4	5.5
			CHO Drink (1.4 MJ)			5.2 ± 0.5	6.6
			Dairy-Based Beverage (1.4 MJ)			4.4 ± 0.2[b]	5.8
Monteyne *et al*. [385] (2018), UK	15 M	Resistance exercise; ~50 min	Protein Drink (0.4 MJ)	0.5	Pasta, tomato sauce, olive oil and cheese 60 min post-beverage	3.7 ± 1.0[a]	4.1
			CHO Drink (0.4 MJ)			4.2 ± 1.1	4.6
Campagnolo *et al*. [8] (2017); Australia	10 M	Cycle; 60 min; 50–65% PPO	Water (0 kJ)	2.1	Assorted snack foods 60 and 120 min post-exercise	7.8 ± 0.9	7.8 ± 0.9
			Water (0 kJ)	2.1		7.6 ± 1.1	7.6 ± 1.1
			CHO + Electrolyte Drink (3.4 MJ)	2.7		6.8 ± 1.8	10.2 ± 1.5
			Milk-Based Formulation (Sustagen Sport®) (7.4 MJ)	1.8		3.2 ± 1.1[c]	10.6 ± 2.2

M: Male Subjects; PPO: Peak Sustainable Power Output. *a*, indicates a significant difference between two experimental treatments; *b*, indicates a significant difference compared to Water or Placebo; *c*, indicate a significant difference compared to Water and CHO + Electrolyte Drink. Statistical comparisons for Total Energy Intake are not shown (data unavailable).

6.4.2. Dietary Behaviour: The Influence of Sex and Exercise Context

Recent evidence suggests that different beverages promote similar levels of fluid recovery but alter energy and nutrient provision in trained males when consumed voluntarily and with food in the initial hours after exercise [8]. However, it is unclear if these effects persist in different participant populations (i.e. trained females) and across different exercise contexts (e.g. between consecutive bouts of activity) – as these could potentially result in different eating and/or drinking behaviours that might (in turn) influence rehydration and nutrient provision.

6.4.2a. Sex Differences in Dietary Behaviour

Very few studies have directly observed the dietary behaviour of trained females during and/or following a period of exercise. However, several have used dietary surveys to quantify and compare energy, nutrient and fluid intakes, food habits and overall diet quality between sexes [386-389]. For instance, Burke*, et al.* [386] undertook a comprehensive survey (i.e. 7 d food record) of elite Australian athletes (*n*=167; 80 females) from a variety of sporting backgrounds (e.g. endurance, team, sprint- or skill-based sports) during a "typical" training period. In line with earlier studies [387-389], the investigation found that females reported lower relative daily energy and macronutrient intakes than their male counterparts; the largest sex differences were among endurance athletes [386]. Of course, given the methodology employed, these data could potentially be skewed by under-reporting [390]. The study also assessed dietary behaviour during and after exercise. While results suggest that both sexes were equally likely to consume an adequate intake of CHO within the first hour of recovery (i.e. ~1–1.2 $g \cdot kg^{-1}$) [386], males were twice as likely to consume CHO and fluid during training sessions – females typically consumed water alone [386]. Focus group research conducted by sports beverage manufacturers suggests that females may be reluctant to consume caloric fluids because of concerns about energy intake [391]. However, empirical research does not necessarily support this; for instance, Minehan*, et al.* [391] found that female athletes voluntarily consumed similar quantities of a regular CHO-electrolyte sports beverage and a flavoured, low-kilojoule electrolyte beverage during training, suggesting that the energy content of a beverage may not be an important determinant of fluid consumption behaviour. Indeed, it is likely that a wide variety of

factors (i.e. not just concern about weight and body image) contribute to sex differences in post-exercise eating and/or drinking behaviour. For example, compared to males, female athletes report a higher prevalence of some GI conditions (e.g. irritable bowel syndrome and celiac disease) [392]; the menstrual cycle and hormonal contraceptives can also affect appetite and eating behaviour [393] (see *6.4.2b Appetite, Eating Behaviour and the Menstrual Cycle*). There is some evidence to suggest that the appetite-suppressing effect of exercise is less pronounced in females and that the importance of taste in determining food choice differs by sex [394]. Collectively, it appears that female athletes may exhibit different eating and/or drinking behaviours than their male counterparts and that further research is required to better characterise the dietary behaviour of this population in the post-exercise period.

6.4.2b. Appetite, Eating Behaviour and the Menstrual Cycle

Sex hormones appear to function in the regulation of appetite, eating behaviour and energy metabolism [395]. Indeed, studies of rodents suggest that oestrogen inhibits food intake whereas progesterone and testosterone may stimulate appetite [395,396]. Research examining dietary behaviour across the human menstrual cycle similarly suggests that energy intake, typically measured using a food diary or a weighed record, is lowest during the periovulatory phase (i.e. higher oestrogen levels) and highest during the premenstrual phase (i.e. higher progesterone levels) [396]. A previous meta-analysis [396] also reported that energy intake was typically lower (~10%) during the follicular phase than the luteal, and that the luteal phase was associated with an increase in resting metabolic rate (~5–10%). These cyclic changes should therefore be considered in controlled trials investigating the dietary behaviour of female athletes.

6.4.2c. Dietary Behaviour Between Exercise Sessions

Participants in the aforementioned study by Campagnolo*, et al.* [8] were free to leave the research laboratory at the end of the 4 h recovery period. However, it is important to consider that rapid rehydration is often used to facilitate recovery between consecutive exercise sessions (i.e. with limited recovery time) and that the anticipation of further exercise might influence dietary behaviour. For instance, individuals may restrict their intake of food and/or fluid in this situation to avoid

experiencing GI problems during the second bout of exercise, or, alternatively, eat and/or drink in a way they believe will optimise recovery and subsequent athletic performance (i.e. self-select specific foods or nutrients). However, the research candidate is not aware of any studies specifically investigating dietary behaviour or factors influencing athletes' food choices within this context.

6.4.3. Post-Exercise Beverage and Subsequent Athletic Performance

Differences in energy and nutrient provision associated with access to different post-exercise beverages could potentially affect aspects of short-term recovery (e.g. glycogen and muscle protein resynthesis), and thus, subsequent athletic performance. However, to the research candidate's knowledge, no studies have investigated the effect of different post-exercise beverages consumed with food on muscle glycogen resynthesis or subsequent athletic performance. Given that participants' CHO intakes were "suboptimal" for rapid refuelling (i.e. ~0.7 $g \cdot kg^{-1} \cdot h^{-1}$ throughout the 4 h recovery period) in Campagnolo*, et al.* [8] when water was administered, a CHO-containing rehydration beverage (e.g. CHO-electrolyte sports beverage or milk/milk-based formulation) may assist individuals to consume an adequate intake of CHO to meet sports nutrition guidelines (i.e. ~1–1.2 $g \cdot kg^{-1}$), and thus, maximise recovery in the initial hours post-exercise. However, experimental studies are needed to determine whether the consumption of CHO-containing beverages in the post-exercise period can enhance subsequent athletic performance when limited recovery time exists between consecutive exercise sessions.

6.4.4. Post-Exercise Beverage and Chronic Exercise–Nutrient Interactions

Clearly, nutritional strategies that maximise recovery ahead of a subsequent exercise session are important. However, because of the high frequency with which some athlete's train, it is also necessary to consider how these short-term strategies affect chronic exercise–nutrient interactions (e.g. body composition changes, metabolic adaptation and energy availability). The results of Campagnolo*, et al.* [8] suggest that caloric beverages increase energy and nutrient provision in the *immediate* (i.e. ≤4 h) post-exercise period (in males). As yet, however, it is unclear whether individuals modify their dietary behaviour across the remainder of the trial day to offset these

differences. This information would assist to inform the selection of an appropriate post-exercise beverage for individuals with specific long-term dietary goals.

6.5. Conclusion

6.5.1. Summary of Literature Review

Given the detrimental effects of hypohydration on physiological function, considerable research has been undertaken to identify factors that affect the restoration of fluid balance after exercise. Results indicate that individuals must consume a volume of fluid greater than that lost to completely restore euhydration since – even in a hypohydrated state – fluid consumption stimulates diuresis. Some evidence also suggests that a reduced rate of fluid ingestion may aid rehydration, although this effect is likely to be subtle. The most well-researched determinant of fluid recovery is beverage composition. Studies indicate that electrolytes (e.g. Na^+ and K^+), CHO and/or protein increase retention of ingested fluid. Thus, beverages with "complex" nutritional profiles (e.g. milk/milk-based formulations) are reported to be more effective post-exercise rehydration agents than those with "simple" nutritional profiles (e.g. water, CHO-electrolyte sports beverages). However, it is important to recognise that the majority of studies investigating the ability of different beverages to rehydrate post-exercise "prescribe" drinking (i.e. administer fixed quantities of fluid) and deny participants access to food. Given that individuals usually control the volume of fluid they consume and often have access to food in the initial hours after exercise, other personal or contextual factors may influence fluid recovery in a free-living environment. Indeed, findings from the small number of studies investigating *ad libitum* fluid consumption behaviour suggests that individuals may consume different beverages in different volumes, potentially influencing their effectiveness as rehydration agents; the co-ingestion of food also seems to improve fluid retention of beverages with simple nutritional profiles.

To date, only Campagnolo*, et al.* [8] has investigated the ability of different beverages to rehydrate when the fluid and a variety of foods are consumed *ad libitum* post-exercise. The study, which involved 10 endurance-trained males, found that different beverages were *equally* effective at replenishing fluid losses, but that the CHO-containing beverages (i.e. a CHO-electrolyte sports beverage and milk-based

formulation) increased total energy and CHO consumption. Such differences could potentially aid muscle glycogen resynthesis and therefore subsequent athletic performance. However, given the preliminary nature of these findings, additional studies employing protocols that better reflect real-life post-exercise conditions are required to improve our understanding of the interaction between fluid, food and nutrients in post-exercise rehydration. In particular, future studies should explore different participant populations (e.g. females) and exercise contexts (e.g. between consecutive bouts of activity) – as these could potentially elicit different eating and/or drinking behaviours that influence rehydration and nutrient provision. Whether or not the nutrient differences associated with access to the different beverages influences recovery and subsequent athletic performance also remains unknown.

6.5.2. Thesis Part II Research Framework

The research framework of this thesis has been described in Chapter 1 (see *1.3 Research Aims*). Briefly, Thesis Part II addresses two research questions and two main research aims. The research aims are as follows:

Aim 3: To determine the effect of consuming different post-exercise beverages *ad libitum* with food on short-term (i.e. ≤4 h) fluid recovery and nutrient provision in females.

Aim 4: To determine the effect of consuming different post-exercise beverages *ad libitum* with food on short-term (i.e. ≤4 h) fluid recovery, nutrient provision and subsequent athletic performance

The two research aims are met by two specific research studies presented in Chapters 7 & 8, respectively.

Chapter 7: Fluid, Energy and Nutrient Recovery via *Ad Libitum* Consumption of Different Commercial Beverages and Food in Female Cyclists

Reader's note:

This chapter includes a co-authored paper. The bibliographic details of the co-authored paper, including all authors are as follows:

McCartney, D., Irwin, C., Cox, GR., Desbrow, B. Fluid, energy and nutrient recovery via *ad libitum* consumption of different commercial beverages and food in female athletes. *Applied Physiology Nutrition and Metabolism*, 2019; 44(1): 37-46.

The research candidate has made the following contributions to this study:

- Developed the study design (on the basis of earlier research conducted by Campagnolo*, et al.* [8])
- Completed the human research ethics application
- Conducted all participant recruitment and data collection
- Conducted analysis of the data
- Prepared the manuscript for submission to a peer-reviewed journal
- Presented the research at an international conference

(Signed) _____ (Date: 20.02.2019)

Danielle McCartney

(Countersigned) _____ . (Date: 20.02.2019)

Supervisor: A/Prof Ben Desbrow

(Countersigned) _____ (Date: 20.02.2019)

Supervisor: Dr Christopher Irwin

7.1. Abstract

Background: Recent evidence suggests that different beverages promote similar levels of fluid recovery but alter energy and nutrient provision in trained males when consumed *ad libitum* with food post-exercise. However, it is unclear if these effects exist in trained females, who may exhibit contrasting dietary behaviours. This study investigated the effect of consuming different beverages *ad libitum* with food post-exercise on fluid, energy and nutrient recovery in trained females. **Method:** On 4 separate occasions, 8 females (BM: 61.8±10.7 kg; VO_{2max}: 46.3±7.5 mL·kg^{-1}·min^{-1}) lost 2.0±0.3% BM cycling at ~75%VO_{2max} before completing a 4 h recovery period with *ad libitum* access to one of 4 beverages: Water, Powerade® (Sports Drink), Up & Go Reduced Sugar ™ (Lower Sugar [LS-MILK]) or Up & Go Energize ™ (Higher Protein [HP-MILK]). Participants also had 2 × 15 min opportunities to access food within the first 2 h of the recovery period. Beverage intake; total water/nutrient intake; and indicators of fluid recovery (BM, urine output, P_{OSM}), appetitive sensations and beverage palatability were assessed periodically. **Results:** While total water intake (from food *and* beverage) (Water: 1918±580 g; Sports Drink: 1809±338 g; LS-MILK: 1458±431 g; HP-MILK: 1523±472 g; p=0.010) and total urine output (Water: 566±314 g; Sports Drink: 459±290 g; LS-MILK: 220±53 g; HP-MILK: 230±117 g; p=0.009) differed significantly by beverage, the quantity of ingested water retained was similar across treatments (Water: 1352±462 g; Sports Drink: 1349±407 g; LS-MILK: 1238±400 g; HP-MILK: 1293±453 g; p=0.691). Total energy intake (from food *and* beverage) increased in proportion to the energy density of the beverage (Water: 4129±1080 kJ; Sports Drink: 5167±643 kJ; LS-MILK: 6019±1925 kJ; HP-MILK: 7096±2058 kJ; p=0.014). **Conclusion:** When consumed voluntarily and with food, different beverages promote similar levels of fluid recovery but alter energy and nutrient provision in trained female cyclists. Providing access to food and understanding the longer-term dietary goals of athletes are important considerations when recommending a recovery beverage.

7.2. Introduction

Athletes regularly complete competitive events and training sessions that result in substantial sweat loss [44]. Typically, the quantity of fluid consumed during exercise is inadequate to replace these losses [6]. Hence, individuals often finish activity in body water deficit. Exercise may also be accompanied by the depletion of endogenous substrate stores and damage to skeletal muscle tissue [397]. Nutritional strategies that maximise recovery ahead of a subsequent exercise session are, therefore, important. Given the frequency with which athlete's train, acute recovery strategies also have the potential to influence chronic exercise-nutrient interactions (e.g. metabolic adaptation and body composition changes). Hence, nutritional recommendations for post-exercise recovery should align with an athlete's broader dietary goals.

Nutritional recommendations designed to optimise recovery after exercise have been published by the ACSM and the Academy of Nutrition and Dietetics [1,2]. These recommendations encourage individuals to ingest fluid (i.e. 1.25–1.50 L·kg BM lost^{-1}) to restore euhydration and consume CHO and protein to promote glycogen and muscle

protein synthesis. The meta-analyses presented in Chapters 3 & 4 also highlight the importance of consuming fluid and CHO intake between exercise sessions to enhance subsequent athletic performance. Given their ability to deliver both fluid *and* nutrients, beverages have received considerable scientific attention in regard to their influence on recovery.

Most studies investigating the effect of beverage type and composition on fluid recovery following exercise have employed "prescribed" drinking protocols, where participants consume a fixed quantity of fluid at a predetermined rate (Chapter 6). Findings generally indicate that beverages with complex nutritional profiles (i.e. milk/milk-based formulations) are more effective rehydration agents than beverages with simple nutritional profiles (i.e. water and CHO-electrolyte sports beverages) [147,331,333,354]. In addition to fluid, these beverages also typically provide additional CHO and protein, making them ideal candidates to aid post-exercise recovery. However, other factors, including thirst, palatability and GI tolerance or other appetite sensations, which are likely to influence the volume of fluid consumed in practice, are overlooked in studies that prescribe drinking. A small body of research investigating *ad libitum* fluid consumption behaviour suggests that individuals consume different fluids in different quantities post-exercise [144,368,371-373], likely influencing their effectiveness as a recovery options. In addition to "prescribing" drinking, many studies also deny participants access to food: an approach with limited ecological validity. Findings from studies that have allowed participants to eat as part of the experimental protocol generally suggest that co-ingesting food and fluid improves rehydration, such that the magnitude of difference between beverages with different nutrient profiles (i.e. in terms of their potential to rehydrate) is reduced [8,365,375-379]. Still, most studies have prescribed a small number of food items that may not reflect the participants' usual dietary behaviour.

To date, one only study [8] has investigated the ability of different beverages to rehydrate participants when the drink *and* a variety of foods are consumed *ad libitum* post-exercise. The study, which involved 10 endurance-trained males, found that fluid recovery was *similar*, regardless of the type of beverage consumed. This suggests that, with the co-ingestion of food, fluid restoration following exercise may not be influenced by beverage choice. The type of beverage did, however, affect nutrient provision during

the post-exercise period. Specifically, the administration of a CHO-electrolyte sports beverage or a milk-based formulation increased total (i.e. food *and* beverage) energy (~2500 kJ) and CHO (~200 g) intake compared to water; the milk-based formulation also increased total protein consumption (~70 g) compared to the other beverages. Hence, choice of post-exercise beverage may affect aspects of recovery and the suitability of certain beverages for athletes with specific dietary goals. Different energy and nutrient requirements, dietary preferences and motives influencing food choice have the potential to limit the generalisability of previous results. Indeed, it is currently unclear if these effects persist in trained females, who may exhibit contrasting dietary behaviours [398]. Furthermore, it not known how beverages with similar sensory characteristics (i.e. taste, aroma, appearance) but contrasting nutrient profiles affect fluid, energy and nutrient recovery following acute exercise, when consumed *ad libitum* and when access to food is provided.

This study aimed to investigate the effect of consuming different commercial beverages (including milk-based beverages with contrasting formulations) with food *ad libitum* post-exercise on fluid, energy and nutrient recovery in trained females. The research candidate hypothesised that, when consumed *ad libitum* with food, these beverages would result in similar levels of fluid recovery, but different energy and nutrient intakes.

7.3. Methods

7.3.1. Participant Characteristics

Female cyclists/triathletes (≥3 h cycling·week^{-1}) aged 18–45 y were eligible to participate in this investigation. Sample size was determined using power calculation software (G*Power Version 3.1.9.2, University Kiel Germany, 2014). A comparable study of male cyclists [8] detected a significant effect of rehydration beverage (Water vs. Sustagen Sport® vs. Powerade®) on energy intake (partial eta squared [η_p^2] =0.62). Using a power (1-β) of 0.95 and α=0.05 with an equivalent effect, we predicted that 8 participants would be required to detect a significant effect. Ten participants were recruited to account for attrition. One participant withdrew after the familiarisation because trials conflicted with her training schedule; a second individual withdrew after the first experimental trial due to poor availability. The 8 remaining participants (age:

33.2±7.4 y; $VO_{2\ max}$: 46.3±7.5 mL·kg^{-1}·min^{-1}; peak sustainable power output [PSPO]: 244±32 W; cycling: 115±60 km·week^{-1}; Mean±SD) completed all 4 experimental trials. This investigation was approved by the University's Human Ethics Committee (GU 2017/730) and procedures were conducted in accordance with principles outlined in the agreement of Helsinki.

7.3.2. Study Design

Experimental procedures are summarised in Figure 7.1. Each participant attended the laboratory on 6 separate occasions to complete one preliminary screening visit, one familiarisation, and 4 repeated-measures experimental trials (≥5 d apart). Trials were counterbalanced for order and scheduled during the first 14 days of the menstrual cycle (i.e. follicular phase) to minimise the confounding influence of hormonal changes on appetite and substrate utilisation [393,399]. Participants using hormonal contraceptives at the time of the investigation (*n*=3) completed testing while taking the active medication. Each experimental trial involved exercise-induced dehydration followed by a 4 h recovery period, with *ad libitum* access to one of 4 beverages: (1) Water, (2) Powerade® Isotonic (Coca Cola Ltd.) (Sports Drink), (3) Up & Go Reduced Sugar ™ (Sanitarium®, Australia) (Lower Sugar [LS-MILK]), and (4) Up & Go Energize ™ (Sanitarium®, Australia) (Higher Protein [HP-MILK]). Drinking was permitted throughout the 4 h recovery period. Participants also had 15 min to access food at the end of the first and second hour. Individuals were unaware that their dietary behaviour was being monitored; they were instead told that the study intended to investigate the influence of different beverages on recovery of cognitive function (sham questions and cognitive function tasks were administered throughout the trials to maintain this deception). Each participant was fully informed of the purpose of the study and given the opportunity to withdraw their data once data collection activities for the entire study were complete.

7.3.3. Eligibility and Participant Screening

On arrival at the initial visit, individuals completed a medical history questionnaire and the Eating Attitudes Test-26 (EAT-26) [400] (Part C only, to deemphasize the importance of dietary behaviour to this study). Those with a history of cardiovascular, metabolic and/or kidney disease, or currently taking medications known to affect

substrate metabolism were ineligible to participate. Individuals were also excluded if their responses to the EAT-26 indicated possible disordered eating, as were volunteers who reported an allergy, intolerance or dislike towards the food items or test beverages used in the investigation. Next, a self-reported BM history (~6 m) was collected and anthropometric measurements were obtained using digital scales (HW-PW200; A&D Company Ltd, Tokyo, Japan) and a stadiometer device. Participants had to be weight stable (i.e. a BM change ≤5% in 6 months) and not followed an energy-restricted diet during the previous ~6 m. Once eligibility was verified, participant's beverage flavour preferences where recorded and they were trained by a dietitian on how to keep a 24 h diet record. Finally, participants completed a graded exercise test on an electronically braked cycle ergometer (Lode Excalibur Sport; Lode BV, Groningen, Netherlands) for determination of VO_{2max}. The exercise protocol began at 50 W and increased in 30 W increments every 2.5 min until volitional exhaustion. Participants' respiratory gases were sampled continuously by breathing into a calibrated gas analysis system (Medgraphic Ultima, MGC Diagnostics and Medisoft, USA). PSPO was also calculated [401] and used to establish the exercise intensity on subsequent trials.

7.3.4. Familiarisation

Participants completed a full familiarisation trial which employed a chocolate-flavoured milk beverage (different from those used in the experimental trials) as the recovery fluid. This allowed individuals to become accustomed to the research protocol and the effects of consuming milk-products post-exercise.

7.3.5. Pre-Trial Procedures

Prior to each trial, participants were instructed to: (1) abstain from alcohol (24 h); (2) avoid caffeine-containing products and moderate–strenuous exercise (12 h); (3) record all food and fluid consumed (24 h); (4) consume a standardised pre-packaged evening meal (~60 $kJ \cdot kg^{-1}$) [8]; and, (5) fast from all food and fluid (including water) for ~10 h (overnight). Food records were submitted to an investigator and analysed (FoodWorks® Version 8, Xyris Software Pty Ltd, Spring Hill, Australia) by a dietitian using the relevant national food database. A copy of the food record was returned to the participant to assist in the replication of the pre-trial dietary intake.

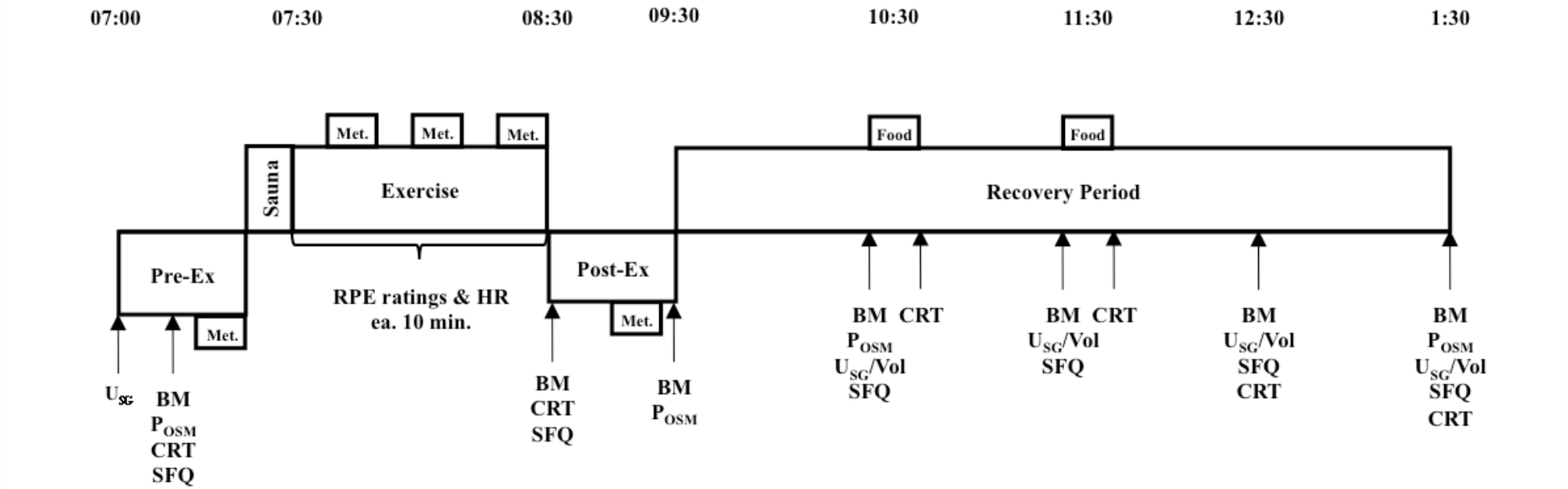

Figure 7.1. Schematic representation of the experimental procedures. BM: Nude body mass; CRT: Choice reaction time cognitive task (for experimental blinding); Food: *ad libitum* access to snack foods for 15 min; Met: metabolic gas measurements; P_{OSM}: Blood collection for plasma osmolality analyses; SFQ: Subjective Feelings Questionnaire; U_{SG}/Vol: Urine collection for measurement of specific gravity and volume.

7.3.6. Experimental Procedures

7.3.6a. Pre-Exercise Period

Participants verbally acknowledged compliance to the pre-trial procedures and self-reported the onset of menstruation on arrival at the laboratory (~7 AM). A urine sample was collected to monitor hydration status (U_{SG}) (Palette Digital Refractometer, ATAGO, USA). One participant had a pre-exercise U_{SG} ≥1.024 on the familiarisation trial, indicating some level of dehydration [402]. This individual was administered water (600 mL) and, when reassessed 30 min later, U_{SG} was <1.024. This practice was repeated on all subsequent trials to ensure consistency. All remaining subjects produced initial U_{SG} samples <1.024 at each attendance. Euhydrated participants rested in the supine position for 15 min prior to respiratory gases (Medgraphic Ultima, MGC Diagnostic Corporation, USA) being measured continuously for 10 min to determine baseline metabolic rate. Following the respiratory measures, participants provided a blood sample, and completed the cognitive function test and subjective feelings questionnaire (Adaptive Visual Analogue Scale (AVAS); Marsh-Richard*, et al.* [403]). Individuals then voided their bladder completely and a pre-exercise nude BM measurement was obtained.

7.3.6b. Dehydration and Exercise Protocol

Dehydration was induced via passive heat exposure (10 min, ~70°C, sauna) followed immediately by exercise on a cycle ergometer (Figures 7.2 & 7.3). Exercise began at a workload of 60% PSPO (24.2±0.9°C; 66±11% RH). However, subjects who expressed likely volitional exhaustion prior to achieving the required BM loss (≥1.8%) could choose to reduce the workload by 5% PSPO after 20 and 40 min of exercise (the minimum allowable workload was 50% PSPO). HR and RPE on the Borg scale (Range: 6–20) [404] were collected at 10 min intervals throughout exercise. Respiratory gases were measured continuously between ~12-20 min, ~32-40 min and ~52-60 min of exercise for determination of energy expended during exercise. Nude BM was measured after 60 min of cycling. If BM loss was <1.8% from baseline, participants were required to continue exercise in 10 min intervals (respiratory gasses were collected across the final 5 min of each additional 10 min block). Once a BM loss ≥1.8% was achieved, exercise ceased. The duration and intensity of the exercise was documented during the familiarisation session and replicated on all subsequent trials. Participants repeated the cognitive function test

and responded to the subjective feelings questionnaire immediately after exercise. They then rested in a supine position and a blood sample was drawn. After resting ~15 min, respiratory gases were collected continuously for a final ~10 min. Individuals then showered and dried themselves thoroughly before a final nude BM measurement was obtained. The change in BM due to fluid loss was calculated by subtracting the final post-exercise BM from the pre-exercise BM.

Figure 7.2. Portable Sauna

Figure 7.3. Exercise-induced dehydration

7.3.6c. Nutrition Recovery Period

Participants completed a 4 h recovery period in an observation room adjacent to the exercise laboratory, where they were allowed to undertake sedentary activities. Participants were given immediate access to one of 4 commercial beverages (Water, Sports Drink [Energy: 103 kJ·dL^{-1}; CHO: 5.8 g·dL^{-1}; Sodium: 28 mg·dL^{1}; Water: 95.0 g·dL^{-1}], LS-MILK [Energy: 279 kJ·dL^{-1}; CHO: 8.9 g·dL^{-1}; Protein: 3.4 g·dL^{-1}; Fat: 1.5 g·dL^{-1}; Sodium: 65 mg·L^{1}; Water: 84.4 g·dL^{-1}], or HP-MILK [Energy: 344 kJ·dL^{-1}; CHO: 9.9 g·dL^{-1}; Protein: 6.7 g·dL^{-1}; Fat: 1.5 g·dL^{-1}; Sodium: 100 mg·L^{1}; Water: 84.4 g·dL^{-1}]) in excess of expected consumption (~3.0 L) . They were told that the two milk-drinks were *different*, but no further product information was provided. (Participants were not permitted to change flavours between milk trials). All beverages were stored in refrigerators (~4°C) in opaque

Jugs (Figure 7.4). Participants were instructed to *"self-serve"* into cups and to "*drink as much as they liked*", as more fluid would be provided upon request. The beverage vessels did not have volume increments. Palatability was evaluated at the onset of drinking and at the conclusion of the recovery period.

Figure 7.4. Beverages are stored in personal fridges

After 1 h, participants completed the subjective feelings questionnaire, collected urine output, provided a blood sample, and measured nude BM. They were then given access to a variety of foods for 15 min. Participants entered a private room containing food and a computer (set up for the cognitive function task). They were allowed to bring their beverage but were asked to refrain from taking other external items. This approach was designed to avoid social interactions which may influence eating behaviours and to reinforce the importance of the cognitive task. Food items included sports bars, fresh fruit, breads and condiments (Figure 7.5; Table 7.1) with participants being instructed to "*eat as much as they liked"* and that *"more of the same food would be provided in 1 h but no food could be removed from the private room due to health and safety regulations*". Participants were also informed that no food would be provided in the third and fourth hours of recovery. In the final 2 min, participants completed the cognitive function test and then returned to the main observation room. These procedures (excluding blood sampling) were repeated at the end of the second hour of the recovery period. At the end of the third and fourth hours, individuals completed the cognitive function test and subjective feelings questionnaire, collected urine output and measured nude BM. Blood sampling was repeated at the end of the fourth hour.

Figure 7.5. Private room containing snack foods

Table 7.1. Nutritional composition of the food items offered (per 100 g).

Food Item	Energy (kJ)	Protein (g)	Fat (g)	CHO (g)	Sodium (mg)	Water (g)
Sports Bar (Apple Berry Crumble), Winners	1480	5.9	5.4	67.4	8	8.5
Sports Bar (Mountain Mix), Winners	1630	7.3	11.6	62.0	8	8.5
Muesli Bar (Yoghurt & Strawberry) Uncle Toby's	1630	7.1	11.1	59.9	19	8.2
Whole Banana	385	1.4	0.3	19.6	0	76.2
Whole Apple	247	0.3	0.4	11.9	0	83.9
Sultanas, Sunbeam®	1290	3.1	0.1	69.0	10	16.3
Raisin Toast, Tip Top®	1150	8.7	2.2	52.9	196	36.0
Multigrain Bread, Woolworths Homebrand	1080	9.3	2.5	47.0	400	37.1
Cheese, Coon™	1690	25.8	33.3	1.0	700	34.0
Plain Rice Crackers, Sakata®	1680	7.4	2.9	86.1	387	4.6
Crunchy Peanut Butter, Kraft	2580	23.7	51.3	13.4	578	1.5
Honey, Woolworths Select	1416	0.3	0.0	83.1	15	16.2
Jam, Fruits of the Forest, IXL®	1110	0.4	0.1	64.1	17	30.7
Yeast Extract Spread, Vegemite	798	25.4	0.9	19.9	3,300	40.9
Margarine, Flora™	2420	0.2	65.0	0.7	590	35.1
Salted Cashews, Woolworths Homebrand	2560	21.3	48.5	22.1	220	1.9

Nutrition values for packaged foods obtained from product nutrition information panel; values for fresh food items and product water content derived from FoodWorks® Version 8 software (Xyris Software Pty Ltd, Spring Hill, Australia).

7.3.7. Post-Trial Procedures

On leaving the laboratory, participants were required to keep a record of the food and fluid they consumed over the remainder of the day and to capture images of each item using a tablet device (iPad, Apple®, Cupertino, USA). Participants were encouraged to include a fiducial marker in each image to assist the investigator in the estimation of portion size. Food records and accompanying images were submitted to an investigator and checked for completeness. Diet records were analysed using the method previously described. A limit of up to two standard alcoholic drinks (20 g alcohol) was imposed on trial days to avoid alcohol's influence on appetitive responses [405].

7.3.8. Study Completion Procedures

At the conclusion of the experiment, participants were asked to indicate whether the purpose of the study was to investigate the effect of consuming different beverages after dehydrating exercise on: (a) mental (i.e. cognitive) function; (b) appetite and food intake; or, (c) muscle soreness and recovery, and, how confident they were that their answer was correct to verify that deception was effective. Individuals also completed the R18 Three-Factor Eating Questionnaire [406] to measure cognitive, behavioural and emotional influencers of eating behaviour.

7.3.9. Data Collection

7.3.9a. Food and Fluid Intake Measures

Energy, macronutrient, sodium and water intake during the recovery period was determined by weighing the food items (covertly) and beverages to the nearest 1 g after each hour. Participants were aware that beverage intake was being monitored, but no information was provided to participants on how much fluid they had consumed. Nutritional values for packaged foods were taken from the product nutrition information panel; values for fresh food items and product water content were derived from the dietary analysis software.

7.3.9b. Estimated Energy Expenditure

Gas exchange data was averaged in 30 s segments over the final ~8 min of each collection period. Estimated energy expenditure (eEE) was then quantified in the following increments: pre-exercise (-20–0 min), exercise blocks 1 (0–20 min), 2 (20–40 min) and 3 (40–60 min), post-exercise (60–80 min), and total trial (100 min). (Where participants continued exercise in 10 min intervals after 60 min, eEE was also quantified at exercise block 4 (60–70 min), such that the total time increased to 110 min). Rates and total substrate oxidation were calculated using the equations of Frayn [407] (assuming negligible protein oxidation). Energy equivalents of 16.75 $kJ \cdot g^{-1}$ of CHO and 37.68 $kJ \cdot g^{-1}$ of fat were utilised to calculate eEE from substrate oxidation [408].

7.3.9c. Urine Sampling and Water Retention

At the end of each hour of the recovery period, participants voided their bladder completely into an empty container for measures of hourly urine output and hourly U_{SG} (Figure 7.6). Participants were permitted to urinate throughout the observation period, and on each occasion, the void was collected and added to the hourly urine output. Total urine loss was calculated as the accumulated urine output from the onset of drinking until the end of the observation period. The proportion of ingested water (i.e. from food and beverage) retained at the end of the 4 h recovery period was calculated using the following formula:

Figure 7.6. Measurement of urine volume and U_{SG}

$$\text{Water Retained (\%)} = \frac{(WI_{Total}(g) - \text{Urine Output (g)})}{WI_{Total}(g)} \times 100$$

Where WI_{Total} represents the total amount of water consumed via food and beverage during the recovery period.

7.3.9d. Body Mass Measurements

Nude BM measures were obtained pre- and ~30 min post-exercise (i.e. after sweating had ceased) and at the end of each hour of the recovery period. All BM measurements were adjusted to account for the non-water mass of the food and beverages consumed.

7.3.9e. Subjective Feelings and Beverage Palatability Questionnaires

Visual analogue scales (VAS) were used to measure subjective feelings of fullness, thirst and fullness pre- and post-exercise and at each hour of the recovery period. To distract participants from the primary outcomes of the study, individuals were also required to indicate feelings of alertness, concentration, muscle soreness and energy levels. VASs were also used to assess beverage palatability (i.e. 'pleasantness') at the onset of drinking and at the conclusion of the recovery period. Beverages were subsequently ranked from most to least palatable (1^{st}–4^{th}) (based on individual participants' preferences) using the average pre/post-recovery palatability rating. All measures were conducted on a 100 mm scale, with 0 mm representing 'not at all' and 100 mm representing 'extremely' using a computerized modifiable software program (AVAS; Marsh-Richard*, et al.* [403]).

7.3.9f. Blood Sampling

Participants rested for ~5 min in a supine position prior to a ~5 mL blood sample being drawn from an antecubital vein. Blood samples were obtained pre-exercise, post-exercise and at the end of the first and fourth hours of the recovery period (Figure 7.7). All samples were collected into pre-treated lithium heparin vacutain-

Figure 7.7. Blood sampling

ers (Becton Dickson vacutainers®) and centrifuged for 10 min (1350 × *g*). Aliquots of plasma supernatant were stored (-80°C) and later analysed in duplicate for P_{OSM} using a calibrated, freezing-point depression osmometer (Osmomat 030, Gonotec, Berlin, Germany).

7.10. Statistical Analysis

Statistical analyses were completed using SPSS Statistics for Windows, Version 21.0 (IBM Corp. 2012, Armonk, N.Y., USA). All measures were examined for normality (Shapiro-Wilk test) and sphericity (Mauchly's test). Where assumptions of sphericity in repeated-measures analyses were violated, the Greenhouse-Geisser statistic was applied. Comparisons between experimental trials for baseline measures (BM, U_{SG}, and P_{OSM}); exercise-induced fluid loss; eEE; previous-day energy/water intake; beverage intake; water intake from beverage ($WI_{Beverage}$), food (WI_{Food}) and beverage plus food (WI_{Total}); energy intake from beverage ($EI_{Beverage}$), food (EI_{Food}) and beverage plus food (EI_{Total}); nutrient intake; urine output; and water retention were performed using one-way repeated measures analysis of variance (ANOVA). The remaining variables were examined using a two-way (Treatment × Time) repeated-measures ANOVA. Pairwise comparisons (paired *t*-tests) were performed where significant main effects were present (Bonferroni). One-way ANOVA (Bonferroni) were used to conduct post hoc comparisons where significant interaction effects were present. Each of the 18 items on the Three-Factor Eating Questionnaire was given a score between 1 and 4 and item scores were summated into raw scores for cognitive restraint, uncontrolled eating, and emotional eating. Raw scale scores were then transformed to a 0–100 scale [((raw score − lowest possible raw score)/possible raw score range) × 100]. Higher scores in the respective scales are indicative of greater cognitive restraint, uncontrolled, or emotional eating [406]. Effect sizes are reported as η_p^2. Significant differences were accepted as $p<0.05$. Data are Mean±SD, unless otherwise indicated.

7.4. Results

7.4.1. Standardisation Procedures

All participants verbally acknowledged compliance to the pre-trial procedures on arrival at the laboratory. Participants' pre-trial records indicated similar energy,

$F_{[3,21]}$=1.78, p=0.182; and water, $F_{[3,21]}$=0.555, p=0.651; intakes 24 h prior to each trial (Water: 9.2±1.8 MJ, 4.5±0.7 L; Sports Drink: 8.3±2.2 MJ, 4.0±1.3 L; LS-MILK: 8.5±2.0 MJ, 4.3±0.5 L; HP-MILK: 9.0±2.6 MJ, 4.3±1.2 L). Pre-exercise values for BM, U_{SG} and P_{OSM} were also similar across treatments (Table 7.2).

7.4.2. Exercise-Induced Dehydration

All participants successfully replicated the same exercise protocol at each experimental trial. For three individuals, the total exercise duration was 70 min; the remainder cycled 60 min to achieve the required BM loss. Gas exchange data indicate that participants exercised at an intensity corresponding to 76±5% VO_{2max}. Neither BM loss nor eEE differed significantly by Treatment (Table 7.2) or by trial order, $F_{[3,21]}$=0.279, p=0.840; $F_{[3,21]}$=0.806, p=0.504, respectively. 4 (Treatment) × 6 (Time) analyses of RPE and HR identified a significant main effect of Time on each variable, $F_{[1.3,9.1]}$=16.79, p=0.002; $F_{[1.8,12.5]}$=16.0, p<0.001, respectively. A significant main effect of Treatment was also observed on HR, $F_{[3,12]}$=3.21, p=0.044; however, pairwise comparisons did not detect any differences across trials (p's>0.05). No main effect of Treatment was observed on RPE, $F_{[3,21]}$=2.07, p=0.134.

Table 7.2. Pre-trial conditions and exercise-induced dehydration

	Water	Sports Drink	LS-MILK	HP-MILK	F-ratio	*p*-value
Pre-exercise U_{SG}	1.012 ± 0.005	1.014 ± 0.007	1.013 ± 0.005	1.014 ± 0.008	$F_{[3, 21]}$=0.547	0.656
Pre-exercise P_{OSM}	286 ± 5	286 ± 8	287 ± 4	287 ± 3	$F_{[1.4, 8.4]}$=0.172	0.770
Pre-exercise BM (kg)	61.98 ± 10.78	61.68 ± 10.86	61.28 ± 10.53	61.56 ± 10.80	$F_{[3, 21]}$=1.69	0.201
BM loss (kg)	1.20 ± 0.19	1.21 ± 0.15	1.22 ± 0.16	1.23 ± 0.10	$F_{[3, 21]}$=0.151	0.928
BM loss (%)	1.96 ± 0.28	2.00 ± 0.43	2.03 ± 0.31	2.03 ± 0.26	$F_{[3, 21]}$=0.388	0.763
Total eEE (kJ)	3241 ± 349	3289 ± 388	3270 ± 457	3164 ± 264	$F_{[3, 21]}$=0.805	0.505

BM: Body mass; eEE: Estimated energy expenditure; HP-MILK: Up & Go Energize™; P_{OSM}: Plasma osmolality (mOsm·kg^{-1}); LS-MILK: Up & Go Reduced Sugar™; U_{SG}: Urine specific gravity. Plasma osmolality values from n=7 participants where blood sampling was performed. Values are Mean±SD.

7.4.3. Water Intake from Food and Beverages

Water and beverage intakes are displayed in Table 7.3. Beverage intake did not differ significantly by Treatment (Table 7.3) or by Trial Order, $F_{[1.8,12.3]}$=0.569, p=0.558. However, $WI_{Beverage}$ was significantly lower with LS-MILK than Sports Drink (p=0.040) and with HP-MILK than Water (p=0.022). While WI_{Total} also differed significantly across

treatments, pairwise comparisons only indicated a trend for a difference in WI_{Total} between HP-MILK and Water (p=0.080); no other differences were observed (p's>0.05). A 4 (Treatment) × 4 (Time) analysis of mean hourly WI_{Total} identified a significant main effect of Time, $F_{[3,21]}$=62.4, p<0.001, η_p^2=0.90; such that WI_{Total} was greater in the first hour of recovery (864±195 g) than at all subsequent time points (p's≤0.001). WI_{Total} did not differ across the second (315±42 g), third (307±119 g) or fourth (191±140 g) hours of recovery (p's>0.05). No significant Treatment × Time interaction was observed, $F_{[9,63]}$=1.66, p=0.118, η_p^2=0.19.

7.4.4. Urine Output and Water Retention

Urine output and water retention data are displayed in Table 7.4; hourly urine volumes and U_{SG} values are indicated in Figure 7.8. Total urine output differed significantly by Treatment. Pairwise comparisons revealed that urine output was elevated with Water compared to HP-MILK (p=0.048) and tended to be elevated compared to LS-MILK (p=0.073); urine output was similar between all other treatments (p's>0.05). A 4 (Treatment) × 4 (Time) analysis of mean hourly urine outputs indicated a main effect of Time, $F_{[3,21]}$=4.544, p=0.013, η_p^2=0.48; such that mean urine output was greater during the fourth hour of recovery than the first hour (p=0.027); but comparable across all other time points (p's>0.05). No Treatment × Time interaction was observed, $F_{[9,63]}$=1.25, p=0.281, η_p^2=0.15. A 4 (Treatment) × 4 (Time) analysis of mean hourly U_{SG} values identified significant main effects of Treatment, $F_{[3,21]}$=17.7, p<0.001, η_p^2=0.72; and Time, $F_{[3,21]}$=5.62, p=0.005, η_p^2=0.45; and a significant Treatment × Time interaction, $F_{[9,63]}$=5.70, p<0.001, η_p^2=0.45. Post hoc comparisons revealed that Water decreased U_{SG} compared to LS-MILK and HP-MILK between the second and fourth hours of recovery (p's<0.05); Sports Drink also decreased U_{SG} compared to LS-MILK (p=0.031) and HP-MILK (p=0.032) during the third hour of recovery, and tended to decrease U_{SG} compared to LS-MILK (p=0.084) and HP-MILK (p=0.054) during the third hour of recovery, although this was not at a level of statistical significance. The proportion (%) of WI_{Total} retained differed significantly across treatments. Pairwise comparisons revealed that water retention increased with LS-MILK compared to Water (p=0.043), but similar across all other treatments (p's>0.05). The overall quantity (g) of WI_{Total} retained at the conclusion of the experimental protocol did not differ significantly by treatment.

Table 7.3. Total water and nutrient intake from food and beverages during the 4 h recovery period and entire trial day (trial *plus* post-trial diet).

	Water	Sports Drink	LS-MILK	HP-MILK	F-ratio	*p*-value	η_p^2
Nutrient Intake from Beverage							
Beverage Intake (g)	1790 ± 540	1801 ± 359	1538 ± 485	1639 ± 587	$F_{[3,21]}$=1.32	0.294	0.16
Water (g)	1790 ± 540^{c}	1711 ± 339^{b}	1299 ± 410	1383 ± 495	$F_{[3,21]}$=5.72	**0.005**	0.45
Energy (kJ)	0 ± 0^{a,b,c}	1874 ± 371^{b,c}	4282 ±1337	5639 ± 2019	$F_{[1.3,9.3]}$=48.8	**<0.001**	0.88
CHO (g)	0 ± 0 ^{a,b,c}	104 ± 21	136 ± 42	162 ± 58	$F_{[3,21]}$=45.8	**<0.001**	0.87
Protein (g)	0 ± 0^{b,c}	0 ± 0^{b,c}	52 ± 16^{c}	110 ± 39	$F_{[3,21]}$=59.6	**<0.001**	0.90
Fat (g)	0 ± 0^{b,c}	0 ± 0^{b,c}	23 ± 7	25 ± 9	$F_{[3,21]}$=63.2	**<0.001**	0.90
Sodium (mg)	0 ± 0^{a,b,c}	504 ± 101^{b,c}	1000 ± 315^{c}	1639 ± 587	$F_{[1.2,\ 8.7)}$=48.3	**<0.001**	0.87
Beverage Mass (kg)	1.79 ± 0.54	1.80 ± 0.36	1.54 ± 0.48	1.64 ± 0.59	$F_{[3,21]}$=1.32	0.294	0.16
Nutrient Intake from Food							
Water (g)	128 ± 59	98 ± 57	160 ± 75	140 ± 78	$F_{[3,21]}$=1.66	0.206	0.19
Energy (kJ)	4129 ± 1080^{b,c}	3292 ± 633^{c}	1737 ± 1171	1457 ± 797	$F_{[3,21]}$=17.4	**<0.001**	0.71
CHO (g)	107 ± 34^{b,c}	75 ± 18^{c}	52 ± 25	42 ± 19	$F_{[3,21]}$=17.0	**<0.001**	0.71
Protein (g)	29 ± 9^{c}	24 ± 8	10 ± 10	8 ± 7	$F_{[3,21]}$=12.4	**<0.001**	0.64
Fat (g)	47 ± 14^{c}	42 ± 13^{c}	17 ± 21	15 ± 15	$F_{[3,21]}$=11.8	**<0.001**	0.63
Sodium (mg)	735 ± 334^{b,c}	738 ± 345^{c}	208 ± 241	205 ± 208	$F_{[1.4,\ 9.7]}$=12.9	**0.003**	0.65
Food Mass (kg)	0.32 ± 0.86^{c}	0.25 ± 0.74	0.25 ± 0.11	0.21 ± 0.88	$F_{([3,21]}$=4.13	**0.019**	0.37
Total Nutrient Intake (Food & Beverage)							
Water (g)	1918 ± 580	1809 ± 338	1458 ± 431	1523 ± 472	$F_{([3,21]}$=4.92	**0.010**	0.41
Energy (kJ)	4129 ± 1080	5167 ± 643	6019 ± 1925	7096 ± 2058	$F_{[1.5,\ 10.4]}$=7.37	**0.014**	0.51
CHO (g)	107 ± 34^{a,b,c}	180 ± 30	188 ± 46	204 ± 47	$F_{[3,21]}$=12.9	**<0.001**	0.65
Protein (g)	29 ± 9^{b,c}	24 ± 8^{b,c}	63 ± 20^{c}	118 ± 41	$F_{[1.3,9.1]}$=33.8	**<0.001**	0.83
Fat (g)	47 ± 14	42 ± 13	40 ± 24	40 ± 19	$F_{[1.4,9.8]}$=0.437	0.590	0.06
Sodium (mg)	735 ± 334^{a,c}	1242 ± 322	1208 ± 298^{c}	1844 ± 488	$F_{[1.5,10.5]}$= 0.7	**0.005**	0.61
Total Mass (kg)	2.11 ± 0.57	2.05 ± 0.35	1.79 ± 0.52	1.85 ± 0.55	$F_{[3,21]}$=2.27	0.110	0.25
Nutrient Intake for Entire Trial Day							
Water (L)	4.0 ± 1.4	4.0 ± 1.1	3.9 ± 1.0	3.7 ± 1.4	$F_{[3,21]}$=0.65	0.592	0.09
Energy (MJ)	8.4 ± 2.4	9.9 ± 2.2	11.0 ± 3.1	10.8 ± 2.5	$F_{[3,21]}$=3.71	**0.028**	0.35
CHO (g)	201 ± 77^{b}	285 ± 65	323 ± 85	295 ± 69	$F_{[3,21]}$=6.83	**0.002**	0.49
Protein (g)	89 ± 33^{c}	93 ± 35^{c}	110 ± 30^{c}	156 ± 41	$F_{[3,21]}$=10.8	**<0.001**	0.61
Fat (g)	86 ± 23	84 ± 29	88 ± 34	73 ± 26	$F_{[3,21]}$=0.98	0.365	0.12
Sodium (g)	2.1 ± 0.3	2.5 ± 0.2	3.3 ± 0.5	3.1 ± 0.3	$F_{[3,21]}$=4.68	**0.012**	0.40

CHO: Carbohydrate; HP-MILK: Up & Go Energize™; LS-MILK: Up & Go Reduced Sugar™. Values are Mean±SD. *a*, mean value significantly different from Sports Drink; *b*, mean value significantly different from LS-MILK; *c*, mean value significantly different from HP-MILK.

Table 7.4. Total urine output and the proportion of WI_{Total} retained on concluding the 4 h recovery period

	Water	Sports Drink	LS-MILK	HP-MILK	F-ratio	*p*-value	η_p^2
Urine Output (g)	566 ± 314^{b,c}	459 ± 290	220 ± 53	230 ± 117	$F_{[2.0, 14.3]}$=6.57	**0.009**	0.48
Water Retention (%)	70.2 ± 13.6^{a}	74.2 ± 18.1	84.1 ± 4.4	83.7 ± 9.5	$F_{[3, 21]}$=5.09	**0.008**	0.42
Water Retained (g)	1352 ± 462	1349 ± 407	1238 ± 400	1293 ± 453	$F_{[3, 21]}$=0.493	0.691	0.07
Net Fluid Balance (g)	151 ± 431	145 ± 488	15 ± 394	64 ± 412	$F_{[3, 21]}$=1.03	0.399	0.13

BM: Body mass; HP-MILK: Up & Go Energize™; LS-MILK: Up & Go Reduced Sugar™. Values are Mean±SD. *a*, mean value significantly different from SF-MILK; *b*, mean value significantly different from HP-MILK; *c*, mean value trending for a significant difference from SF-MILK (p<0.100); *d*, mean value trending for a significant difference from HP-MILK (p<0.100).

7.4.5. Body Mass Changes

Net BM changes are displayed in Figure 7.9. All trials concluded with participants in a state of negative net BM relative to pre-exercise values (Water: -91±430 g; Sports Drink: -93±470 g; LS-MILK: -260±450 g; HP-MILK: -179±419 g). A 4 (Treatment) × 6 (Time) analysis of BM changes identified a significant main effect of Time, $F_{[1.4,\ 10.0]}$=51.0, p<0.001, η_p^2=0.88; and a trend for a significant Treatment × Time interaction, $F_{[15,105]}$=1.65, p=0.074, η_p^2=0.19. However, post hoc comparisons failed to detect a difference between treatments at any time points (p's>0.05).

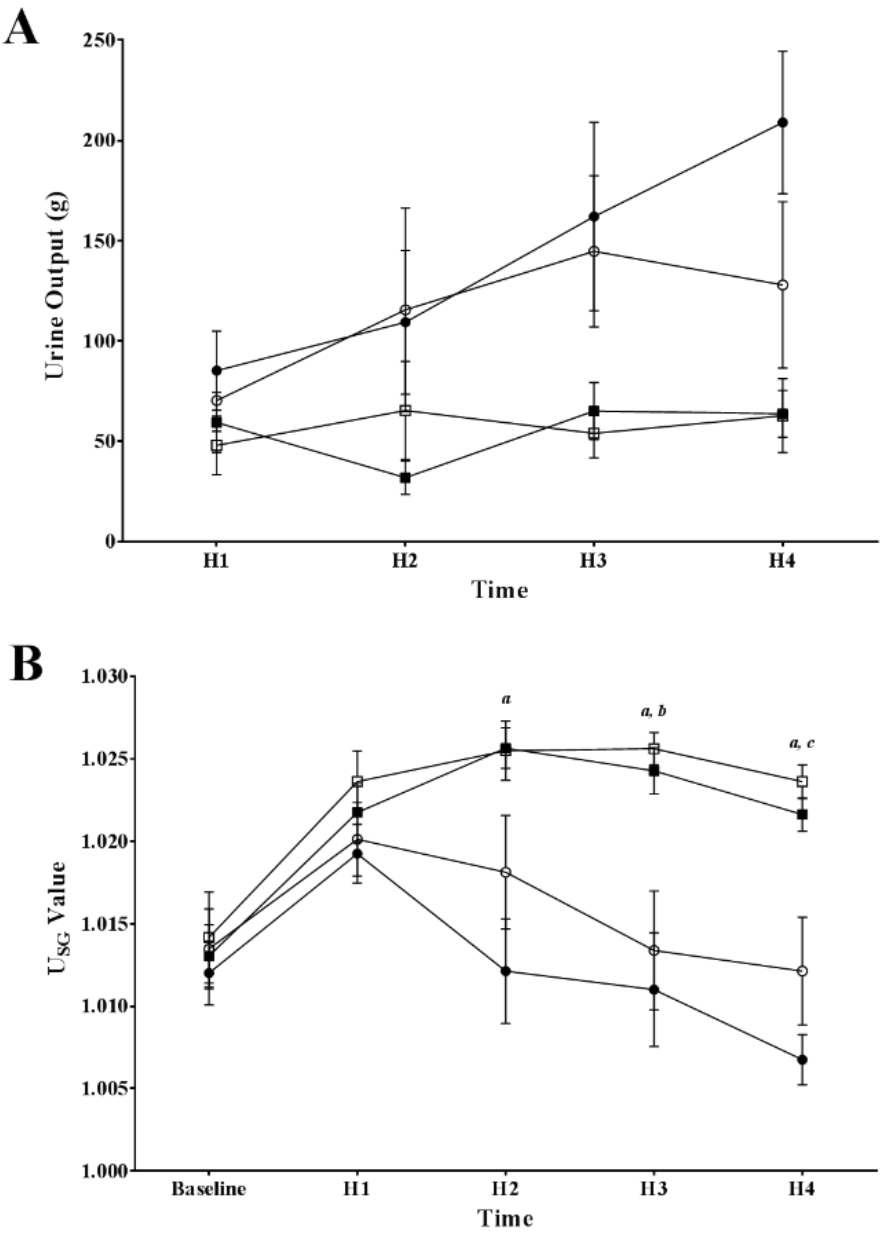

Figure 7.8. Hourly urine output (A) and U_{SG} values (B) under each of the experimental treatments. Values are Mean±SEM for Water (●); Sports Drink (○); LS-MILK (■); HP-MILK (□). a, Water significantly different to LS-MILK and HP-MILK; b, Sports Drink significantly different to LS-MILK and HP-MILK; c, Sports Drink trending for a significant difference from LS-MILK and HP-MILK (p<0.100). H1–4: Hours 1 to 4 of recovery.

7.4.6. Plasma Osmolality

A 4 (Treatment) × 4 (Time) analysis of P_{OSM} identified significant main effects of Treatment, $F_{[3,18]}$=6.41, p=0.004, η_p^2=0.52; and Time, $F_{[3,18]}$=46.7, p<0.001, η_p^2=0.89; and a significant Treatment × Time interaction, $F_{[9,54]}$=15.5, p<0.001, η_p^2=0.72. Pairwise comparisons indicated that P_{OSM} increased following the exercise-dehydration protocol (287±5 vs. 297±5 mOsm·kg^{-1}, p<0.001). Post hoc comparisons suggested that Water elicited lower P_{OSM} values than LS-MILK (p=0.001) and HP-MILK (p=0.008) after the first

hour of recovery; Sports Drink also reduced P_{OSM} compared to LS-MILK (p=0.012) at this time (Water: 287±5 mOsm·kg^{-1}; Sports Drink: 291±8 mOsm·kg^{-1}; LS-MILK: 298±7 mOsm·kg^{-1}; HP-MILK: 296±4 mOsm·kg^{-1}). After the fourth hour of recovery, Water again elicited lower P_{OSM} values than LS-MILK (p=0.013) and HP-MILK (p=0.004); Sports Drink also reduced P_{OSM} compared to HP-MILK (p=0.021) and tended to reduce P_{OSM} compared in LS-MILK (p=0.066) at this time (Water: 288±5 mOsm·kg^{-1}; Sports Drink: 288±5 mOsm·kg^{-1}; LS-MILK: 295±5 mOsm·kg^{-1}; HP-MILK: 299±4 mOsm·kg^{-1}).

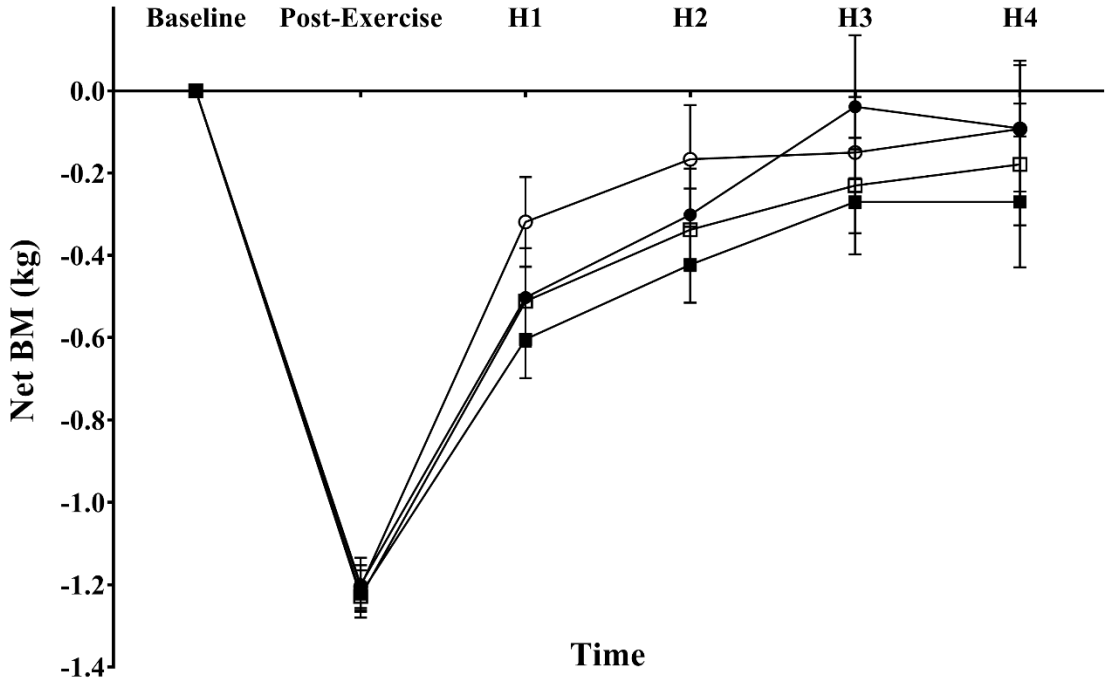

Figure 7.9. Net BM responses under each of the experimental treatments. Values are Mean±SEM for Water (●); Sports Drink (○); LS-MILK (■); HP-MILK (□). H1–4: Hours 1 to 4 of the recovery period.

7.4.7. Food and Nutrient Intakes

Nutrient intakes during the 4 h recovery period and across the entire trial day are displayed in Table 7.3. Mean energy intakes (food, beverage and total) differed significantly by Treatment during the 4 h recovery period. Pairwise comparisons revealed that EI_{Food} was significantly lower with HP-MILK compared to Water (p=0.005) and Sports Drink (p=0.006); EI_{Food} was also lower with LS-MILK than Water (p=0.033) and tended to suppress intake compared to Sports Drink (p=0.086). EI_{Food} did not differ significantly between Water and Sports Drink (p>0.05). Although not statistically significant, EI_{Total} tended to be increased with HP-MILK compared to Water (p=0.071) and Sports Drink (p=0.085). Total CHO and protein intakes also differed significantly across treatments

during the 4 h recovery period. Pairwise comparisons revealed that Sports Drink (p=0.004), LS-MILK (p=0.026) and HP-MILK (p=0.014) increased total CHO intake compared to Water; CHO intake was not different between the other beverages (p's>0.05). LS-MILK and HP-MILK also increased total protein intake compared to Water and Sports Drink (p's<0.05); protein intake was also higher with HP-MILK compared to LS-MILK (p=0.006). Participants' post-trial intake of energy, water, CHO, fat and sodium did not differ significantly by treatment (p's>0.05); only dietary protein intake was influenced by the beverage consumed post-exercise. Pairwise comparisons revealed a trend for decreased protein consumption post-trial with HP-MILK compared to Sports Drink (p=0.065). Nonetheless, 24 h protein intake with HP-MILK was higher than with all other beverages (p's<0.05). 24 h energy, CHO and sodium intakes differed significantly by treatment (p's<0.05). Although pairwise comparisons did not detect differences in total energy intake between trials (p's>0.05), LS-MILK and HP-MILK appeared to increase intake in comparison to Water and Sports Drink. Sports Drink (p=0.076), LS-MILK (p=0.022) and HP-MILK (p=0.087) all tended to increase 24 h CHO intake compared to Water.

7.4.8. Appetitive Sensations

A 4 (Treatment) × 6 (Time) analysis of subjective hunger ratings identified a significant main effect of Time, $F_{[5,35]}$=11.5, p<0.001, η_p^2=0.62; and a significant Treatment × Time interaction, $F_{[5,105]}$=2.27, p=0.008, η_p^2=0.25. Post hoc comparisons revealed that LS-MILK and HP-MILK decreased hunger compared to Water and Sports Drink during the first hour of recovery (p's<0.05). Hunger ratings were not significantly different at any other point in time (p's>0.05). 4 (Treatment) × 6 (Time) analyses revealed significant main effects of Treatment on thirst, $F_{[1.4,9.7]}$=34.4, p<0.001, η_p^2=0.83; and fullness ratings, $F_{[3,21]}$=3.81, p=0.025, η_p^2=0.35; significant main effects of Time on thirst, $F_{[5,35]}$=22.1, p<0.001, η_p^2=0.76; and fullness ratings, $F_{[5,35]}$=27.9, p<0.001, η_p^2=0.80; and significant Treatment × Time interactions on thirst, $F_{[15,105]}$=10.4, p<0.001, η_p^2=0.60; and fullness ratings, $F_{[15,105]}$=2.12, p=0.014, η_p^2=0.23. Post hoc comparisons demonstrated that LS-MILK and HP-MILK increased subjective fullness compared to Water during the first hour of recovery (p's<0.05). Fullness ratings were not significantly different between treatments at any other point in time (p's>0.05). Ingestion of LS-MILK

and HP-MILK suppressed thirst less than Water and Sports Drink at all stages of recovery (p's<0.05). No significant main effects or interaction effects were detected on ratings of bloatedness (p's>0.05).

7.4.9. Beverage Palatability

A 4 (Treatment) × 2 (Time) analysis of beverage pleasantness ratings failed to indicate a significant main effect of beverage, $F_{[1.5,10.6]}$=1.97, p=0.150, η_p^2=0.22; although, pleasantness ratings were generally increased with Water (79±30 mm) and Sports Drink (70±33 mm) compared to LS-MILK (50±16 mm) and HP-MILK (56±15 mm). The analysis identified a significant main effect of time, $F_{[3,7]}$=10.4, p=0.015, η_p^2=0.60; such that pleasantness decreased from the onset to the conclusion of the drinking period (67±9 mm vs. 60±10 mm). Total beverage intake also differed by palatability, $F_{[3,21]}$=10.6, p<0.001, η_p^2=0.60; such that the participants consumed a significantly greater quantity of their most preferred beverage than their least- (p=0.007) and second-least (p=0.004) preferred beverages (1st: 2057±498 g; 2nd: 1662±370 g; 3rd: 1471±389 g; 4th: 1515±496 g). Participants' beverage preferences are displayed in Figure 7.10. All participants identified either water or Sports Drink as their "most preferred" fluid; though, beyond this, individual preferences were extremely varied. The overall quantity of WI_{Total} retained also tended to be greater when participants received their most- than least-preferred beverage (p=0.059) (1435±434 vs. 1204±412 g). Average ratings for the 1st, 2nd, 3rd and 4th most palatable beverages were 95±5 mm, 72±19 mm, 52±14 mm and 34±12 mm, respectively.

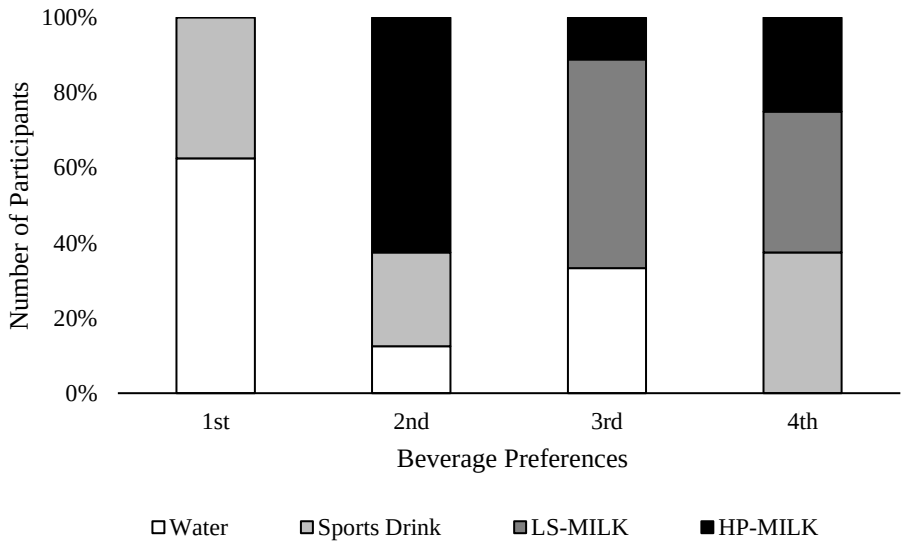

Figure 7.10. Participants' beverage preferences. Data represent the percentage (%) of total participants indicating a given beverage was their 1st, 2nd, 3rd and 4th most preferred.

7.4.10. Post-Study Survey

All 8 participants indicated that the purpose of the study was to investigate the effect of consuming different beverages on recovery of cognitive function. Of these, 7 indicated they were either *absolutely* or *very confident* in this response; one participant was *somewhat confident*. Mean±SD (Range) scores for Uncontrolled Eating, Cognitive Restraint and Emotional Eating on the R18 Three-Factor Eating Questionnaire were: 33±11% (19–53%), 23±18% (0–50%), and 25±26% (0–75%), respectively. No participant exceeded clinical thresholds for any of these characteristics.

7.5. Discussion

This study investigated the effect of consuming different commercial beverages (including milk-based beverages with contrasting formulations) with food *ad libitum* post-exercise on fluid, energy and nutrient recovery in females. Overall, results indicate that, when consumed voluntarily and with food, different beverages are likely to elicit similar levels of fluid recovery. However, caloric beverages, particularly milk-based formulations, appear to increase energy consumption and alter nutrient provision compared to when water is consumed. Findings from this study suggest that, when food is available post-exercise, the type of recovery beverage ingested will influence daily nutrient intake and nutrient intake immediately post-exercise, rather than acute measures of fluid recovery.

Previous research investigating the impact of beverage type on fluid recovery post-exercise generally indicates that milk/milk-based formulations are more effective rehydration agents than water and CHO-electrolyte sports beverages [147,331,333,354]. However, the majority of studies have prescribed fluid intake (i.e. fluid volume and rate) and denied participants access to food: an approach with limited ecological validity. The current investigation administered beverages and a variety of foods *ad libitum* in an attempt to simulate real-life post-exercise conditions. Under these circumstances, the different commercial beverages promoted *similar* levels of fluid recovery (Water: 1352±462 g; Sports Drink: 1349±407 g; LS-MILK: 1238±400 g; HP-MILK: 1293±453 g); despite differences in the quantity of water consumed and the proportion of water retained between treatments. Notably, however, P_{OSM} was higher at the conclusion of the recovery period following consumption of the LS-MILK and HP-MILK than either Water or Sports Drink, possibly indicating that a proportion of the fluid derived from these beverages still remained inside the GI tract (i.e. unabsorbed) at this time. Thus, although milk-based beverages are effective rehydration agents, they do not appear to restore fluid losses quickly, potentially limiting their utility is situations where rapid recovery is required. The only other study employing comparable methodology [8] demonstrated similar effects in male participants (fluid recovery was not influenced by the choice of water, a CHO-electrolyte sports beverage [Powerade®] or a milk-based formulation [Sustagen Sport®] when food and fluid were consumed *ad libitum*). This suggests that post-exercise rehydration is complex and likely to be influenced by the type of beverage provided and the availability of food during recovery. However, additional studies employing protocols that better reflect real-life post-exercise conditions are required to improve our understanding of the interaction between fluid, food and nutrients in rehydration and recovery after exercise. Indeed, one factor that warrants further consideration is the fact that participants in this study were aware they were hypohydrated, and therefore, motivated to drink. In many applied contexts, athletes may not know they are dehydrated, and, as such, could exhibit different drinking behaviours.

Previous research suggests that CHO-electrolyte sports beverages, water and milk-based formulations are typically ingested in different quantities when individuals are permitted to consume these beverages *ad libitum* [8,367,372,373,378]. Indeed, studies

consistently report that sports beverages increase voluntary post-exercise fluid consumption by ~30–42% compared to water [8,367,372,378] and by >50% compared to milk-based formulations [8,373]. Each of the different test beverages in the present study were consumed in roughly similar volumes. The inconsistency in findings between the current and previous reports may reflect sex differences. That is, the former studies have predominantly been conducted using male participants and some evidence (albeit in older individuals and/or during exercise) suggests sex differences exist in voluntary fluid consumption for different beverages [409,410]. Baker*, et al.* [409] observed that females consumed more water but not CHO-electrolyte sports beverage than males relative to BM. Furthermore, males consumed significantly more CHO-electrolyte sports beverage than water, but intakes between the two beverages for females were not different. While this study examined fluid intake during rest periods interspersed throughout an exercise protocol (and is therefore not directly comparable to the present study), similar behaviours may apply post-exercise.

While beverage intakes were similar *on average* in this study, it is worthwhile noting that volumes varied somewhat at an individual-level. This may be due to participants having different taste preferences. On examining the data, we can see that participants who indicated Sports Drink was "more pleasant" than Water (*n*=2) did in fact, consume ~40% more Sports Drink; while those who perceived Sports Drink as "more pleasant" than LS-MILK (*n*=5) and HP-MILK (*n*=5) also consumed ~40% more of the preferred beverage. The influence of palatability is further demonstrated by the fact that participants consumed a greater amount of their most preferred beverage than their least- and second-least preferred beverages. These data indicate that palatability is an important determinant of fluid intake and that taste preferences can vary greatly amongst individuals. The importance of thirst and beverage palatability as factors influencing fluid intake post-exercise is further supported by results of the current study, where HP-MILK and LS-MILK were consumed in similar volumes. These two beverages had similar sensory characteristics (i.e. flavour, mouth-feel, appearance, aroma), yet contrasting nutrient profiles. Clearly, physiological and psychological cues provided when consuming similar tasting beverages with different nutrition are too subtle or participants are unable to detect them to moderate drinking behaviour. Thus,

individuals likely revert to consuming the beverage to relieve thirst and/or on the basis that they find it palatable, which influences intake volume.

Findings from this study indicate that food plays an important role in mediating fluid recovery following exercise when beverages with simple nutrient profiles are consumed. Although the present investigation did not incorporate a "beverage only" trial for direct comparisons, the available evidence suggests that if food is *not* consumed, just ~45±10% of water and ~~55±16% of CHO-electrolyte sports beverage is typically retained post-ingestion (≥1.0 L·kg BM lost^{-1}; Figure 6.1). Values indicated in the current investigation are noticeably higher (Water: 70±14%; Sports Drink: 74±18%). This observation is consistent with results from previous experiments suggesting that the consumption of food enhances fluid retention [375,377] by delaying gastric emptying and attenuating osmotic diuresis [144]. It is important to note that the male subjects in Campagnolo et al. (2017) retained a *very similar* proportion of ingested water (Trial #1: 72±8%; Trial #2: 73±11%) and sports beverage (74±17%) as the female participants in the current study, even though females derived roughly half the amount of energy, CHO, protein and sodium (relative to BM) from food across on all beverage treatments. This suggests that water may, in fact (contrary to popular belief), be an appropriate rehydration beverage when co-ingested with food; particularly, if the calories delivered in sports beverages are not desired. Collectively, these data suggest that consuming food *ad libitum* after exercise is likely to enhance fluid retention similarly in males and females, despite differences in their dietary behaviour.

Results from the present study indicate that the choice of recovery beverage can have a profound effect on overall dietary intake. Total energy intake during the 4 h recovery period increased proportionately to the energy-density of the experimental beverage (i.e. even despite an opposing stepwise reduction in energy consumed from food). Though, it should be noted these post hoc comparisons failed to reach statistical significance, suggesting the study may have been underpowered to detect clear effects on dietary behaviour. An analysis of the participants' post-trial diet records revealed that individuals did not modify their dietary behaviour over the remainder of the day to offset this difference in energy consumption. While the caloric beverages increased CHO intake post-exercise (Water: 0.4±0.2 g CHO ·kg^{-1}·h^{-1}; all other treatments: 0.8±0.2 g·kg^{-1}·h^{-1}), it is worth noting that *ad libitum* intake of food and any beverage included in this study

failed to meet recommended CHO intakes for "rapid refuelling" after exercise; although total daily intakes were within the recommended range (~3.0–5.0 g CHO·kg^{-1}·d^{-1}) [2]. Protein consumption during the first 2 h after exercise was in excess of recommendations (0.25–0.30 g·kg^{-1}); particularly, when the milk-based beverages were consumed (Water: 0.47±0.17 g·kg^{-1}; Sports Drink: 0.39±0.09 g·kg^{-1}; LS-MILK: 0.81±0.28 g·kg^{-1}; HP-MILK: 1.49±0.41 g·kg^{-1}) [2]. Total daily protein intakes were generally appropriate (i.e. ~1.2–2.0 g·kg^{-1}·d^{-1}), but exceeded recommended levels on the HP-MILK (2.6±0.8 g·kg^{-1}·d^{-1}) [2]. Hence, the type of beverage consumed may influence aspects of post-exercise recovery and the suitability of certain beverages for individuals with specific dietary goals). For instance, caloric beverages may facilitate rehydration, substrate repletion and positive energy balance in circumstances where weight gain or large nutritional intakes are required. Whereas, the consumption of water (with food) should be encouraged to facilitate rehydration without excessive caloric intake in circumstances where weight loss or maintenance is desirable.

This investigation (and results from Campagnolo*, et al.* [8]) provide evidence that different commercial beverages promote similar levels of fluid recovery after exercise. However, it is important to consider that "rapid rehydration" is typically utilised to facilitate recovery *ahead of a subsequent exercise session*. Under these circumstances, individuals may restrict their intake of food/fluid to avoid experiencing GI problems during exercise; potentially affecting fluid and nutrient recovery. Additional research is required to determine the behaviours of athletes given access to *ad libitum* food and different beverages when they are required to perform a subsequent exercise session; and impact of these behaviours on post-exercise recovery and subsequent exercise performance.

7.6 Conclusion

In summary, this study demonstrates that different beverages are similarly effective at replenishing exercise-induced sweat loss, but result in different energy/nutrient intakes, when consumed *ad libitum* with food. An athlete's acute nutritional requirements to support recovery, broader dietary goals, taste preferences and access to food should therefore inform selection of the most appropriate recovery beverage.

Chapter 8: The Effect of Different Post-Exercise Beverages with Food on *Ad Libitum* Fluid Recovery, Nutrient Provision and Subsequent Endurance Cycling Performance

Reader's note:

This chapter includes a co-authored paper. The bibliographic details of the co-authored paper, including all authors are as follows:

McCartney, D., Irwin, C., Cox, GR., Desbrow, B. The effect of different post-exercise beverages with food on *ad libitum* fluid recovery, nutrient provision and subsequent athletic performance. *Physiology & Behavior*, 2019; 201: 22-30.

The research candidate has made the following contributions to this study:

- Developed the study design (on the basis of earlier research conducted by Campagnolo*, et al.* [8])
- Completed the human research ethics application
- Conducted all participant recruitment and data collection
- Conducted analysis of the data
- Prepared the manuscript for submission to a peer-reviewed journal
- Applied to present the research at an upcoming international conference

(Signed) _____ (Date: 20.02.2019)

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8.1. Abstract

Background: Recent evidence suggests that different beverages promote similar fluid recovery but alter nutrient provision when consumed voluntarily with food post-exercise. However, when preparing to undertake a subsequent bout of exercise, individuals may exhibit different dietary behaviour (e.g. to reduce GI distress or optimise performance). This study investigated the effect of consuming either Water or a CHO-electrolyte sports beverage ('Sports Drink') *ad libitum* with food during a 4 h post-exercise recovery period on fluid restoration, nutrient provision and subsequent endurance cycling performance. **Method:** On two occasions, 16 endurance-trained cyclists; 8 males (age: 31±9 y; VO_{2max}: 54±6 mL·kg^{-1}·min^{-1}) and 8 females (age: 33±8 y; VO_{2max}: 50±7 mL·kg^{-1}·min^{-1}); lost 2.3±0.3% and 1.6±0.3% of their BM (respectively) during 1 h of fixed-intensity cycling. Participants then had *ad libitum* access to either Water or Sports Drink and food for the first 195 min of a 4 h recovery period. At the conclusion of the recovery period, participants completed a cycling performance test consisting of a 45 min fixed-intensity pre-load and an incremental ride to volitional exhaustion (peak power output, PPO). Beverage intake; total water/nutrient intake; and indicators of fluid recovery (BM, urine output, P_{OSM}) were assessed periodically throughout trials. **Results:** Participants returned to a similar state of net positive fluid balance prior to recommencing exercise, regardless of the beverage provided (Water: +0.4±0.5 L; Sports Drink: +0.3±0.3 L, p=0.529). While Sports Drink increased post-exercise energy (M: +1.8±1.0 MJ; F: +1.3±0.5 MJ) and CHO (M: +114±31 g; F: +84±25 g) intake (i.e. total from food and beverage) (p's<0.001), this did not enhance subsequent endurance cycling performance (Water: 337±40 W [M] and 252±50 W [F]; Sports Drink: 340±40 W [M] and 258±47 W [F], p=0.242). **Conclusion:** Recovery beverage recommendations should consider the post-exercise environment (i.e. the availability of food), an individual's tolerance for food and fluid pre-/post-exercise, the immediate requirements for refuelling (i.e. CHO demands of the activity) and the athlete's overall dietary goals.

8.2. Introduction

The body's fluid and substrate stores (e.g. muscle and liver glycogen) are progressively depleted during prolonged exercise [1,2]. Endurance athletes may be required to complete consecutive exercise sessions with short periods of rest between bouts. In these situations, individuals have a limited opportunity to recover lost nutrients and are at risk of carrying residual CHO and/or fluid deficits from one activity to the next [411]. To rapidly restore losses, individuals are encouraged to consume ~1.25–1.50 L H_2O·kg lost^{-1} and 1.0–1.2 g CHO·kg^{-1}·h^{-1} for 4 h beginning ≤30 min post-exercise [1,2]. These recommendations exist as dehydration [3] and low CHO availability [4] can impair endurance performance. Given their ability to deliver fluid and nutrients, beverages may assist athletes to rehydrate and refuel simultaneously after exercise.

Numerous studies have investigated the ability of different beverages to influence acute recovery from exercise (i.e. restore fluid and substrate losses) and improve

subsequent performance. Collectively, the available evidence suggests: (1) beverages with "complex" nutritional profiles (e.g. milk/milk-based formulations) are more effective rehydration agents than beverages with "simple" nutritional profiles (e.g. water and CHO-electrolyte sports beverages) [147,331,333,354]; and that (2) the consumption of CHO-containing beverages in the post-exercise period improves muscle glycogen resynthesis [292] and subsequent endurance performance (Chapter 4). However, these studies typically "prescribe" drinking and deny participants access to food. Given that individuals usually control the volume of fluid they consume and often have access to food during the post-exercise period, personal and/or contextual factors are likely to alter fluid/nutrient recovery and influence subsequent performance.

Two recent studies (one presented in Chapter 7) have investigated how the provision of different beverages influences post-exercise fluid/nutrient recovery when consumed voluntarily and with access to food. These studies, which involved endurance-trained males (*n*=10) [8] and females (*n*=8) (see Chapter 7), gave participants *ad libitum* access to one type of beverage (i.e. water, a CHO-electrolyte sports beverage or various milk-based formulations) and a variety of foods during a 4 h recovery period. In both instances, the different beverages were similarly effective at replenishing fluid losses. This effect was attributed to: (1) the water and sports beverage being ingested in larger volumes than the milk-based formulations, and (2) the co-ingested food improving retention of these "simple" fluids [375,377]. Importantly, however, the consumption of different beverages did influence energy and CHO intake. More specifically, the administration of a CHO-electrolyte sports beverage increased energy (M: ~2.5 MJ, or ~32%; F: ~1.0 MJ, or ~25%) and CHO (M: ~186 g, or ~95%; F: ~73 g, or ~68%) intake, compared to water. The milk-based formulations also increased energy and nutrient provision (to a similar extent observed with the CHO-electrolyte sports beverage for males and to an even greater extent in females); however, these tended to increase ratings of GI discomfort in earlier studies [8,373]. Given that in both previous investigations participants were free to leave the laboratory at the end of the recovery period, the extent to which anticipation of a subsequent exercise session influences dietary behaviour requires clarification. In addition, whether the nutrient differences associated with access to the different beverages influences subsequent performance remains unknown.

The aim of this study was to investigate the effect of consuming either a CHO-electrolyte sports beverage or water *ad libitum* with food during a 4 h post-exercise recovery period on fluid restoration, nutrient provision and subsequent endurance cycling performance in trained individuals. It was hypothesised that both beverages would be effective at replenishing fluid losses. Furthermore, it was anticipated that access to a CHO-electrolyte sports beverage would increase CHO intake and therefore improve subsequent endurance cycling performance.

8.3. Method

8.3.1. Participant Characteristics

Competitive, endurance-trained cyclists/triathletes aged 18–45 y were eligible to take part in this investigation. Sample size was determined using power calculation software (G*Power Version 3.1.9.2, University Kiel Germany, 2014). Previous studies investigating post-exercise *ad libitum* food and fluid consumption behaviour have demonstrated significant beverage effects (i.e. Water vs. Sports Drink) on total CHO intake in males (Cohen's d_Z=2.7) [8] and females (d_Z=2.1) (Chapter 7). Using a power (1-β) of 0.95, an α=0.01 and a more conservative effect size (d_Z=2.0), we anticipated that 8 male and 8 female participants would be required to create a significant difference in CHO ingestion (i.e. within each sub-group). Twenty-four participants were recruited to account for attrition. Seven withdrew after completing one or both of the familiarisation sessions (2 were lost to follow-up and 5 withdrew due to unavailability); an eighth participant was lost to follow-up after their first experimental trial. Thus, 16 participants; 8 males (age: 31±9 y; VO_{2max}: 54±6 mL·kg^{-1}·min^{-1}; PSPO: 384±22 W; cycling: 214±124 km·week^{-1}) and 8 females (age: 33±8 y; VO_{2max}: 50±7 mL·kg^{-1}·min^{-1}; PSPO: 248±52 W; cycling: 204±87 km·week^{-1}), completed both trials. This investigation was approved by the University's Human Ethics Committee (GU 2017/969) and procedures were conducted in accordance with the principles outlined in the agreement of Helsinki.

8.3.2. Study Design

The experimental procedures are summarised in Figure 8.1. Participants attended the laboratory on 5 occasions to complete a preliminary screening, two familiarisation sessions, and two repeated-measures experimental trials. Experimental trials were

separated by 4–14 d and counterbalanced for order. Females completed both experimental trials during the same menstrual phase (i.e. either the follicular or the luteal) to reduce the confounding influence of hormonal changes on appetite [393] and substrate utilisation [399]; those using hormonal contraceptives (*n*=3) completed testing while taking the active medication. Each experimental trial involved an initial standardised exercise bout on a stationary cycle ergometer, followed by a 4 h recovery period and a cycling performance test. Participants had *ad libitum* access to one test beverage – either Water or Powerade® Isotonic (Coca Cola Ltd.) ('Sports Drink') – and food for the first 195 min of the recovery; no further food or fluid was consumed from 45 min prior to the performance test.

8.3.3. Eligibility, Preliminary Screening and Familiarisation

On arrival at the initial visit, individuals completed a medical screening questionnaire and the EAT-26 [400]. Those with a history of cardiovascular, metabolic and/or kidney disease, or currently taking medications known to influence substrate metabolism were ineligible to participate. Individuals were also excluded if their responses the EAT-26 indicated possible disordered eating, as were volunteers who reported an allergy, intolerance or dislike toward multiple food items or either test beverage used in the investigation. A self-reported BM history (~6 months) was then collected. To participate, individuals had to be weight stable stable (i.e. a BM change ≤5% in 6 months) and not followed an energy-restricted diet in the previous ~6 months. Once eligibility was verified, participants Sports Drink flavour preferences (Mountain Blast, Lemon-Lime or Berry Blast) were recorded. Participants then completed a graded exercise test on an electronically-braked cycle ergometer (Lode Excalibur Sport; Lode BV, Groningen, Netherlands) for determination of VO_{2max} using previously described protocols for males [8] and females (Chapter 7). Participants' respiratory gases were sampled continuously by breathing into a calibrated gas-analysis system (Medgraphics Ultima, MGC Diagnostics and Medisoft, USA). PSPO was also calculated [401] and used to set the exercise intensity on all trials. Following the preliminary screening visit, all participants undertook two familiarisation sessions (as advised by Hopkins*, et al.* [259]) where they practiced the cycling performance test before completing the experimental trials.

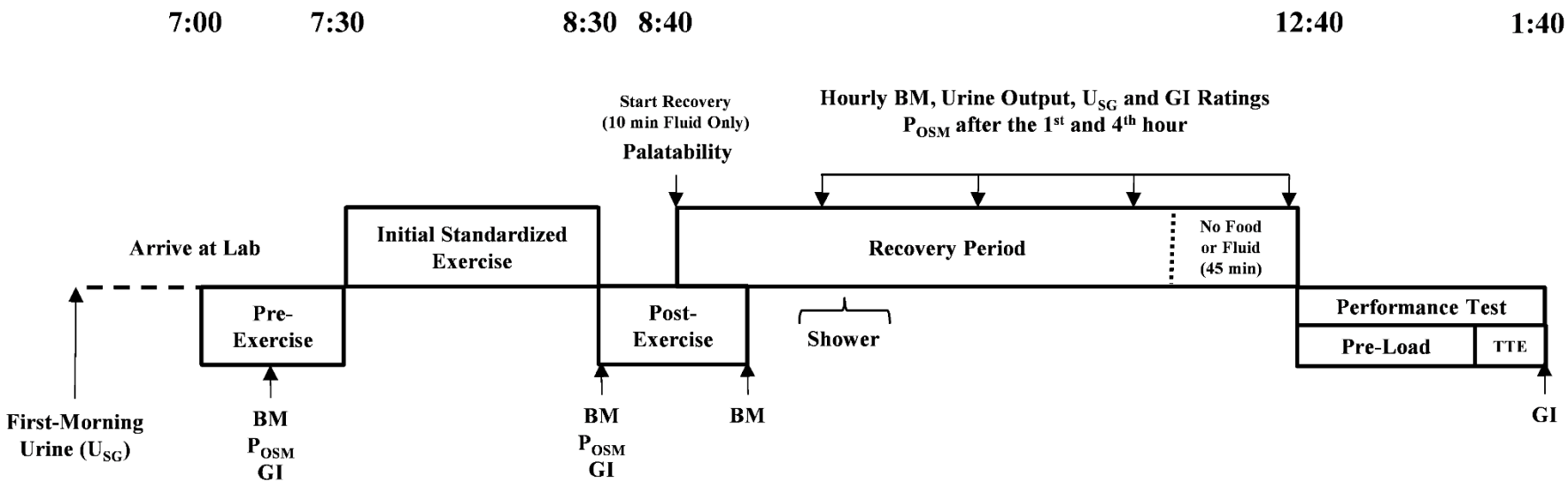

Figure 8.1. Schematic representation of the experimental procedures. BM: Nude body mass; GI: Gastrointestinal symptoms questionnaire; Palatability: Beverage palatability questionnaire; P_{OSM}: Blood collection for plasma osmolality analyses; U_{SG}: Urine specific gravity; TTE: Incremental cycling test to volitional exhaustion.

8.3.4. Pre-Trial Procedures

Prior to trials, participants were instructed to: (1) abstain from alcohol and moderate–strenuous exercise (>24 h); (2) avoid caffeine-containing products (>12 h); (3) keep a record of all food and beverages consumed (24 h); (4) consume a standardised pre-packaged evening meal (~60 $kJ \cdot kg^{-1}$) [8]; (5) fast overnight (~10 h); and (6) collect a first-morning urine sample and consume 200 mL of water before arriving at the laboratory. Each participant received a copy of their diet record (after their initial trial) and was instructed to replicate their behaviour ahead of the subsequent trial.

8.3.5 Experimental Procedures

8.3.5a. Pre-Exercise Period

Participants arrived at the laboratory fasted (~5–7 AM) and verbally acknowledged compliance to the pre-trial procedures; females also reported the onset of menstruation to determine menstrual phase. First-morning urine samples were analysed to determine U_{SG} (Palette Digital Refractometer, ATAGO, USA). If U_{SG} was ≥1.024, indicating likely dehydration [402], a second sample was collected, and U_{SG} was re-assessed (all samples had U_{SG}'s<1.024). Euhydrated participants provided a blood sample and completed the appetite questionnaire, before voiding their bladders completely and taking a pre-exercise nude BM measurement (HW-PW200; A&D Company Ltd, Tokyo, Japan).

8.3.5b. Initial Standardised Exercise

The initial standardised exercise consisted of 6 consecutive 10 min 'blocks' performed on a cycle ergometer (Lode Excalibur Sport; Lode BV, Groningen, Netherlands) (Figure 8.2). Blocks involved 8 min *steady-state* cycling, 1 min *high-intensity* cycling, and a 1 min *recovery* cycling (M: 125 W; F: 75W) period. For males, the steady-state and high-intensity phases were completed at 55% (211±12 W) and 70% (269±15 W) of their PSPO, respectively; while females exercised at 50% (124±26 W) and

Figure 8.2. Initial Standardised Exercise

70% (173±37 W) of their PSPO. Fluid consumption was not permitted during exercise. HR (Ambit3 Peak, Suunto®, Vantaa, Finland) and RPE on the Borg scale (Range: 6–20) [404] were recorded at the end of each steady-state and high-intensity phase. Following activity, participants collected an initial post-exercise nude BM measurement, completed the GI questionnaire and provided a blood sample.

Figure 8.3. 195 min post-exercise eating/drinking period

8.3.5c. Post-Exercise Recovery Period

The 4 h recovery period began ~10 min post-exercise. For the first 5 min (while continuing to sweat), participants were restricted to consuming their assigned beverage only – Water (sodium: 3 mg·L^{1}) or Sports Drink (Energy: 103 kJ·dL^{-1}; CHO: 5.8 g·dL^{-1}; Sodium: 28 mg·dL^{1}; Water: 95.0 g·dL^{-1}) – they then recorded a second post-exercise nude BM measurement. Exercise-induced fluid loss was calculated as the initial change in BM (i.e. pre- to post-exercise) plus the post-exercise change in BM (i.e. the initial post-exercise BM minus the second post-exercise BM, after accounting for the volume of beverage consumed). Participants were then taken to an observation room, where they were able to continue drinking their beverage and given access to a variety of foods (Figure 8.3). The food items were similar to those in Study 3 (Chapter 7) and included sports bars, fresh fruit, breads and condiments (Table 8.1). Participants were instructed to "*self-serve*" and to "*eat and drink as much as they liked"*, as more would be provided upon request. The "eating/drinking period" lasted 195 min; participants were informed

that no food or fluid would be available in the final 45 min of the recovery period. The beverage vessels were opaque and did not have volume increments; all beverages were stored in personal refrigerators (4°C). After 1 h of recovery, participants completed the appetite questionnaire, collected their urine output and provided a blood sample, before showering and returning to the observation room. Individuals repeated the GI questionnaire and urine collection at the end of the second, third and fourth hours of the recovery period; blood sampling was repeated at the end of the fourth hour.

8.3.5d. Cycling Performance Test

The cycling performance test (Figure 8.4) consisted of a 45 min pre-load culminating in an incremental ride to volitional exhaustion (Lode Excalibur Sport; Lode BV, Groningen, Netherlands). Male and female participants cycled at 55% and 50% of PSPO, respectively (in front of a fan) during the 45 min pre-load; HR and RPE were recorded at 15 min intervals throughout. The incremental test consisted of a 1 Watt increase every 6 s until volitional exhaustion; a similar protocol has previously demonstrated sensitivity to different doses of CHO [293]. Exhaustion was determined when the participant voluntarily ceased exercise or when their pedal cadence dropped below a threshold level (M: 75 rpm; F: 70 rpm) for a third time (individuals received two initial "warnings"). Maximum HR attained and PPO were recorded at the conclusion of exercise. Participants did not receive any feedback on elapsed time or encouragement during the test; investigators stood quietly behind the participant (i.e. outside their line of view). A financial incentive was provided to encourage engagement in the performance test. Specifically, the male and female participants with the "best" cycling performance (i.e. highest average PPO·kg^{-1} across trials) received a $200 prize. Participants completed the appetite questionnaire shortly after exercise ceased.

Figure 8.4. Cycling performance test

Table 8.1. Nutritional composition of the food items offered (per 100 g).

Food Item	Energy (kJ)	Protein (g)	Fat (g)	CHO (g)	Sodium (mg)	Water (g)
Sports Bar (Apple Berry Crumble), Winners	1480	5.9	5.4	67.4	8	8.5
Sports Bar (Mountain Mix), Winners	1630	7.3	11.6	62.0	8	8.5
Muesli Bar (Yoghurt & Strawberry), Uncle Toby's	1630	7.1	11.1	59.9	19	8.2
Banana	385	1.4	0.3	19.6	0	76.2
Apple	247	0.3	0.4	11.9	0	83.9
Sultanas, Sunbeam®	1290	3.1	0.1	69.0	10	16.3
Dried Apricots, Angas Park®	947	2.4	0.1	48.8	72	29.6
Raisin Toast, Tip Top®	1150	8.7	2.2	52.9	196	36.0
Multigrain Bread, Woolworths Homebrand	1080	9.3	2.5	47.0	400	37.1
Crumpets, Golden	750	5.7	0.9	35.5	600	51.8
Cheese, Coon™	1690	25.8	33.3	1.0	700	34.0
Plain Rice Crackers, Sakata®	1680	7.4	2.9	86.1	387	4.6
Crunchy Peanut Butter, Kraft	2580	23.7	51.3	13.4	578	1.5
Honey, Woolworths Select	1416	0.3	0.0	83.1	15	16.2
Jam, Fruits of the Forest, IXL®	1110	0.4	0.1	64.1	17	30.7
Yeast Extract Spread, Vegemite	798	25.4	0.9	19.9	3,300	40.9
Margarine, Flora™	2420	0.2	65.0	0.7	590	35.1
*Fruit-Flavoured Yoghurt, Yoplait®	375	4.7	1.9	13.2	50	77.4
*Orange Juice, Just Juice	170	0.6	<0.1	9.0	8	94.5
Salted Cashews, Woolworths Homebrand	2560	21.3	48.5	22.1	220	1.9

*Participants were only given 200 g of these items to consume due to their high fluid content. Nutritional values for packaged foods were taken from the product nutrition information panel; values for fresh food items and product water content were taken from FoodWorks® Version 8 (Xyris Software Pty Ltd, Spring Hill, Australia).

8.3.6. Study Completion Procedures

At the conclusion of the second trial, participants completed a questionnaire evaluating their knowledge and beliefs regarding certain aspects of sports nutrition (e.g. post-exercise rehydration and CHO consumption) and food composition (i.e. the CHO content of the food items offered); questions were based on those used in a previous questionnaire [412]. Participants indicated their expectations regarding the ability of different beverages to influence cycling performance, and whether (or not) they consciously altered their dietary behaviour as a result of trial order or the type of beverage provided. Lastly, participants completed the R18 Three-Factor Eating Questionnaire [406] to assess cognitive, behavioural and emotional influencers of eating behaviour.

8.3.7. Data Collection

8.3.7a. Food and Fluid Intake Measures

Energy, macronutrient, sodium and water consumption during the recovery period was estimated by weighing all food and beverages to the nearest 1 g pre-ingestion and then again after 90 min and 195 min (i.e. approximately halfway and at the end of recovery). Individuals were aware that their dietary behaviour was being monitored. Nutritional values for packaged foods were taken from the product nutrition information panel; values for fresh food items and product water content were obtained from FoodWorks® Version 8 dietary analysis software (Xyris Software Pty Ltd, Spring Hill, Australia).

8.3.7b. Urine Sampling and Water Retention

Participants voided their bladder completely into an empty container at the end of each hour of the recovery period for measures of hourly and total urine loss. Individuals were permitted to urinate throughout the observation period, and on each occasion the void was collected and added to the hourly urine output. Total urine loss was calculated as the accumulated urine output from the onset of drinking until the end of the observation period. Each hourly urine sample was also analysed to determine U_{SG} (Palette Digital Refractometer, ATAGO, USA). The proportion of ingested water (i.e. from food and

beverage) retained at the end of the 4 h recovery period was calculated using the following formula:

$$\text{Water Retained (\%)} = \frac{(WI_{Total}(g) - \text{Urine Output (g)})}{WI_{Total}(g)} \times 100$$

Where WI_{Total} represents the total amount of water consumed via food and beverage during the recovery period. Net fluid balance was also estimated as follows:

$$\text{Net Fluid Balance (g)} = \text{Fluid Loss (g)} + (WI_{Total}\ (g) - \text{Urine Output (g)}\ )$$

8.3.7c. Appetitive Sensation and Beverage Palatability Questionnaires

VASs were used to evaluate appetitive sensations (i.e. hunger, thirst, fullness and nausea) pre- and post-exercise, at the end of each hour of the recovery period and immediately after the performance test. VASs were also used to assess beverage palatability (i.e. 'pleasantness') at the onset of drinking. All measures were completed on a 100 mm scale, with 0 mm representing 'not at all' and 100 mm representing 'extremely' using a computerised modifiable software program (Adaptive Visual Analog Scale; Marsh-Richard*, et al.* [403]).

8.3.7d. Blood Sampling

For collection of blood samples, participants rested in a supine position prior to a 5 mL blood sample being drawn from an antecubital vein. All samples were collected into pre-treated lithium heparin vacutainers (Becton Dickson vacutainers®) and centrifuged for 10 min (1350 × *g*). Aliquots of plasma supernatant were stored at -80°C and later analysed in duplicate to determine P_{OSM} on a calibrated (300 mOsm·kg^{-1}) osmometer (Osmomat 030, Gonotec, Berlin, Germany).

8.2.8. Statistical Analysis

Statistical analyses were completed using SPSS Statistics for Windows, Version 25.0 (IBM Corp. 2012, Armonk, N.Y., USA). All measures were examined for normality (Shapiro-Wilk test) and sphericity (Mauchly's test). Where assumptions of sphericity in repeated-

measures analyses were violated, the Greenhouse-Geisser statistic was applied. Comparisons between experimental trials for baseline measures (BM, U_{SG}, and P_{OSM}); exercise-induced BM loss; fluid retention and net fluid balance; $WI_{Beverage}$, WI_{Food}, and WI_{Total}; total nutrient intake; total urine output; beverage palatability; and PPO and maximum HR attained on the incremental cycling test; were conducted using Treatment × Sex split-plot ANOVAs. Treatment × Time × Sex split-plot ANOVAs were used to investigate HR and RPE values; hourly urine outputs and U_{SG} values; and subjective GI ratings. Pairwise comparisons (Bonferroni) were completed where significant main effects were present. Paired and independent *t* tests (i.e. for within- and between-subject analyses) were used to conduct post hoc comparisons on significant interaction effects. Paired *t* tests were also used to compare the temperature and RH across experimental trials and test for trial order effects on select variables. Each of the 18 items on the Three-Factor Eating Questionnaire was given a score between 1 and 4 and item scores were summed into raw scores for cognitive restraint, uncontrolled eating, and emotional eating. Raw scale scores were then transformed to a 0–100 scale [((raw score − lowest possible raw score)/possible raw score range) × 100]. Higher scores in the respective scales are indicative of greater cognitive restraint, uncontrolled, or emotional eating [406]. Effect sizes were calculated as η_p^2. Significant differences were accepted as $p<0.05$. Data are Mean±SD, unless otherwise indicated.

8.4. Results

8.4.1. Standardisation Procedures

All 16 participants verbally acknowledged compliance to the pre-trial procedures on arrival at the laboratory. Pre-exercise values for BM and P_{OSM} differed significantly by Sex (Table 8.2); however, no main effects of Treatment or Treatment × Sex interactions were observed (*p*'s>0.05); pre-exercise U_{SG} values did not differ by Treatment, by Sex, or indicate a Treatment × Sex interaction (*p*'s>0.05) (Table 8.2). Temperature, $t(15)=0.368$, $p=0.718$; and RH, $t(15)=1.11$, $p=0.287$; were also similar on each trial (Water: 25±1°C, 61±10%; Sports Drink: 25±1°C, 63±8%).

8.4.2 Initial Standardised Exercise

Neither HR nor RPE differed significantly by Treatment during either the *steady-state*, $F_{[1,14]}$=0.091, p=0.767; $F_{[1,14]}$=3.29, p=0.096, respectively; or *high-intensity*, $F_{[1,14]}$=0.265, p=0.615; $F_{[1,14]}$=0.469, p=0.505, respectively; exercise phases and no Treatment × Time interactions were observed (p's>0.05). On average, males and females cycled at 80±6% HR_{max} (RPE: 13±1) and 75±6% HR_{max} (RPE: 12±1) in the *steady-state phase*; and 85±7% HR_{max} (RPE: 15±1) and 82±7% HR_{max} (RPE: 14±1) in the *high-intensity phase*, respectively. BM loss did not differ by Treatment or indicate a Treatment × Sex interaction; though males lost a greater proportion of their pre-exercise BM than females (Table 8.2).

8.4.3. Water Intake, Output and Retention

8.4.3a. Beverage Intake and Water Intake from Food and Beverages

Fluid consumption data are summarised in Table 8.3. Total beverage intake, $WI_{Beverage}$, WI_{Food} and WI_{Total} did not differ significantly by Treatment or indicate a Treatment × Sex interaction; beverage intake and $WI_{Beverage}$ were also similar by Sex, although male participants consumed a greater quantity of WI_{Food} and, subsequently, WI_{Total} than females (Nb., however, that beverage intake, t(15)=3.31, p=0.005 [Trial #1: 1.8±0.5 L; #2: 2.1±0.6 L]; and WI_{Total}, t(15)=3.16, p=0.006 [Trial #1: 2.1±0.6 L; #2: 2.4±0.6 L]; indicated significant trial order effects). An analysis of fluid ingestion over time revealed that participants consumed a greater quantity of WI_{Total} during the first half of the eating/drinking period (Males: 1624±299 g; Females: 1157±299 g) than the second half (Males: 992±275 g; Females: 810±275 g), $F_{[1,14]}$=32.4, p<0.001, η_p^2=0.70; no other differences were detected in this analysis (p's>0.05). Beverage pleasantness ratings did not differ by Treatment, $F_{[1,14]}$=3.16, p=0.097; by Sex, $F_{[1,14]}$=1.34, p=0.267; or indicate a Treatment × Sex interaction, $F_{[1,14]}$=3.51, p=0.082; participants indicated a relatively high degree of liking for both beverages (Water: 86±13 mm; Sports Drink: 74±27 mm).

Table 8.2. Pre-trial conditions and exercise-induced dehydration

	Male Participants		Female Participants		Treatment Effect			Sex Effect		
	Water	Sports Drink	Water	Sports Drink	$F_{[1,14]}$	*p*-value	η_p^2	$F_{[1,14]}$	*p*-value	η_p^2
Pre-Exercise U_{SG}	1.016 ± 0.008	1.016 ± 0.006	1.014 ± 0.003	1.012 ± 0.004	0.610	0.448	0.04	1.45	0.249	0.05
Pre-Exercise P_{OSM}	294 ± 4	293 ± 4	290 ± 4	289 ± 4	0.907	0.358	0.07	7.76	**0.015**	0.37
Pre-Exercise BM	80.4 ± 6.9	80.2 ± 6.6	60.5 ± 8.0	60.7 ± 7.3	0.000	0.995	<0.01	29.8	**<0.001**	0.68
BM Loss (kg)	1.80 ± 0.15	1.79 ± 0.15	0.95 ± 0.21	0.96 ± 0.20	0.003	0.957	<0.01	112	**<0.001**	0.89
BM Loss (%)	2.25 ± 0.17	2.25 ± 0.35	1.57 ± 0.29	1.59 ± 0.32	0.032	0.860	<0.01	25.9	**<0.001**	0.91

BM: Body mass; P_{OSM}: Plasma osmolality ($mOsm \cdot kg^{-1}$); U_{SG}: Urine specific gravity. P_{OSM} from *n*=8 male and *n*=7 female participants where blood sampling was successful. No significant Treatment × Sex interaction effects (*p*'s>0.05). Values are Mean±SD.

Table 8.3. Water intake, output and retention during the 4 h recovery period

	Male Participants		Female Participants		Treatment Effect			Sex Effect		
	Water	Sports Drink	Water	Sports Drink	$F_{[1,14]}$	*p*-value	η_p^2	$F_{[1,14]}$	*p*-value	η_p^2
Fluid Consumption										
Beverage Intake (g)	2306 ± 564	2150 ±716	1680 ± 620	1770 ± 400	0.074	0.790	0.01	3.89	0.069	0.22
$WI_{Beverage}$ (g)	2306 ± 564	2043 ± 680	1680 ± 620	1682 ± 380	1.25	0.283	0.08	3.95	0.067	0.22
WI_{Food} (g)	438 ± 55	448 ± 209	333 ± 106	239 ± 67	1.42	0.254	0.09	9.14	**0.009**	0.40
WI_{Total} (g)	2743 ± 571	2491 ± 389	2012 ± 539	1921 ± 389	2.12	0.168	0.13	8.00	**0.015**	0.36
BM Loss Replaced (%)	155 ± 42	139 ± 24	217 ± 57	206 ± 44	2.63	0.127	0.16	10.4	**0.006**	0.43
Fluid Output and Retention										
Urine Output (g)	543 ± 255	418 ± 307	709 ± 440	635 ± 311	1.66	0.219	0.11	1.66	0.218	0.01
Water Retention (%)	80 ± 9	84 ± 8	67 ± 15	68 ± 9	0.945	0.347	0.06	9.94	**0.007**	0.42
Water Retained (g)	2200 ± 530	2073 ± 360	1303 ± 342	1286 ± 144	0.587	0.456	0.04	28.0	**<0.001**	0.67
Net Fluid Balance (g)	+397 ± 616	+284 ± 278	+355 ± 259	+329 ±240	0.417	0.529	0.03	<0.001	0.993	<0.001

$WI_{Beverage}$: Water intake from beverage; WI_{Food}: Water intake from food; WI_{Total}: Water intake from food and beverage. No significant Treatment × Sex interaction effects (*p*'s>0.05). Values are Mean±SD.

8.4.3b. Urine Output and Water Retention

Urine output and water retention data are summarised in Table 8.3. Total urine output did not differ significantly by Treatment, by Sex, or indicate a Treatment × Sex interaction. Hourly urine outputs indicated a main effect of Time, $F_{[1.8,24.7]}$=7.30, p=0.004, η_p^2=0.34; such that urine output tended to be greater during the final hour of recovery (207±136 g), than during the first (105±76 g, p=0.071) and second (96±52 g, p=0.009) hours. No other significant differences were detected in this analysis (p's>0.05); analysis of hourly U_{SG} values also failed to indicate any significant effects (p's>0.05). Neither the proportion (%) or quantity (g) of WI_{Total} retained at the end of the recovery period differed by Treatment or indicated a Treatment × Sex interaction; though males typically retained a greater proportion and absolute quantity than females. Regardless of the beverage consumed, participants were in a similar state of net positive fluid balance at the conclusion of the recovery period.

8.4.3c. Plasma Osmolality

P_{OSM} values indicated a significant main effect of Time, $F_{[3,39]}$=57.1, p<0.001, η_p^2=0.82. Pairwise comparisons revealed that P_{OSM} increased from pre- to post-exercise (291±3 vs. 302±3 mOsm·kg^{-1}, p<0.001), and then decreased from post-exercise to the end of the first hour of recovery (298±3 mOsm·kg^{-1}, p=0.004), and from the end of the first to the end of the fourth hour of recovery (294±4 mOsm·kg^{-1}, p=0.005). Except for a main effect of Sex, F1,13)=21.1, p<0.001, η_p^2=0.62; indicating that, on average, females had a lower P_{OSM} than males (294±3 vs. 299±3 mOsm·kg^{-1}), no other significant differences were observed in this analysis (p's>0.05).

Table 8.4. Energy and nutrients consumed via food and beverage during the 195 min eating/drinking period

	Male Participants		Female Participants		Treatment Effect			Sex Effect		
	Water	Sports Drink	Water	Sports Drink	$F_{[1,14]}$	*p*-value	η_p^2	$F_{[1,14]}$	*p*-value	η_p^2
Beverage Nutrient Intake										
Energy (kJ)	0 ± 0	2257 ± 752	0 ± 0	1859 ± 420	183	**<0.001**	0.93	183	0.212	0.11
CHO (g)	0 ± 0	125 ± 42	0 ± 0	103 ± 23	183	**<0.001**	0.93	1.71	0.212	0.11
Sodium (mg)	69 ± 17	602 ± 201	50 ± 16	496 ± 112	165	**<0.001**	0.92	2.08	0.171	0.13
Food Nutrient Intake										
Energy (kJ)	8105 ± 1761	7661 ± 1909	5515 ± 1053	4958 ± 874	5.01	**0.042**	0.26	14.3	**0.002**	0.51
CHO (g)	235 ± 53	225 ± 56	164 ± 47	145 ± 46	3.48	0.083	0.20	9.83	**0.007**	0.62
Protein (g)	58 ± 16	54 ± 17	39 ± 10	37 ± 6	1.62	0.224	0.10	8.83	**0.010**	0.39
Fat (g)	80 ± 38	75 ± 29	53 ± 11	48 ± 6	1.36	0.262	0.09	5.55	**0.034**	0.28
Sodium (mg)	1609 ± 693	1490 ± 778	1172 ± 411	1246 ± 417	0.112	0.743	0.13	1.37	0.262	0.09
Half-Time Nutrient Intake (Food + Beverage)										
Energy (kJ)	4920 ± 1964	5061 ± 1048	3705 ± 1410	3965 ± 993	0.254	0.622	0.02	4.61	**0.050**	0.25
CHO (g)	147 ± 33	192 ± 41	109 ± 51	135 ± 41	11.7	**0.004**	0.45	6.65	**0.022**	0.32
Protein (g)	35 ± 14	26 ± 10	26 ± 12	23 ± 6	3.09	0.100	0.18	1.80	0.201	0.11
Fat (g)	47 ± 33	32 ± 15	36 ± 17	31 ± 8	2.99	0.106	0.18	0.458	0.510	0.03
Sodium (mg)	979 ± 300	861 ± 321	850 ± 337	965 ± 290	<0.001	0.988	<0.01	0.011	0.920	<0.01
Full-Time Nutrient Intake (Food + Beverage)										
Energy (kJ)	8105 ± 1761	9918 ± 1376	5515 ± 1053	6817 ± 1078	59.8	**<0.001**	0.81	19.6	**0.001**	0.58
CHO (g)	235 ± 53	349 ± 30	164 ± 47	248 ± 58	201	**<0.001**	0.94	14.0	**0.002**	0.25
Protein (g)	58 ± 16	54 ± 17	39 ± 10	37 ± 6	1.63	0.222	0.10	8.84	**0.010**	0.39
Fat (g)	80 ± 38	75 ± 29	53 ± 11	48 ± 6	1.37	0.262	0.09	5.55	**0.034**	0.28
Sodium (mg)	1678 ± 696	2092 ± 741	1223 ± 419	1742 ± 444	39.7	**<0.001**	0.74	1.97	0.183	0.04

Half-Time: Total intake from food and beverage during the first 90 min of the eating/drinking period; Full-Time: Total intake from food and beverage during the 195 min eating/drinking period. No significant Treatment × Sex interaction effects (*p*'s>0.05). Values are Mean±SD.

8.4.4. Energy and Nutrient Consumption

Energy and nutrient intakes during the 195 min eating/drinking period are summarised in Table 8.4. On average, males consumed 2.2±0.8 MJ, 125±42 g CHO and 0.6±0.2 g sodium via Sports Drink; while females consumed 1.9±0.4 MJ, 103±23 g CHO and 0.5±0.1 g sodium; these intakes did not differ significantly by Sex. While the quantity of CHO, protein, fat and sodium consumed via food was not significantly affected by the beverage provided; an accumulation of small reductions in macronutrient consumption resulted in a significant decrease in energy intake from food when Sports Drink was consumed. Overall, Sports Drink had a significant effect to increase total (i.e. from food and beverage) CHO consumption during the first half of the eating/drinking period and total CHO, energy and sodium intake across the entire recovery period. Total energy, $t(15)=1.51$, $p=0.153$ (Trial #1: 7.3±2.0 MJ; #2: 7.9±2.2 MJ); energy via food, $t(15)=1.05$, $p=0.312$ (Trial #1: 6.4±1.8 MJ; #2: 6.7±2.2 MJ); total CHO, $t(15)=0.796$, $p=0.438$ (Trial #1: 239±82 g; #2: 259±82 g); and CHO via food, $t(15)<0.001$, $p=1.000$ (Trial #1: 192±60; #2: 192±66 g) did not differ by order of trials.

8.4.5. Cycling Performance Test

PPO attained on the incremental test to exhaustion did not differ by Treatment, $F_{[1,14]}=1.49$, $p=0.242$; or indicate a significant Treatment × Sex interaction, $F_{[1,14]}=0.24$, $p=0.630$ (Figure 8.5). Overall, males attained a PPO of 337±40 W on Water and 340±40 W on Sports Drink; females achieved 252±50 W and 258±47 W on each respective treatment. Individuals elicited a maximum HR equal to 96±6% HR_{max} during the performance test; this value did not differ by Treatment, $F_{[1,14]}=1.871$, $p=0.193$; by Sex, $F_{[1,14]}=0.064$, $p=0.805$; or indicate a Treatment × Sex interaction, $F_{[1,14]}=0.043$, $p=0.838$. Neither PPO, $t(15)=1.60$, $p=0.131$ (Trial #1: 294±61 W; #2: 299±60 W); nor the maximum HR attained, $t(15)=0.324$, $p=0.751$ (Trial #1: 96±7% HR_{max}; #2: 96±6% HR_{max}) differed by trial order. HR and RPE did not differ by Treatment, $F_{[1,14]}=0.897$, $p=0.360$; $F_{[1,14]}=0.860$, $p=0.370$, respectively; or indicate a Treatment × Time interaction, $F_{[2,28]}=0.135$, $p=0.874$; $F_{[2,28]}=1.48$, $p=0.245$, respectively; during the 45 min pre-load. On average, male and female participants cycled at 79±6% HR_{max} (RPE: 13±1) and 74±6% HR_{max} (RPE: 12±1) throughout the pre-load, respectively.

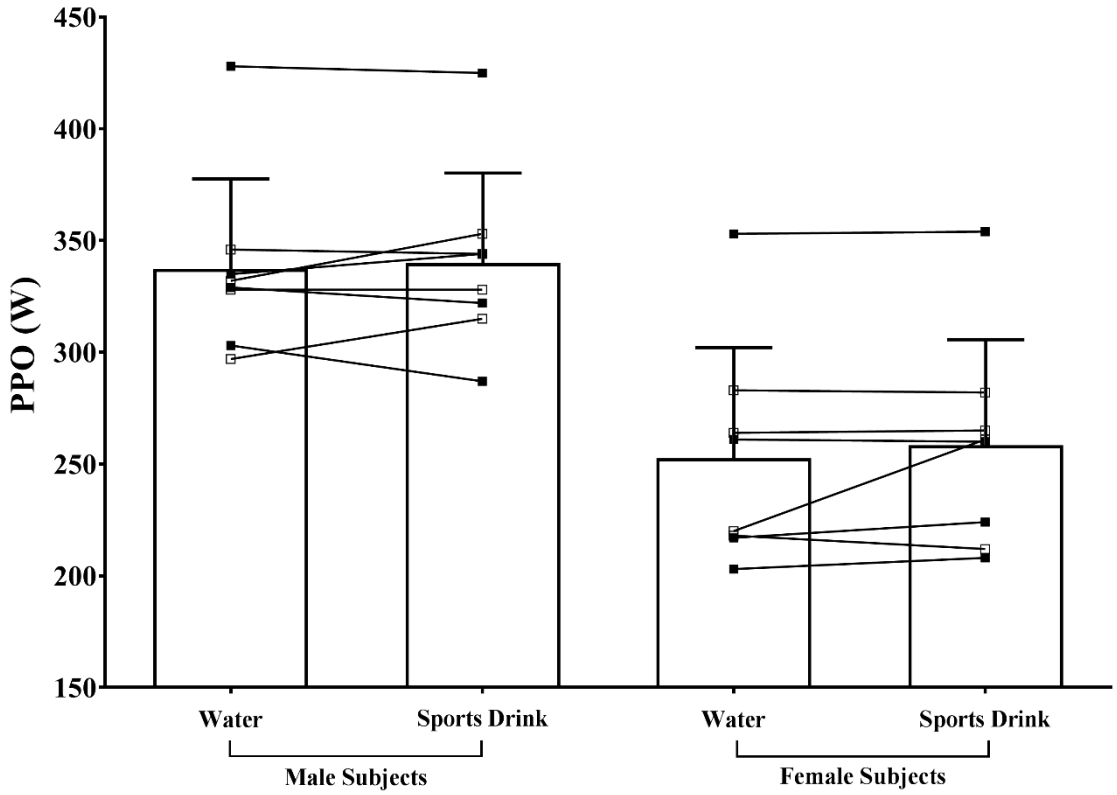

Figure 8.5. PPO attained on average (bars) and by individual participants (lines) on the incremental test to volitional exhaustion under each experimental treatment. Values are Mean±SD.

8.4.6. Appetitive Sensations

Subjective ratings of thirst, $F_{[2.6,36.3]}$=54.9, p<0.001, η_p^2=0.78; hunger, $F_{[2.2,31.1]}$=24.3, p<0.001, η_p^2=0.63; and fullness, $F_{[3.3,46.7]}$=29.6, p<0.001, η_p^2=0.68; differed significantly across Time; hunger also indicated a significant Time × Sex interaction, $F_{[6,84]}$=2.40, p=0.037, η_p^2=0.14. Pairwise comparisons revealed higher thirst ratings after the initial standardised exercise (81±14 mm) and the cycling performance test (68±20 mm) than at all other time points (24±12 mm, p's<0.001). Participants also reported lower levels of fullness and higher levels of hunger on arrival (hunger: 44±24 mm; fullness: 20±18 mm) and after the initial standardised exercise (hunger: 54±28 mm; fullness: 14±13 mm) than at all other time points (hunger: 17±12 mm; fullness: 50±16 mm, p's<0.030). Males reported higher levels of hunger than females after the first (29±19 vs. 11±10 mm, p=0.037), second (25±15 vs. 10±9 mm, p=0.030) and third (26±13 vs. 8±8 mm, p=0.004) hours of the recovery period. No other significant differences were detected in these analyses (p's>0.05). Subjective ratings of nausea also differed significantly across Time, $F_{[2.1,28.8]}$=3.97, p=0.029, η_p^2=0.22; however, pairwise comparisons failed to identify any significant differences between time points (p's>0.05).

8.4.7. Post-Study Survey

8.4.7a. Three-Factor Eating Questionnaire

Mean±SD (Range) transformed scores for Uncontrolled Eating, Cognitive Restraint, Emotional Eating were 55±14% (28–71%), 53±13% (29–79%) and 44±18% (25–75%), respectively; these did not differ significantly by Sex, $t(9.8)=0.683$, $p=0.511$; $t(14)=0.378$, $p=0.711$; $t(14)=1.28$, $p=0.222$. No participant exceeded clinical thresholds for any of these characteristics.

8.4.7b. Sports Nutrition Knowledge and Beliefs

All 16 participants believed fluid consumption was important and that recommencing exercise in a hypohydrated state could impair endurance performance. However, only 4 were aware of the amounts they should consume in accordance with current fluid replacement guidelines (i.e. 1.25–1.50 L· kg BM loss^{-1}); most believed a smaller volume (e.g. *n*=7, 1.0–1.25 L· kg BM loss^{-1}) would be sufficient to rehydrate. The majority of participants believed CHO was the most important macronutrient to replace after endurance exercise (*n*=13) and that the optimum time to eat (i.e. to enhance recovery) was within 30 min of activity (*n*=12). However, only 3 knew that commercial sports beverages contained 4–8% CHO; most believed them to contain more (e.g. *n*=7, 20–25% CHO). The vast majority of participants believed that sports bars (*n*=15), muesli bars (*n*=14), bananas (*n*=15), raisin bread (*n*=15), bread (*n*=16) and crumpets (*n*=15) were "high CHO foods"; apples (*n*=10), sultanas (*n*=12), dried apricots (*n*=12), rice crackers (*n*=12), honey (*n*=11), jam (*n*=11) and orange juice (*n*=11) were usually (but less frequently) perceived as "high CHO foods". Participants typically indicated that cheese (*n*=13), peanut butter (*n*=11), yeast extract spread (*n*=9), margarine (*n*=12), cashew nuts (*n*=13) and fruit-flavoured yoghurt (*n*=12) were "low CHO foods".

8.4.7c. Participant Expectations

Four out of the 16 participants (all female) expected to "perform better" on the incremental cycling test after consuming Sports Drink; two of these individuals did. The remainder believed they would perform similarly on both experimental trials. One participant reported deliberately altering their dietary behaviour during the second trial (Sports Drink) because of their previous experience; specifically, he reported making a

conscious effort to consume more fluid. Another participant reported deliberately altering their dietary behaviour based on the available beverage; specifically, she reported making "less of an effort" to select high CHO foods when Sports Drink was available. No other participants indicated a deliberate alteration in their food and/or beverage intake between trials.

8.5. Discussion

This study investigated the effect of consuming either Water or Sports Drink *ad libitum* with food during a 4 h post-exercise recovery period on fluid restoration, nutrient provision and subsequent endurance cycling performance in trained males and females. In line with our hypothesis, results indicate that both beverages were effective at replenishing fluid losses between consecutive exercise (i.e. cycling) sessions, and that Sports Drink increased total energy and CHO intake during the recovery period (irrespective of sex). However, in contrast to our hypothesis, this additional CHO did not translate to an improvement in subsequent cycling performance. Findings from this study suggest that, in a laboratory setting with readily available access to fluid and food, the consumption of a CHO-electrolyte sports beverage between consecutive exercise sessions influences nutritional intake, rather than acute measures of fluid recovery or exercise performance. Post-exercise beverage recommendations should consider the post-exercise environment (i.e. availability of food), an individual's tolerance for food and fluid pre-/post-exercise, the immediate requirements for refuelling (i.e. CHO demands of the activity) and the athlete's overall dietary goals.

8.5.1. Fluid Restoration

Previous research generally indicates that beverages with "simple" nutritional profiles (e.g. water and CHO-electrolyte sports beverages) are poorly retained post-ingestion, often leaving individuals with a residual fluid deficit [147,331,333,354]. However, these studies typically prescribe fluid consumption (i.e. fluid volume and rate) and deny participants access to food: an approach with limited ecological validity. In contrast, the current investigation provided access to one beverage and a variety of food items *ad libitum*. In this context, these "simple" beverages were effective at replenishing fluid losses, returning participants to net positive fluid balance prior to

recommencing exercise (Water: +0.4±0.5 L; Sports Drink: +0.3±0.3 L). Two recent studies [8] (one presented in Chapter 7) employing comparable methodology demonstrated similar effects; specifically, that water and CHO-electrolyte sports beverages were as effective as "complex" fluids (i.e. milk-based formulations) at replenishing fluid losses after exercise. These observations may be explained by two factors; first, that individuals in these studies voluntarily consumed the "simple" beverages in relatively large quantities (i.e. >1.25 L· kg BM lost^{-1}); and second, that the co-ingested food enhanced fluid retention (i.e. by delaying gastric emptying and attenuating hemodilution) [375,377]. Unlike the current investigation, however, neither of the aforementioned studies incorporated a subsequent exercise session; participants instead left the laboratory at the end of the recovery period. This distinction is important because the anticipation of subsequent activity might influence dietary behaviour, and consequently rehydration. Indeed, individuals could possibly restrict their intake of food and/or fluid in this situation to avoid experiencing GI problems during the second bout of exercise [413]. While the current study did not incorporate a "no exercise" trial to determine exactly *how* or *if* the subsequent activity influenced dietary behaviour, the present data suggest that individuals were willing to consume *enough* food and fluid to support rehydration. In fact, participants consumed relatively similar amounts of fluid and energy as individuals in the previous investigations (i.e. Chapter 7 and Campagnolo*, et al.* [8]). Considering the large volumes of fluid ingested (i.e. ~1.4–2.2 L·kg BM lost^{-1}), it is possible that the subsequent exercise session actually motivated drinking behaviour (particularly as the participant's nutrition knowledge questionnaires indicated a belief that dehydration could impair subsequent athletic performance). Thus, the present data improve our understanding of post-exercise fluid recovery, indicating that beverages with "simple" nutritional profiles are likely to be effective at replenishing fluid losses when consumed voluntarily and with food between consecutive cycling sessions.

8.5.2. Nutrient Provision and Timing

Results indicate that the choice of recovery beverage can influence post-exercise nutritional intake. In keeping with the hypothesis, the current investigation found that participants consumed 25±13% (Hedges' *g*=0.77) more energy, 54±24% (*g*=1.44) more

CHO, and 41±33% (*g*=0.75) more sodium when Sports Drink was provided during the recovery period. The intakes of other nutrients were not affected by the beverage treatment. For instance, the quantity of protein consumed was consistent with recommendations for post-exercise recovery (i.e. ~0.25–0.30 $g \cdot kg^{-1}$ ≤2 h post-exercise [2]), regardless of the beverage provided (Water: 0.42±0.14 $g \cdot kg^{-1}$; Sports Drink: 0.35±0.11$g \cdot kg^{-1}$). The timing of nutrient consumption also differed between beverages. Specifically, Sports Drink increased intakes of energy, CHO and sodium to a greater extent during the second-half of the eating/drinking period (1st half [% increase compared to Water], Energy: +12±33%, *g*=0.13; CHO: +38±47%, *g*=0.72; Sodium: +9±42%, *g*=0.01; 2nd half, Energy: +99±141%, *g*=0.87; CHO: +166±237%, *g*=1.30; Sodium: +236±295%, *g*=0.73). This result possibly reflects a reduction in solid food intake (but continued drinking) over the latter half of the eating/drinking period. Consequently, it is possible that some of this additional CHO may not have been completely absorbed prior to recommencing exercise.

8.5.3. Subsequent Endurance Cycling Performance

Previous studies demonstrating that the consumption of CHO-containing beverages in the post-exercise period enhances subsequent endurance performance typically deny participants access to food; meaning that the control condition often receives no CHO (Chapter 4). While participants in the current study consumed significantly *more* CHO during the recovery period with Sports Drink, they still consumed some CHO on the Water trial. Under these circumstances, the type of beverage provided did not influence subsequent endurance cycling performance. One possible explanation for the lack of a performance effect is that CHO consumption was already close to recommended levels for "rapid refuelling" (1.0–1.2 g $CHO \cdot kg^{-1} \cdot h^{-1}$ [2,160,165]) on the Water trial. In other words, participants voluntarily consumed sufficient CHO from food alone to facilitate muscle glycogen re-synthesis in this laboratory environment (i.e. where it was readily available post-exercise). Indeed, males and females consumed 0.91±0.22 and 0.85±0.25 g $CHO \cdot kg^{-1} \cdot h^{-1}$ during the 195 min eating/drinking period, respectively. Indeed, Ivy*, et al.* [163] failed to demonstrate a difference between similarly "sub-optimal" (e.g. 0.75 $g \cdot kg^{-1} \cdot h^{-1}$) and "optimal" CHO doses in terms of their effect on muscle glycogen re-synthesis ~4 h post-exercise. Furthermore, there is limited evidence to

suggest that consuming >1.2 g CHO·kg^{-1}·h^{-1}, as was the case in the present study when Sports Drink was provided (M: 1.35±0.14; F 1.26±0.28 g CHO·kg^{-1}·h^{-1}) accelerates glycogen re-synthesis further [160,165]. Additionally, given that protein has the capacity to enhance muscle glycogen re-synthesis when CHO intakes are sub-optimal [160]; and that the additional CHO consumed via Sports Drink may not have been completely absorbed prior to the performance test (see *Nutrient Provision and Timing*) [414], the observed result is not unexpected based on the profile of the observed nutrient intakes.

While the current study did not detect a benefit of consuming Sports Drink on subsequent endurance cycling performance, it is important to consider this outcome in context. First, while we attempted to provide exercise tasks that utilised CHO, it is possible that the protocols employed lacked sufficient duration/intensity to seriously deplete substrate stores and elicit a CHO-mediated performance change. In addition, participants in this study were given *ad libitum* access to a variety of foods (including many high-CHO options) during a dedicated 195 min eating/drinking period that began almost immediately (i.e. within ~15 min) post-exercise. In actuality, a wide selection of foods is not always so readily available following exercise and it is possible that a reduction or delay in the availability of food could alter the outcome of this study. Furthermore, other factors (e.g. nutrition knowledge and beliefs, food preferences, food cost, social environment and time of day) and the likelihood of subsequent GI problems (e.g. running vs. cycling [205]) may influence an individual's dietary behaviour during recovery in a free-living environment [415]. The post-exercise environment, type of activity and an athlete's immediate requirements for refuelling (i.e. the duration and intensity of the initial and subsequent sessions [416,417] are important contextual features when interpreting the findings of this study.

The present data also suggest that an athlete's overall dietary goals (e.g. body composition aspirations) should be considered when recommending a suitable post-exercise recovery beverage. In practical terms, the additional energy delivered via Sports Drink (M: +1.8±1.0 MJ; F: +1.3±0.5 MJ) is likely to represent a meaningful proportion (i.e. ~5–15%) of a competitive cyclist/triathletes' "typical" daily energy intake (M: ~14.5–22.9 MJ [418,419]; F: ~12.0–14.9 MJ [420]). For some athletes, regular consumption of CHO-electrolyte sports beverages post-exercise may assist in the maintenance of energy balance, particularly when exercise loads are high. Conversely, frequent consumption of

CHO-electrolyte sports beverages during periods of reduced physical activity or by individuals with lower training-demands (e.g. recreational athletes) may promote excess energy intakes and could be counterproductive to body composition aspirations. This is particularly important given that individuals do not usually modify their dietary behaviour to compensate for additional energy consumed in caloric rehydration beverages [374] (also, Chapter 7).

This study does contain some limitations. First, a blinded experimental design was not employed, since artificially-sweetening and/or flavouring the Water (i.e. to create a realistic placebo) could have altered drinking behaviour and fluid recovery. Thus, expectancy effects may have influenced cycling performance. To reduce bias, all participants received the same study overview, which intended to create uncertainty regarding the outcome of the investigation; specifically, they were told that "*CHO-electrolyte sports beverages have been demonstrated to improve endurance performance*" but that "*studies had not tested their effects in combination with food*". We must also acknowledge the challenges associated with measuring "subsequent" endurance performance. Indeed, research suggests that prior exercise can reduce the reliability of a performance measurement and increase the risk of Type II error [293]. To improve sensitivity, the current study employed an incremental test (rather than a TT), thereby avoiding the need for participants to select an appropriate pacing strategy. Finally, the fact that participants voluntarily ingested ~300 mL less fluid on their first than second trial suggests that "learning" (or prior experience) may have affected their drinking behaviour in this study. Future research employing comparable methodology should therefore consider familiarising participants to the rehydration component of the trial.

8.6. Conclusion

This study demonstrated that: (1) beverages with "simple" nutritional profiles were effective at replenishing fluid losses, when consumed *ad libitum* and with food between consecutive cycling sessions; and (2) that cyclists in the laboratory environment voluntarily derived enough CHO from food alone to meet post-exercise refuelling recommendations; meaning that the additional nutrition consumed during the Sports

Drink trial did not benefit subsequent endurance cycling performance. Findings from the current investigation suggest that before spontaneously providing a CHO-containing fluid during recovery, consideration of the post-exercise environment, an individual's tolerance for food/fluid, the immediate requirements for refuelling and the athlete's overall dietary goals is required.

Chapter 9: Summary of Thesis Part II

The first experiment in Thesis Part II (Study 3; presented in Chapter 7) investigated how different post-exercise beverages affect fluid restoration and nutrient provision when consumed *ad libitum* and with food by trained females. Overall, results were consistent with the earlier study of trained males by Campagnolo*, et al.* [8], indicating that while different beverages are *equally* effective at replenishing fluid losses under these conditions, the consumption of CHO-containing products (i.e. a CHO-electrolyte sports beverage and milk-based formulation) increases energy and CHO intake (both immediately post-exercise and across the entire day). Specifically, the study indicated that energy intake increased in proportion to the energy-density of the recovery beverage. Of course, one limitation of Study 3 (and the earlier investigation [8]) is that participants were not required to perform a "subsequent bout of exercise". Thus, the second experiment in Thesis Part II (Study 4; presented in Chapter 8) investigated how different post-exercise beverages affect fluid restoration, nutrient provision and subsequent athletic performance when consumed *ad libitum* and with food between exercise sessions with limited recovery time. Study 4 did not include a milk/milk-based formulation as these tended to elicit GI discomfort in earlier investigations [8,373] and this may have affected participant retention. Overall, the study found that: (a) both water and a CHO-electrolyte sports beverage were effective at replenishing fluid losses between consecutive exercise sessions; and (b) that despite increasing CHO intake, access to a sports beverage did not enhance subsequent endurance performance. One possible explanation for the lack of a performance effect was that participants' CHO intakes were already close to recommended levels for "rapid refuelling" (i.e. ~0.9 vs. 1.0–1.2 $g \cdot kg^{-1} \cdot h^{-1}$ [2]) when water was provided. In other words, it appears that individuals voluntarily consumed sufficient CHO from food alone to attenuate any CHO or glycogen mediated cycling performance changes in this laboratory setting.

Collectively, the research presented in Thesis Part II suggests that, when food is readily available post-exercise, the type of beverage consumed is more likely to influence nutrient provision than acute measures of fluid restoration or subsequent endurance performance. Hence, an athlete's overall dietary goals (e.g. energy needs

and body composition aspirations) should be considered before spontaneously providing a CHO-containing post-exercise beverage. Other factors to consider when recommending a suitable post-exercise beverage include an individual's ability to tolerate food and fluid pre-/post-exercise, taste preferences (i.e. beverage palatability) and immediate requirements for refuelling.

Chapter 10: General Discussion and Conclusions

10.1. Overview of Findings

The overall objective of this thesis was to develop a better understanding of how different beverages affect fluid restoration, nutrient provision and subsequent athletic performance when consumed between exercise sessions with limited recovery time (e.g. ≤4 h). The thesis aims and main findings from each study are summarised in Table 10.1.

Table 10.1. Overview of the thesis aims and main findings of each research study.

Research Study	Research Aim	Main Findings
Study #1: The effect of fluid intake following dehydration on subsequent athletic and cognitive performance: A systematic review and meta-analysis	To explore the effect of fluid consumption following dehydration on subsequent (i.e. ≤4 h recovery) athletic performance	1. Fluid (water) intake following dehydration improved continuous exercise performance. The magnitude of this improvement tended to increase as environmental temperature and exercise duration increased. 2. The effect of fluid intake volume on subsequent athletic performance was unclear and requires further investigation. 3. Evidence indicating a beneficial effect of fluid consumption on intermittent, resistance, sport-specific and balance exercise and on cognitive function was also unclear and requires further investigation.
Study #2: Post-exercise ingestion of carbohydrate, protein and water: A systematic review and meta-analysis for effects on subsequent athletic performance	To explore the effect of consuming CHO and protein with water following exercise on subsequent (i.e. ≤4 h recovery) athletic performance	1. Water co-ingested with CHO during and/or following an initial bout of exercise improved subsequent endurance perf-ormance (compared to water alone). The magnitude of this improvement decreased when participants were fed ≤4 h before the experiment as opposed to fasted overnight. 2. The addition of protein to a CHO-containing post-exercise beverage did not enhance subsequent endurance perf-ormance. The beneficial effect of protein reported in some studies may be a consequence of the additional energy delivered in the nutrient, not the 'protein' per se.
Study #3: Fluid, energy and nutrient recovery via *ad libitum* consumption of different commercial beverages and food in female athletes.	To determine the effect of consuming different post-exercise beverages *ad libitum* with food on short-term fluid, energy and nutrient recovery in trained females.	1. Water, a CHO-electrolyte sports beverage, and two different milk-based beverages were equally effective at replenishing fluid losses when consumed *ad libitum* and with food after exercise. 2. The caloric beverages increased energy consumption and altered nutrient provision immediately post-exercise and across the entire trial day compared to when water was consumed.
Study #4: The effect of different post-exercise beverages with food on *ad libitum* fluid recovery, nutrient provision and subsequent athletic performance.	To determine the effect of consuming different post-exercise beverages *ad libitum* with food on short-term fluid recovery, nutrient provision and subsequent athletic performance.	1. Both water and a CHO-electrolyte sports beverage were effective at replenishing fluid losses when consumed *ad libitum* and with food between exercise sessions with limited recovery time. 2. Despite increasing energy and CHO consumption between consecutive exercise sessions, access to a CHO-electrolyte sports beverage did not enhance subsequent endurance performance.

Initially, Study 1 determined that fluid consumption during and/or following dehydration is likely to improve performance on a subsequent bout of prolonged, continuous exercise (Figure 3.3); particularly, when exposed to heat-stress conditions (Figure 3.4). Of note is that the meta-analysed studies typically administered fluid to replace only ~90% (range: 50–115%) of participants' sweat losses (Table 3.1); none administered a quantity large enough to completely restore euhydration (i.e. 1.25–1.50 L·kg BM lost^{-1} [1,2]). Thus, it appears that fluid, even if it is consumed in quantities that are inadequate for complete rehydration, is important for individuals undertaking consecutive bouts of dehydrating exercise and attempting to optimise performance on the subsequent session. Still, further research is required to better understand how larger quantities of fluid are tolerated between exercise sessions with limited recovery time and influence subsequent athletic performance (bearing in mind that this might depend on the specific circumstances involved, e.g. the magnitude of the fluid deficit, composition of the beverage consumed and/or the environmental conditions). Research investigating the effect of fluid intake on intermittent exercise, resistance exercise, sport-specific technical skills and cognition was relatively limited, and a narrative synthesis of the available data failed to indicate a clear improvement. Thus, well-controlled studies to clarify the importance of fluid consumption for those individuals performing these sports or forms of exercise are also needed.

With Study 1 identifying a benefit of fluid intake, Study 2 was conducted to determine whether the addition of other nutrients; specifically, CHO and protein, to a post-exercise beverage could enhance subsequent athletic performance *further*. In this investigation, fluid (water) co-ingested with CHO during and/or following an initial bout of exercise was found to improve subsequent endurance performance compared to fluid ingested alone (Figure 4.3). The administration of CHO also improved subsequent anaerobic exercise performance (Figure 4.5). Notably, the average amount of CHO delivered in the meta-analysed studies was only marginally less than that required to optimise muscle glycogen resynthesis (i.e. ~0.8 vs. 1.0–1.2 g·kg^{-1}·h^{-1} [2]) (Table 4.3); suggesting that CHO delivered at the recommended rate between exercise sessions is likely to be tolerated. On the other hand, protein added to a CHO-containing post-exercise beverage did not influence subsequent endurance performance (Figure 4.6). In fact, the performance-enhancing effect of protein demonstrated in some studies

appeared to be a consequence of the additional energy it delivered (i.e. compared to an isocarbohydrate control), rather than an effect of protein per se (Figure 4.7). Overall, the research presented in Thesis Part I suggests that athletes who have limited opportunity for nutritional recovery between consecutive bouts of dehydrating exercise (e.g. ≤4 h) should prioritise CHO and water consumption to enhance subsequent athletic performance.

Given their ability to deliver fluid and CHO simultaneously, it was anticipated that CHO-containing beverages might assist athletes to rehydrate and refuel between exercise sessions with limited recovery time. While numerous studies have previously investigated the ability of different beverages to influence short-term post-exercise recovery (i.e. restore fluid and substrate losses) and improve subsequent athletic performance, most have "prescribed" drinking (i.e. fluid volume and rate) and denied participants access to food. On the contrary, individuals usually control the volume of fluid they consume and often have access to food in the initial hours after exercise. Hence, Thesis Part II examined how different post-exercise beverages affect fluid restoration, nutrient provision and subsequent athletic performance when consumed *ad libitum* and with food between exercise sessions with limited recovery time.

Both studies in Thesis Part II investigated the ability of different beverages to rehydrate when consumed *ad libitum* and with food post-exercise. The first, Study 3, determined that, under these conditions, water, CHO-electrolyte sports beverages and milk-based formulations are all likely to be effective at replenishing exercise-induced fluid losses in trained females (Table 7.4). The only other study employing comparable methodology [8] observed similar effects in trained males; suggesting that different beverages consumed *ad libitum* with food are likely to promote similar levels of fluid recovery in both sexes, despite the fact that, compared to males, females seem to voluntarily derive smaller amounts of energy, CHO, protein and sodium (per kg BM) from food. In contrast, studies that prescribe drinking and deny participants access to food typically find that beverages with "complex" nutritional profiles (e.g. milk/milk-based formulations) are more effective rehydration agents than those with "simple" nutritional profiles (e.g. water and CHO-electrolyte sports beverages) [147,331,333,354]. Of course, one limitation of Study 3 (and the earlier investigation [8]) is that participants were not required to perform a "subsequent bout of exercise". This is important because the

anticipation of further exercise could potentially influence dietary behaviour, and therefore, rehydration. For instance, individuals might restrict their intake of food and/or fluid to avoid experiencing GI problems that hinder subsequent athletic performance. However, Study 4 found that "simple" beverages (i.e. water and a CHO-electrolyte sports beverage) were, in fact, still effective rehydration agents when consumed *ad libitum* with food between consecutive exercise sessions (Table 8.3); in other words, it appeared that individuals were willing to consume enough food and fluid to support rehydration without concern they would experience GI problems. Considering the large volumes of fluid consumed (i.e. ~1.4–2.2 L·kg BM lost^{-1}), it is possible that the subsequent exercise session actually *motivated* drinking behaviour. A further point of interest is that while Studies 3 and 4 suggest that different post- exercise beverages are likely to promote similar levels of fluid recovery in a free-living environment, Study 3 did observe some influence of palatability, in that participants tended to restore a greater proportion of their fluid losses when given access to their most preferred, rather than least preferred, beverage. As such, it appears that individuals attempting to maximise fluid restoration between exercise sessions with limited recovery time should select a beverage they consider to be palatable.

In addition to fluid restoration, Studies 3 and 4 also examined the ability of different beverages consumed *ad libitum* and with food to influence post-exercise nutrient provision – particularly, CHO provision, since this is an important determinant of glycogen resynthesis and subsequent endurance performance (Study 2); Study 2 also identified a beneficial effect of dietary energy (in general) on subsequent endurance performance. Study 3 demonstrated that access to a caloric beverage (i.e. either a CHO-electrolyte sports beverage or a milk-based formulation), in the initial post-exercise period is likely to increase both short-term and total daily energy and CHO consumption in trained females (Table 7.3). This result was again consistent with the aforementioned study of trained males by Campagnolo*, et al.* [8]; suggesting that individuals, regardless of their sex, are unlikely to reduce their solid food intake sufficiently to offset the additional nutrition delivered in caloric post-exercise beverages. Indeed, Study 4 also found that access to a CHO-electrolyte sports beverage increased post-exercise energy (i.e. ~25%) and CHO (i.e. ~55%) consumption (Table 8.4). However, this additional nutrition did not translate to an improvement in

subsequent endurance cycling performance (Figure 8.5). One possible explanation for the lack of a performance effect is that CHO consumption was already close to recommended levels for "rapid refuelling" (i.e. ~0.9 vs. 1.0–1.2 $g \cdot kg^{-1} \cdot h^{-1}$ [2]) when water was provided (energy intakes were also reasonably high, irrespective of the beverage consumed). In other words, it appears that participants voluntarily consumed sufficient CHO from food alone to facilitate muscle glycogen resynthesis in this laboratory environment (i.e. where it was readily available). Collectively, the present data suggest that, when food is available post-exercise, the type of beverage consumed is more likely to influence nutrient provision than fluid restoration or subsequent athletic performance. Hence, an athlete's overall dietary goals (e.g. energy needs and body composition aspirations) should be considered before spontaneously providing a CHO-containing post-exercise beverage.

It is important to acknowledge the limitations that exist within this body of research. First, participants in Studies 3 & 4 were restricted to consuming *one type* of beverage, only; in reality, individuals may consume more than one type of post-exercise beverage. These beverages were also served in large opaque jugs, whereas caloric products are typically sourced in single-serve packages. Second, participants in Studies 3 & 4 were permitted to drink *ad libitum* throughout the entire 4 h recovery period. In some circumstances, however, individuals may rehydrate using a combination of prescribed and *ad libitum* drinking strategies. Third, it is possible that the exercise sessions used Study 4 lacked sufficient duration and/or intensity to seriously deplete substrate stores and thus elicit a CHO-mediated performance change. Fourth, participants in Studies 3 & 4 were given *ad libitum* access to a wide variety of foods. A reduction or delay in the availability of food could therefore yield different results. Finally, it is important to recognise that a wide variety of factors (e.g. nutrition knowledge and beliefs, food preferences, food cost, the social environment and/or time of day and the likelihood of subsequent GI problems, e.g. running vs. cycling [205]) have the potential influence an individual's dietary behaviour in a free-living environment. The reader should refer to 10.3 *Limitations and Recommendations for Future Research*, for further discussion on these limitations.

Overall, it appears that while individuals undertaking consecutive bouts of dehydrating and fuel-demanding exercise with limited recovery time (e.g. ≤4 h)

should consume CHO and fluid to optimise subsequent athletic performance, the utility of a CHO-containing post-exercise beverage (specifically) may depend on the circumstances involved. Indeed, the research in this thesis indicates that: (a) CHO-containing beverages are no more effective than water at promoting fluid restoration when consumed *ad libitum* and with food between exercise sessions; and that (b) although CHO-containing beverages increase CHO delivery (i.e. under these circumstances), the additional nutrition may not enhance subsequent athletic performance, since individuals can derive enough CHO from food alone to meet post-exercise refuelling recommendations. It is, however, important to recognise that access to a CHO-containing beverage in the initial post-exercise period may increase total daily energy consumption and therefore has the potential to influence chronic exercise–nutrient interactions. Thus, if food is available, an athlete's overall dietary goals (e.g. energy needs and body composition aspirations) should be considered before spontaneously providing a CHO-containing post-exercise beverage. Other factors to consider when recommending a suitable post-exercise beverage include an individual's ability to tolerate food and fluid pre-/post-exercise, taste preferences (i.e. beverage palatability) and immediate requirements for refuelling.

10.2. Implications of the Research

The current results would indicate that when food is available post-exercise, the type of beverage consumed is more likely to influence nutrient provision than fluid restoration or subsequent athletic performance. Indeed, in practical terms, the additional energy delivered in caloric post-exercise beverages (e.g. +1.8±1.0 MJ in males or +1.3±0.5 MJ in females when a sports beverage is consumed) is likely to represent a meaningful proportion (i.e. ~5–15%) of a competitive cyclist or triathletes' "typical" daily energy intake (M: ~14.5–22.9 MJ [418,419]; F: ~12.0–14.9 MJ [420]). Hence, an athlete's overall dietary goals should be considered when recommending a suitable post-exercise beverage. In circumstances where weight gain or maximising nutrient intake (e.g. to maintain energy balance during periods of heavy training) are desired, the consumption of a CHO-containing post-exercise beverage (e.g. CHO-electrolyte sports beverages or milk/milk-based formulations) with food is likely to facilitate rehydration, refuelling and intake of additional energy. Conversely, the

consumption of water with food is sufficient to promote rehydration and refuelling without excessive energy intake in situations where weight loss or maintenance is required. Frequent consumption of caloric beverages during periods of reduced physical activity (e.g. during injury, offseason or retirement) or by individuals with lower training-demands (e.g. recreational athletes) may promote excess energy intakes and could be counterproductive to body composition aspirations.

10.3. Limitations and Recommendations for Further Research

A number of recommendations for further research can be made:

1. Firstly, while Studies 3 and 4 indicate that access to a caloric beverage in the initial post-exercise period increases both short-term and total daily energy consumption, it is important to recognise that participants were restricted to consuming *one type* of beverage, only. This approach was used as these investigations were among the first of their kind and it was important to understand the impact of each in isolation before moving forward in this field of study. In reality, However, individuals may consume more than one type of post-exercise beverage; for instance, a caloric beverage in combination with water – since it is readily available and, anecdotally, participants often reported feeling "thirsty for water" on these treatments. Beverages were also served in large opaque jugs, but caloric beverages are typically sourced in single-serve packages. Both the availability of water (or other beverages) and the container a beverage is served in have the potential to influence consumption behaviour of caloric beverages. Thus, further research is required to determine if caloric post-exercise beverages effect energy and nutrient provision when served in their original packaging with *ad libitum* access to food and water.
2. Participants in Studies 3 and 4 were permitted to drink *ad libitum* throughout the entire 4 h recovery period. In some circumstances, however, individuals may rehydrate using a combination of prescribed and *ad libitum* drinking strategies. For instance, individuals may be instructed (e.g. by a coach) to consume a fixed volume of fluid immediately post-exercise (e.g. a "priming" dose), and then left to restore the remainder of their fluid losses *ad libitum* over the next few hours. Thus, further research is required to determine how

different post-exercise beverages affect fluid restoration when a combination of prescribed and *ad libitum* drinking strategies are employed post-exercise.

3. While milk-based formulations, like CHO-electrolyte sports beverages, had an effect to increase post-exercise energy and nutrient consumption in Study 3, the ability of these beverages to influence subsequent athletic performance was not investigated in Study 4. The decision not to include milk/milk-based formulations in Study 4 was based on their likelihood to induce GI problems [8,373] (i.e. this might have negatively impacted participant retention). The acceptability of these beverages might, however, be improved if they were administered alongside additional water (i.e. as described above). Thus, research to clarify the manner in which milk/milk-based post-exercise beverages impact on subsequent athletic performance when consumed with *ad libitum* access to food and water is warranted.
4. Despite increasing total energy and CHO consumption between exercise sessions, Study 4 found that the provision of a CHO-electrolyte sports beverage did not enhance subsequent endurance cycling performance. It is important to recognise that although the research candidate attempted to provide fuel-demanding exercise sessions, it is possible that the protocols employed lacked sufficient duration and/or intensity to seriously deplete substrate stores and elicit a CHO-mediated performance change. Thus, additional studies are required to determine if CHO-electrolyte sports beverages (as opposed to water) consumed *ad libitum* and with food are likely to benefit individuals undertaking more fuel-demanding exercise sessions.
5. Lastly, it is important to consider that participants in Studies 3 and 4 were given *ad libitum* access to a wide variety of foods during a dedicated eating and drinking period. In reality, a wide selection of foods is not always so readily available following exercise and it is possible that a reduction or delay in the availability of food could affect post-exercise recovery and subsequent athletic performance. Other factors (e.g. nutrition knowledge and beliefs, food preferences, food cost, the social environment and/or time of day) and the likelihood of subsequent GI problems (e.g. running vs. cycling [205]) might also influence an individual's dietary behaviour in a free-living environment. Further research is therefore required to clarify how these different factors

influence post-exercise recovery, subsequent athletic performance, and thus, post-exercise beverage recommendations.

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