SENSORY PERCEPTION OF AN ORAL REHYDRATION SOLUTION THROUGHOUT EXERCISE IN THE HEAT

A thesis presented in partial fulfilment of the requirements for the degree of

Masters of Science

In

Nutrition and Dietetics

Massey University, Albany

New Zealand

Olivia Kitson

2020

ABSTRACT

Background Oral rehydration solutions (ORS) are formulated specifically to replenish fluid and electrolytes lost through diarrhoea and sweat. Regardless of poor palatability ratings in relation to traditional sports drinks or water, ORS may be effective at optimising hydration during prolonged exercise in the heat.

Aim To assess the palatability of an ORS at rest and throughout 60 min of moderate intensity exercise in the heat.

Methods Twenty-seven recreationally active participants (n=13 males; n=14 females) completed sensory analysis of an ORS, a traditional sports drink (TS) and a flavoured water placebo (PL) at rest and throughout 60 min (3 x 20-min bouts) of cycling exercise at 70% age-predicted maximum heart rate (HR_{max}) at 30-35°C. Before and every 20 min after exercise, drinks were rated based on liking of sweetness, liking of saltiness, thirst-quenching ability, and overall liking on a 9-point hedonic scale. Hydration status was assessed by changes in semi-nude body mass, urine osmolality (U_{Osm}), urine specific gravity (U_{SG}), urine colour (U_{Col}), saliva osmolality (S_{Osm}), and saliva total protein concentration (S_{PC}).

Results After 60 min of exercise, participants had lost an average of 1.36 ± 0.39 % of body mass and there was an increase in S_{Osm}, S_{PC}, U_{SG} and U_{Col} ($p<0.05$) but no change in U_{Osm} ($p>0.05$). At all time points, liking of sweetness, saltiness, thirst-quenching ability and overall liking was higher for the TS and PL compared to the ORS ($p<0.05$). However, the saltiness liking and thirst-quenching ability of the ORS increased significantly after 60 min of exercise compared to before exercise ($p<0.05$). There was also a significant change in predictors of overall liking with pre-exercise ratings mostly determined by liking of sweetness, saltiness and thirst-quenching ability ($p<0.001$); whereas only liking of saltiness predicted overall liking post-exercise ($R^2=0.751$; $p<0.001$).

Conclusions There appears to be a hedonic shift during exercise in which the perception of saltiness becomes the most important predictor of overall liking. This finding supports the potential use of an ORS as a valuable means of hydration during the latter stages of prolonged and/or intense exercise in the heat.

Keywords: dehydration, electrolytes, palatability, saltiness, sports drinks, thirst

ACKNOWLEDGEMENTS

I must express my greatest appreciation to everyone who made this research possible.

Firstly, I would like to thank my main academic supervisor, Assoc Prof Ajmol Ali for your inspiration, wisdom and patience over the last couple of years. I would also like to thank my co-supervisor, Assoc Prof Kay Rutherfurd-Markwick, for your guidance and education in the art of writing concisely and ensuring quality, consistency and accuracy throughout this thesis. Thank you both for your continuous support and encouragement especially given the uncertain and challenging circumstances this year.

Thank you to Dr Charles Diako for helping with the sensory evaluation and to Prof Marie Wong for helping with the development of the ORS. Thank you also to Finn Cadman for the development of the initial ORS solution that was used in this study and to Dr Daniel Walsh for your statistics help during the COVID lockdown.

I would also like to thank Darrien Holten and Mathilde Cancalon for carrying out participant recruitment and data collection. This helped me immensely to complete this thesis in a timely manner.

Thank you so much to all the participants for your time, sweat and effort. It takes a lot of mental toughness to cycle in a sweat suit for 60 min in the heat!

Lastly, thank you to my family, friends and fiancé for your incredible support and encouragement over the years, I could not have done it without you.

TABLE OF CONTENTS

ABSTRACT .... **II**

ACKNOWLEDGEMENTS .... **III**

LIST OF TABLES .... **VI**

LIST OF FIGURES .... **VII**

LIST OF ABBREVIATIONS AND SYMBOLS .... **VIII**

CHAPTER 1: INTRODUCTION **1**

1.1 SUMMARY & JUSTIFICATION FOR RESEARCH 5
1.2 PURPOSE OF THE STUDY 5
1.3 AIMS AND OBJECTIVES 5
1.4 THESIS STRUCTURE 6
1.5 CONTRIBUTIONS OF RESEARCHERS 6

CHAPTER 2: LITERATURE REVIEW **7**

2.1 INTRODUCTION 7
2.2 DEFINING HYDRATION TERMS 8
2.3 METHODS FOR ASSESSING HYDRATION STATUS 8
2.4 THERMOREGULATION, CARDIOVASCULAR RESPONSES AND PERFORMANCE 10
2.5 TRADITIONAL SPORTS DRINKS 11
2.6 ORAL REHYDRATION SOLUTIONS 12
2.7 KEY DRIVERS OF VOLUNTARY FLUID INTAKE 13
2.8 THIRST PERCEPTION 13
2.9 BEVERAGE PALATABILITY 14
2.10 THEORY OF PHYSIOLOGICAL USEFULNESS 21
2.11 PERCEPTION OF FLUID OSMOLALITY 21
2.12 PERCEPTION OF SWEETNESS 22
2.13 PERCEPTION OF SALTINESS 23
2.14 PSYCHOLOGICAL FACTORS 25
2.15 SUMMARY 27

CHAPTER 3: SENSORY PERCEPTION OF AN ORAL REHYDRATION SOLUTION THROUGHOUT EXERCISE: A RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY **28**

3.1 ABSTRACT 28
3.2 INTRODUCTION 29
3.3 METHODS 30
3.4 RESULTS 33
3.5 DISCUSSION 39
3.6 CONCLUSION 43

CHAPTER 4: CONCLUSIONS 44

4.1 OVERVIEW AND ACHIEVEMENT OF STUDY AIMS AND OBJECTIVES 44
4.2 FINDINGS AND CONCLUDING REMARKS 45
4.3 RESEARCH CONTRIBUTION 45
4.4 STRENGTHS AND LIMITATIONS 45
4.5 DIRECTIONS FOR FUTURE RESEARCH 47
4.6 FINAL RECOMMENDATIONS 48

REFERENCES 49

APPENDICES 62

APPENDIX A. PARTICIPANT INFORMATION SHEET 62
APPENDIX B. HEALTH SCREENING QUESTIONNAIRE 65
APPENDIX C. PARTICIPANT CONSENT FORM 67
APPENDIX D. LETTER OF ETHICS APPROVAL FROM MASSEY UNIVERSITY HUMAN ETHICS COMMITTEE 68
APPENDIX E. SUPPLEMENTARY METHODS 69
APPENDIX F. SUPPLEMENTARY RESULTS 77

LIST OF TABLES

Table 1.1. List of contributions of researchers 6

Table 2.1. Summary of literature investigating the effects of exercise on sensory perception and hedonic response 16

Table 3.1. Composition of the drinks used for this study 31

Table 3.2. Physiological and perceptual responses throughout exercise 34

Table 6.1. Sample codes 69

Table 6.2. Sensory ratings for each fluid at each time point throughout exercise 77

LIST OF FIGURES

Figure 2.1. Search strategy..7

Figure 3.1. Overview of the exercise and sensory protocol..31

Figure 3.2. Liking of saltiness of drinks. 35

Figure 3.3. Liking of sweetness of drinks 36

Figure 3.4. Thirst-quenching ability of drinks. 37

Figure 3.5. Overall liking of drinks...38

Figure 6.1. Sample presentation order matrix..69

Figure 6.2. Sensory 9-point hedonic scale 69

Figure 6.3. Urine colour scale..70

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation or symbol	**Definition**
~	Approximately
%	Percentage
<	Less than
=	Equal to
>	Greater than
±	Plus and/or minus
≤	Equal to or less than
≥	Equal to or greater than
°C	Degrees Celsius
ANOVA	Analysis of variance
$[AVP]_p$	Plasma arginine vasopressin
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BML	Body mass loss
CHO	Carbohydrate
CHO-e	Carbohydrate-electrolyte
CI	Confidence interval
d	Cohen's effect size index
df	Degrees of freedom
e	Electrolyte
e.g.	For example
et al.	and others
F	F-ratio
FAS	Felt Arousal Scale
FS	Feeling Scale
h	Hour
HR	Heart rate
HR_{max}	Age-predicted maximum heart rate (220-age)
i.e.	That is
IQR	Interquartile range

kg	Kilogram
mg	Milligram
min	Minute
ml	Millilitre
mmol	Millimole
mOsmol	Milliosmole
MSc	Master of Science
MUHEC	Massey University Human Ethics Committee
N	Population size
n	Sample size
Na	Sodium
NaCl	Sodium chloride (salt)
NZ	New Zealand
ORS	Oral Rehydration Solution
PL	Placebo
P_{osm}	Plasma osmolality
PPE	Personal protective equipment
PubMed	Public/Publisher MEDLINE
PV	Plasma volume
p-value	The probability of rejecting the null hypothesis when it is true
r	Pearson's product-moment correlation coefficient
r^2	Coefficient of determination (r-squared)
RCT	Randomised control trial
RH	Relative humidity
RPE	Rating of Perceived Exertion
SD	Standard deviation
SOP	Standard operating procedures
SPSS	Statistical Package for the Social Sciences
Temp	Temperature
TS	Traditional Sports drink
U_{Col}	Urine colour
U_{osm}	Urine osmolality
U_{SG}	Urine specific gravity

VAS	Visual analogue scale
$\dot{V}O_2$	Maximal oxygen uptake
wk	Week
y	Years

CHAPTER 1: INTRODUCTION

Hydration involves a dynamic balance between fluid intake and loss which is maintained through physiological and behavioural responses (Perrier et al., 2015). Nonetheless, a body water deficit, termed dehydration or hypohydration, occurs when fluid losses (i.e. through excessive sweating) exceed fluid intake (James et al., 2019). Even mild levels of dehydration, as low as 1-2% loss of body mass, have shown to impair thermoregulation (Casa et al., 2012), cardiovascular and metabolic functions, and exercise performance (Sawka et al., 2015). At higher levels (≥2% loss of body mass), cognitive function may be impaired, and the risk of heat illness becomes critical (Adan, 2012; Ganio et al., 2011). Thus, when exercise is prolonged (≥2 h) and accompanied by high sweat losses, the quantity and composition of fluid replacement is vital to preserve physiological functions and optimize performance (Adan, 2012; Evans et al., 2017).

Sports drinks, comprised of water, carbohydrate (CHO) and electrolytes (sodium, chloride, and potassium) are particularly valuable in situations where solid food is not available or desired by the athlete, or when dehydration is a major concern (i.e. when sweat losses are excessive; Murray and Stofan, 2001; Rodriguez et al., 2009; Sawka et al., 2007). Most traditional sport drinks contain around 6-9% CHO in an effort to delay fatigue by maintaining blood glucose levels and high CHO oxidation (Murray and Stofan, 2001; Rodriguez et al., 2009; Sawka et al., 2007). Higher CHO concentrations (≥10% CHO) delay the rate of gastric emptying and compromise intestinal absorption which reduces water availability and may cause gastrointestinal distress during exercise (Desbrow et al., 2014). Thus, it is not surprising that endurance athletes are known to water-down their sports drinks or opt for beverages with a reduced energy content (1-3% CHO) for consumption during exercise (Ali et al., 2011; Romijn et al., 1993).

During prolonged exercise, the provision of electrolytes, particularly sodium (Na) becomes crucial to replace losses through sweat (Evans et al., 2017; Leshem et al., 1999). Sodium is the most abundant cation in sweat, thus when sweat losses are excessive, Na loss will be correspondingly high (Maughan and Shirreffs, 1997), and providing water alone may increase risk of developing hyponatremia (serum Na concentration <130 mmol/L; Montain et al., 2001). Furthermore, Na contributes to the stimulation of thirst by keeping plasma osmolality high and maintaining extracellular volume (Evans et al., 2017; Meyer et al., 1992). Sodium is usually added to sports drinks in the form of sodium chloride (NaCl) at concentrations ranging from 20-25 mmol/L (1.2-1.5 g/L;

Noakes et al., 1985; Sawka et al., 2007). Despite substantial variation in individual sweat and Na loss, the Na concentration of traditional sports drinks is generally equal to or lower than those of sweat (Meyer et al., 1992). Indeed, a study involving a large population of athletes determined fluid and Na losses through sweat to range from 0.3-5.7 L/h and 18.2-70.8 mmol/L, respectively (Baker et al., 2016). These numbers therefore suggest that the Na content of traditional sports drinks is insufficient to restore electrolyte balance for athletes exhibiting high sweat losses.

Oral rehydration solutions (ORS) are intended for the treatment of diarrhea-induced dehydration which contain relatively high Na (30-90 mmol/L; 1.8-2.0 g/L) and low CHO (2-3%) concentrations (Evans et al., 2017; Leshem et al., 1999). Consequently, ORS tend to receive poor palatability ratings; nonetheless, these hypotonic, medically proven formulations are consistent with the need to ensure rapid gastric emptying and intestinal absorption to replace the large amounts of electrolytes that can be lost through sweat and diarrhea (Murray and Stofan, 2000). Indeed, clinical trials using ORS clearly show that hypotonic solutions with no more than 13.3 g/L glucose achieve faster and more effective rehydration in children and adults with acute diarrhea than either water alone or ORS with higher glucose concentrations (Binder et al., 2014). Furthermore, the addition of Na at a concentration equal to or greater than that of the sweat lost is required to maintain positive fluid balance and typically these concentrations are higher than those found in traditional sports drinks (Evans et al., 2017). Thus, regardless of poor palatability ratings, ORS may be more efficient for replacing fluid and electrolyte losses during prolonged exercise, especially in the heat (Evans et al., 2017; Schleh and Dumke, 2018).

Despite the wide range of sports beverages available, athletes rarely consume sufficient fluids to replace sweat losses resulting in a condition termed ‘voluntary dehydration’ (Baker et al., 2016). Indeed, athletes typically only drink up to approximately 50% of their fluid losses often resulting in adverse effects on performance and health (Passe et al., 2007). Thus, in order to guide more effective hydration interventions, exercise scientists have investigated the key drivers of fluid intake; those identified include thirst perception, palatability and sensory properties of the beverage such as temperature, flavour and carbonation, as well as social, cultural and psychological factors (Passe et al., 2000; 2009).

Thirst, defined as a desire to consume fluids as a result of body water deficit, has an important role in driving fluid intake (Cheuvront et al., 2013). The physiological onset of thirst appears to be triggered by a 1-2% loss of body mass corresponding with a 2% rise in plasma osmolality (~5 mmol/kg; Cheuvront et al., 2013; Hughes et al., 2018). Nonetheless, the threshold for thirst is highly variable (Cheuvront et al., 2013) and thirst mechanisms can be affected by a number of factors unrelated to water balance (Hughes et al., 2018). Indeed, oropharyngeal cues and gastrointestinal factors such as stomach distension have been shown to alleviate thirst before complete rehydration can be achieved (Figaro and Mack, 1997; Greenleaf, 1992). Current recommendations advocate using thirst to guide hydration practices in order to reduce risk of exertional hyponatremia (Cheuvront et al., 2013); however, evidence suggests that relying solely on thirst may prevent the full restoration of body water losses, thus leading to voluntary dehydration (Arnaoutis et al., 2018). Furthermore, a number of studies comparing 'planned drinking' with '*ad libitum* drinking' have shown thirst to be unreliable at maintaining body fluid balance (Adams et al., 2019; Ayotte, 2018; Bardis et al., 2017; Lopez et al., 2016).

Beverage palatability has been recognised as an important determinant of voluntary fluid intake with higher palatability ratings consistently associated with greater fluid intake (Maughan and Shirreffs, 1997; Passe et al., 2000). For instance, a reciprocal relationship between hedonic liking and fluid intake has been reported, with the most-liked beverages consumed in the greatest quantities (Passe et al., 2000). Further studies have identified a positive effect on voluntary fluid intake when beverages were cooled (15°C) and further benefits when flavour was added (Hubbard et al., 1984; Szylk et al., 1987; Wilk and Bar-Or, 1996). These findings support the role of sensory characteristics such as taste and temperature on palatability and subsequent fluid intake (Hubbard et al., 1984; Szylk et al., 1987; Wilk and Bar-Or, 1996).

Furthermore, exercise appears to elicit a hedonic shift such that a least-preferred beverage at rest gradually becomes more acceptable throughout exercise (Ali et al., 2011; Passe et al., 2000). Although the exact physiological mechanism is unknown, an increase in liking of low-osmolality fluids (Ali et al., 2011; Appleton, 2005; Takamata et al., 1994), sweetness (Horio, 2004; Horio and Kawamura, 1998; Narukawa et al., 2009), and/or saltiness (Leshem et al., 1999; Takamata et al., 1994; Wald and Leshem, 2003) has been observed throughout exercise. These findings are consistent with the physiological need to replace lost fluid, nutrients, and electrolytes (Cabanac, 1971; Passe et al., 2000).

Immediately after exercise, significant increases in pleasantness ratings for low osmolality fluids have been observed, particularly in individuals exhibiting high sweat losses (Ali et al., 2011; Appleton, 2005; Takamata et al, 1994). Due to the fundamental importance of maintaining body water balance for cardiovascular and thermoregulatory functions, it has been proposed that any food or fluid item of high-water content will become increasingly pleasant as fluids are lost through sweat (Ali et al., 2011; Appleton, 2005; Takamata et al, 1994).

Nonetheless, an increase in pleasantness of sweetness has been related to the need to replenish depleting glycogen stores (Horio and Kawamura, 1998; Horio, 2004). Interestingly, this preference for sweetness has only been observed with solutions containing simple CHO or natural sweeteners with a similar structure (Horio and Kawamura, 1998). The artificial sweetener saccharin does not have the same effect on palatability possibly due to its synthetic nature and inability to provide energy (Horio and Kawamura, 1998). Furthermore, the duration and intensity of exercise appears to influence sweetness perception, perhaps in response to changing CHO requirements (Narukawa et al., 2009; 2010).

Liking of saltiness has also been shown to improve with exercise, particularly when sweat losses are high and Na is not replaced (Leshem et al., 1999; Takamata et al., 1994; Wald and Leshem, 2003). Indeed, a ‘sodium appetite’, whereby solutions of relatively high NaCl concentration increase in palatability, has been observed after exercise that is prolonged (≥2 h), intense and/or in the heat (Leshem et al., 1999; Takamata et al., 1994; Wald and Leshem, 2003). Moreover, the intensity and duration of exercise, amount of sweat lost, Na content of any replacement fluids, and the time at which sensory analyses were completed appears to influence the preference for salt (Meyer et al., 1992).

There also appears to be an important psychological component contributing to the hedonic response, with cognitive drivers such as familiarity (Pliner, 1982) and experience of subsequent beneficial effects (Rogers, 1999; Yeomans et al., 2000) found to play an important role. A group of regular exercisers were found to restrict the amount of sweetener added to their tea as they believed that sweet items were ‘fattening’ (Leshem et al., 1999). Furthermore, training status, familiarity with sports beverages and perhaps tolerance to a hypohydration stimulus may alter expectations and regulatory responses to hypertonic hypovolemia (Merry et al., 2010). Thus, it is difficult to compare the results from published studies due to differences in subject characteristics (gender, age, athletic ability) as well as the method and extent of dehydration achieved (i.e. exercise type, duration, body mass loss).

1.1 Summary & Justification for Research

Voluntary dehydration occurs when *ad libitum* fluid intake is insufficient to match losses often leading to impaired performance and adverse health outcomes (Adan, 2012; Casa et al., 2012; Evans et al., 2017; Sawka et al., 2015). A wide range of sports drinks have been designed to provide fluid, CHO and electrolytes during exercise (Shirreffs, 2009), although their electrolyte concentration appears to be insufficient to match losses through sweat for many endurance athletes (Baker et al., 2016). Thus, an ORS with relatively lower CHO and higher electrolyte concentrations may be more effective for promoting fluid and electrolyte balance during prolonged, intense exercise (Evans et al., 2017). The issue with these hypotonic solutions, however, is that they often receive poor palatability ratings due to their low level of sweetness and high intensity of saltiness. However, there is strong evidence for a hedonic shift towards an increase in liking for saltiness with exercise perhaps related to its physiological usefulness (Leshem et al., 1999; Takamata et al., 1994; Wald and Leshem, 2003). Indeed, it appears that palatability of higher NaCl concentrations increases in proportion to the amount of Na lost through sweat (Takamata et al., 1994). We have designed an ORS that has been shown to meet consumer acceptability at rest, therefore, we aim to test sensory perception of this beverage throughout different stages of exercise in the heat.

1.2 Purpose of the study

The overall purpose of this study was to test and compare the palatability of an ORS with a traditional sports drink-based solution and a flavoured-water placebo at rest and throughout various levels of dehydration induced by 60 min of exercise in the heat. This study offers practical information for sensory and behavioural sciences and the development of sports beverages suitable for hydration during prolonged exercise in the heat.

1.3 Aims and Objectives

Aim:

The aim of this study is to investigate the palatability of an ORS at rest and throughout various levels of dehydration induced by 60 min of exercise in the heat.

Objectives:

- To assess changes in hydration status using changes in body mass and analysis of urine and saliva samples.

- To determine whether exercise and/or exercise-induced fluid loss has an effect on sensory perception and overall liking of the ORS in comparison to the TS and PL.

Hypothesis:

We hypothesise that liking of saltiness and thus, overall liking of the ORS will increase progressively with level of dehydration throughout exercise in the heat.

1.4 Thesis Structure

Chapter 1 introduces this study by outlining aims and objectives and providing research justification. **Chapter 2** is a review of relevant literature, covering the background to the study and key concepts, such as voluntary dehydration, sports beverages and perceived palatability. **Chapter 3** is a manuscript which outlines the methods, results, discussion and conclusion of the main study. Lastly, **Chapter 4** includes a brief summary of the results and conclusions, discusses study strengths and limitations and provides final recommendations from this study. **Supplementary appendices** include additional methods and study protocol.

1.5 Contributions of Researchers

Table 1.1. List of contributions of researchers

Researcher	Contribution
Olivia Kitson MSc Nutrition and Dietetic Student	Primary author of this thesis; data collection; data analysis; writing manuscript
Associate Professor Ajmol Ali Academic Supervisor	Supervision of the entire research process
Associate Professor Kay Rutherfurd-Markwick Academic Supervisor	Supervision throughout research process
Mathilde Cancalon	Participant recruitment; data collection
Darien Holten	Participant recruitment; data collection
Assoc Prof Andrew Foskett	Designing project; manuscript review
Assoc Prof Jason Lee	Designing project; manuscript review

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter reviews literature on voluntary dehydration and key drivers of fluid intake including thirst, beverage palatability, and psychological factors. The current understanding will yield implications for both sports beverage development and behavioural and sensory science. From October 2019 – August 2020, potential research studies were identified by searching the online databases PubMed (MEDLINE), Google Scholar and Scopus. A combination of search terms were determined from the study's purpose, aims and objectives. To manage large search returns, filters were applied and reference lists from relevant articles were screened. Only full-text English journal articles published up to April 2020 were reviewed.

Date searched: October 2019 – October 2020

Search terms:
Dehydration OR hypohydration
AND hydration
AND fluid intake
AND thirst
AND palatability OR sensory perception
AND sports drink
AND oral rehydration solution OR ORS
AND athlete
AND physical activity OR exercise

Filters: past 5 years, past 10 years

Electronic Databases: PubMed (MEDLINE), Google Scholar, Scopus

Figure 2.1. Search strategy

2.2 Defining hydration terms

Hydration involves a dynamic balance between fluid intake and loss which is maintained through physiological and behavioural responses (Perrier et al., 2015). An excess of body water, termed hyperhydration, can result in serious health consequences including cardiopulmonary disorders, hyponatremia, oedema and gastrointestinal dysfunction (El-Sharkawy et al., 2015; Evans et al., 2017; Hew-Butler et al., 2017). On the other hand, dehydration is the process in which fluid output exceeds fluid intake which results in a state of hypohydration (Evans et al., 2017). Due to the transient nature of water during exercise, these terms are often used interchangeably (James et al., 2019) and can lead to profound implications for human health (Armstrong et al., 1985; Evans et al., 2017; Sawka, 1992) and performance (Sawka et al., 2007). Nonetheless, hydration status can be difficult to assess in a field setting (Armstrong et al., 2016; Smith et al., 2012).

2.3 Methods for assessing hydration status

A range of methods have been used to assess and monitor hydration status (Villiger et al., 2018). Defining dehydration outside of the laboratory, however, presents a major challenge due to limitations regarding accuracy and applicability across settings (Adams et al., 2019). Commonly used methods involve haematological markers such as plasma osmolality (P_{Osm}), urinary markers such as urine osmolality (U_{Osm}), urine colour (U_{Col}), and urine specific gravity (U_{SG}), as well as changes in body mass (Villiger et al., 2018). Other techniques may involve testing biochemical markers present in “freely accessible” bodily fluids such as saliva, sweat and tears (Fortes et al., 2011). Most of these techniques are low-cost, easy-to-apply and reliable, however, each have limitations and there is ongoing debate as to which method(s) most accurately reflects hydration status throughout exercise (Armstrong et al., 2016).

Plasma osmolality is often considered a criterion measure of dehydration (Thomas et al., 2008). Osmolality refers to the number of solute particles (such as Na, K, Cl) contributing to osmotic pressure (Osmol) per kg of solvent (Koeppen and Stanton, 2013). Thus, when body mass is lost through sweat, plasma osmolality is expected to rise in a graded manner (~5 mOsmol/kg for every 2% loss of body mass; Popowski et al., 2001; Smith et al., 2012). Plasma osmolality levels within the range of 275-295 mOsmol/kg are generally classified as euhydrated, 295-300 mOsmol/kg as impeding dehydration, and >300 mOsmol/kg as current dehydration (Cheuvront et al., 2010; Thomas et al., 2008). This method is not well suited to field assessments, however, as it involves taking invasive blood samples (Hew-Butler et al., 2017). Furthermore, due to the body’s compensatory mechanisms to maintain extracellular volume, P_{Osm} does not tend to change with moderate levels of dehydration (<2% loss of body mass; Sawka et al., 1985; Smith et al., 2012; Walsh et al., 2004a). Indeed, progressive dehydration induced by moderate intensity cycling exercise and heat stress did not significantly affect P_{Osm} until 2.1% body mass loss (BML; Walsh et al., 2004a). A previous study found no change in P_{Osm} at 3% BML, but a significant increase was observed at 7% BML (Sawka et al., 1985).

Alternatively, urinary measures are quick, simple and easy to use in both laboratory and field settings (Hew-Buter et al., 2018; Sawka et al., 2007). In response to dehydration, an increase in plasma osmotic pressure causes an increase in levels of antidiuretic hormone (ADH) which stimulates vasoconstriction and reabsorption of water in the kidneys (Thornton, 2010). Alternatively, a decrease in plasma volume/pressure contributes to an increase in blood levels of angiotensin II which causes vasoconstriction, stimulates thirst, and the release of aldosterone to promote sodium reabsorption (Atlas, 2007; Thornton, 2010). It has also been proposed that sympathetic nerve stimulation in response to exercise causes vasoconstriction of the arteries leading to the kidneys (Perrier et al., 2015); all of which, result in the excretion of smaller amounts of darker and more concentrated urine (Perrier et al., 2015; Thornton, 2010). The American College of Sports Medicine define dehydration as a $U_{SG} \geq 1.020$ and/or $U_{Osm} \geq 700$ mOsmol/kg (Sawka et al., 2007). However, urinary markers often correlate poorly with "gold standards" such as P_{Osm} and fail to reliably track documented changes in body mass (Kovacs et al., 1999; Popowski et al., 2001; Walsh et al., 2004a). For instance, dehydration induced by exercise and heat stress evoked a significant increase in U_{Osm} at 2.1% BML compared to pre-exercise values, although there was no significant change in U_{Osm} between 2.1% and 3% BML (Walsh et al., 2004a). Similarly, another study reported no significant correlation between urinary markers and P_{Osm} in collegiate athletes who were instructed to decrease body mass by 3% through controlled dehydration (Sommerfield et al., 2016). Such discrepancies may be the result of inconsistent sampling methodologies as urine concentration measurements are subject to timing and uniformity and can also be confounded by diet (Zubac et al., 2018).

Saliva has also been analysed as a potential marker of hydration status due to its ease of collection and a composition similar to extracellular fluid (Martinez, 1990; Walsh et al., 2004b). Several characteristics of saliva have been found to be related to dehydration, including decreased salivary flow rate (S_{FR}; Bishop et al., 2000; Walsh et al., 1999; 2004b), increased saliva total protein concentration (S_{PC}; Walsh et al., 2004b), and increased saliva osmolality (S_{Osm}; Smith et al., 2012; Walsh et al., 2004b). A role for hypohydration has been proposed together with an increase in sympathetic nervous system activity that causes vasoconstriction of blood vessels to the salivary glands leading to the secretion of smaller amounts of more concentrated saliva (Walsh et al., 2004b). During progressive acute dehydration, S_{Osm} and S_{PC} have been shown to correlate strongly with percent BML (Smith et al., 2012; Walsh et al., 2004b). Indeed, S_{Osm} and S_{PC} increased from 50 ± 11 mOsmol/kg and 0.47 ± 0.19 mg/ml, respectively, to 105 ± 41 mOsmol/kg and 1.80 ± 0.82 mg/ml at 3% BML (Walsh et al., 2004b). Furthermore, both S_{Osm} and S_{PC} have been shown to correlate strongly with P_{Osm} (Walsh et al., 2004a). Nonetheless, large inter-individual variation in saliva parameters has been observed, particularly saliva flow rate as a result of inconsistent sample collection practices, sample collection following food or fluid intake, and differences in the rate and/or speed of breathing leading to dehydration of fluid in the mouth (Cheuvront et al., 2010; Ely et al., 2011; Walsh et al., 2004b).

Semi-nude body mass, measured nude and with skin towelled dry before and after exercise, can also indicate a state of dehydration (Cheuvront and Kenefick, 2014). Indeed a BML of $\geq$2% generally marks the threshold for implications on physical and cognitive performance (Sawka et al., 2007). However, BML is only a proxy for water loss, as some mass loss occurs due to respiratory water loss and loss due to substrate metabolism (Hoffman et al., 2018; Nuccio et al., 2017). Furthermore, over longer periods, body mass can be influenced by food and fluid intake, metabolic water formation, faecal losses, and urine production, thus limiting this technique for assessment of hydration (Maughan et al., 2007). As no single hydration index has shown to be valid in all circumstances, the use of at least two markers is recommended when serial measurements are made (i.e. BML and U_{Osm} or BML and P_{Osm}; Cheuvront et al., 2014; Munoz et al., 2013; Sawka et al., 2007).

2.4 Thermoregulation, cardiovascular responses and performance

Dehydration induced by exercise and heat stress evokes substantial physiologic strain due to a reduction in plasma volume (hypovolemia) and subsequent increase in P_{Osm} (hyperosmolality) as sweat is hypotonic relative to body fluids (Baker et al., 2016; Cramer and Jay, 2016; Kenefick, 2018). This state of hyperosmotic hypovolemia impairs the body’s ability to transfer heat from contracting muscles to the skin surface where heat can be dissipated to the environment (Gonzalez-Alonso et al., 1998; Sawka et al., 2007). Furthermore, sweat production is reduced, thus limiting evaporative heat loss which accounts for 80% of heat loss in a hot-dry environment (Logan-Sprenger, 2019). The extent of dehydration seems to determine the thermal and cardiovascular impact with core temperature rising by 0.15-0.2°C for every 1% of body mass loss (Montain and Coyle, 1992). Furthermore, a decrease in stroke volume due to hypovolemia provokes a compensatory increase in heart rate to maintain a given cardiac output leading to severe cardiovascular strain (Gonzalez-Alonso et al., 2000). Thus, exercise performance that is dependent upon the cardiovascular and thermoregulatory systems, such as aerobic exercise in the heat, can be substantially impaired by dehydration (see reports by Campa et al., 2020; and Cheuvront et al., 2010 for further information regarding mechanisms of performance impairment with heat stress and dehydration).

There is a considerable body of work showing that the threshold of ≥2% BML impairs athletic performance across a range of exercise modalities and durations (Cheuvront and Kenefick, 2014; Holland et al., 2017; Sawka et al., 2007), particularly in hot environments. Furthermore, a loss of ≥2% body mass has been shown to impair attention, memory and psychomotor tasks (Adan, 2012; Ganio et al., 2011). On the other hand, there are concerns around gains in body mass ≥2% as a result of voluntary hyperhydration (Hew-Butler et al., 2017; Noakes et al., 2005). The ingestion of excessive amounts of hypotonic fluids can cause hyponatremia (serum Na concentration <130 mmol/L), a condition associated with serious health consequences including seizures, coma and death (Hew-Butler et al., 2017; Noakes et al., 2005). Thus, a number of factors must be considered when designing fluid replacement strategies such as the intensity and duration of the exercise task, ambient temperature and humidity, the characteristics of the individual (i.e. fitness, acclimatization and hydration status) and their exercise goals (Hew-Butler et al., 2017; Kenefick, 2018; Noakes et al., 2005).

2.5 Traditional sports drinks

Sports drinks are available in a range of flavours with water, carbohydrate (CHO) and electrolytes (sodium, chloride, and potassium) being the main ingredients (Evans et al., 2017; Shirreffs, 2009). Generally, plain water is sufficient for exercise lasting <1 h; however, when exercise is prolonged, especially in the heat, the provision of CHO and electrolytes becomes important (Murray and Stofan, 2001; Rodriguez et al., 2009; Sawka et al., 2007). During prolonged exercise it is recommended for CHO to be ingested at a rate of 30-60 g/h to maintain blood glucose concentrations and enhance carbohydrate oxidation to delay fatigue (Sawka et al., 2007; Temesi et al., 2011). This can be achieved by ingesting fluids containing 4-8% CHO at a rate of 600-1200 ml/h (Sawka et al., 2007; see reports by Cole et al., 2018; and Dumke et al., 2007 for further information regarding mechanisms behind delayed fatigue through carbohydrate supplementation). Most traditional sports drinks contain around 6-9% CHO; at higher CHO concentrations (≥10%), the rate of gastric emptying is slowed proportionally, thus compromising fluid absorption and water availability from the small intestine (Desbrow et al., 2014; Kwiatek et al., 2009). Studies in humans have shown that water absorption from the small bowel is impaired when luminal glucose concentration is higher than 80 mmol/L (Rolston et al., 1990). There is also a high risk of gastrointestinal distress associated with consumption of higher CHO concentrations (>10%) during exercise (Evans et al., 2009; Tsintzas et al., 1995). For these reasons, flavoured-water drinks with reduced energy (1-3% CHO) content may be a preferred hydration choice for many endurance athletes (Ali et al., 2011; Romijn et al., 1993).

Most sports drinks contain low levels of electrolytes, particularly sodium (Na) to help replace losses through sweat (Evans et al., 2017; Leshem et al., 1999). Sodium is a major cation in extracellular fluid (ECF) involved in maintaining cellular homeostasis and regulating fluid and electrolyte balance. Sodium also has an important role in the excitability of muscle and nerve cells and stimulates thirst by keeping plasma osmolality (Evans et al., 2017; Meyer et al., 1992). Sodium is usually added as sodium chloride (NaCl), in concentrations ranging from 20-25 mmol/L (1.2-1.5 g/L; Noakes et al., 1985). Despite substantial variation in individual sweat and Na loss, the electrolyte portion of sports drinks is generally equal to or lower than those of sweat (Meyer et al., 1992). One study involving a large population of athletes determined fluid and Na losses through sweat to range from 0.3-5.7 L/h and 18.2-70.8 mmol/L, respectively (Baker et al., 2016). These numbers suggest that the Na content of traditional sports drinks is insufficient to restore electrolyte balance for athletes with large sweat losses (Evans et al., 2017).

2.6 Oral rehydration solutions

Oral rehydration solutions (ORS), intended for the treatment of diarrhea-induced dehydration, have been shown to be effective in maintaining P_{Osm} and preserving physiological function during exercise (Schleh and Dumke, 2018). In order to replace the large amounts of electrolytes that can be lost through sweat and diarrhea, ORS have a relatively low CHO (2-3%) and high Na (30-90 mmol/L; 1.8-2.0 g/L) content consistent with the need to ensure rapid gastric emptying and intestinal absorption to maintain positive fluid balance (Evans et al., 2017; Murray and Stofan, 2000; Schleh and Dumke, 2018). Clinical trials using ORS clearly show that hypotonic solutions with no more than 13.3 g/L glucose achieve faster and more effective rehydration in children and adults with acute diarrhea than either water alone or ORS with higher glucose concentrations (Binder et al., 2014). Several studies have systematically investigated the effect of the Na concentrations from rehydration drinks on the restoration of fluid balance (Maughan and Leiper, 1995; Merson et al., 2008; Shirreffs and Maughan, 1998), clearly demonstrating that urine output decreases as drink Na concentration increases. It appears that the addition of Na at a concentration equal to or greater than that of the sweat lost is required to maintain positive fluid balance (Evans et al., 2017). However, ORS often receive poor palatability ratings due to their high intensity of saltiness and relatively low level of sweetness (Murray and Stofan, 2000).

2.7 Key drivers of voluntary fluid intake

In order to address voluntary dehydration and guide more effective hydration interventions, the key drivers of voluntary fluid intake have been investigated. Those identified include thirst perception; beverage palatability and sensory properties such as temperature, flavour and carbonation, as well as social, cultural and psychological factors (Passe et al., 2000; 2009).

2.8 Thirst perception

Thirst is a key psychological and physiological driver of fluid intake in response to a body water deficit (Cheuvront et al., 2013; Greenleaf, 1992; McKinley and Johnson, 2004). In most research studies, thirst is treated as a sensory experience and measured using visual analogue scales (VAS; Passe, 2000). The physiological onset of thirst, however, is stimulated by fluid losses corresponding to a 1-2% loss of body mass and a subsequent 2% rise in plasma osmolality (~5 mmol/kg; Cheuvront et al., 2013; Hughes et al., 2018). Threshold increases in plasma osmolality (≥2%) triggers a number of compensatory responses including increases in arginine vasopressin (AVP) secretion, renal water conservation and thirst (James et al., 2019). While there is some evidence to support an association between perceived thirst and voluntary fluid intake (Appleton, 2005; Westerp-Platenga et al., 1997; Wilk and Bar-Or, 1996), exercise seems to reduce the sensitivity and reliability of the thirst response to maintain euhydration (Kenefick, 2018).

In order to reduce the risk of exertional hyponatremia, some recommendations advocate for using thirst to guide hydration practices (Hew-Butler et al., 2015). Alternatively, recommendations by the American College of Sports Medicine (Rodriguez et al., 2009; Hew-Butler et al., 2015) and the National Athletic Trainers' Association (McDermott et al., 2017) encourage individualised replacement of fluid losses to prevent dehydration-mediated loss of more than 2% of body mass during exercise. As a result, there are two schools of thought related to guidelines for fluid intake during exercise, namely 'programmed drinking' (i.e. the use of a pre-established and individualised drinking plan) and 'thirst-driven drinking' or '*ad libitum* drinking' (Kenefick, 2018).

The perception of thirst is often not closely linked to the physiological need for fluid, particularly during prolonged exercise. Athletes typically lose 1-2% of their body mass during exercise despite fluids being freely available (Baker et al., 2016; Passe et al., 2007). This phenomenon, known as 'voluntary dehydration', was identified in early studies when men marching in the desert did not report a sensation of thirst until they had incurred a water deficit of about 2% of body mass (Adolph et al., 1947). Furthermore, premature alleviation of thirst following exercise has been attributed to oropharyngeal cues which trigger thirst satiation before volume is fully restored (Brunstrom et al., 2000; Figaro and Mack, 1997; Greenleaf and Sargent 1965). More recently, an analysis of group means from 14 marathon studies conducted in a range of environments (10-28°C), with runners of a wide range of abilities, showed that *ad libitum* drinking commonly led to excessive dehydration (>2% BML; Cheuvront et al., 2007). Similarly, a group of cyclists practicing prescribed drinking versus *ad libitum* drinking during a 30 km cycle in the heat, dehydrated by 0.5% to 1.8% of their body weight, respectively (Bardis et al., 2017). Thus, thirst appears to be an unreliable stimulus to fluid replacement during prolonged exercise and should not be used solely to guide hydration practices.

2.9 Beverage palatability

Beverage palatability is a key determinant of drinking behaviour with enhanced palatability contributing to significantly greater fluid intake (Maughan et al., 1996; Passe et al., 2000). A summary of the literature investigating the effects of exercise on sensory perception and hedonic response is presented in Table 2.1. Studies commonly used a 9-point hedonic scale to evaluate palatability (Hubbard et al., 1984; Passe et al., 2000; Yeomans, 1998); this acknowledges the contribution of sensory characteristics such as temperature, taste/flavour, and mouthfeel to form an overall hedonic experience (Passe et al., 2000). Indeed, temperature has been shown to be important, with cooler beverages (≤15°C) consistently associated with higher palatability ratings and greater voluntary fluid intake (Hubbard et al., 1984; Szlyk et al., 1987, 1989). Perhaps this preference is further influenced by their ability to help reduce body core body temperature during exercise in the heat (Burdon et al., 2012).

Throughout a 14.5 km simulated desert walk, fluid intake was found to increase by approximately 50% when water was chilled (15°C), 40% with the addition of cherry-flavouring, and 80% when water was both chilled and flavoured (Hubbard et al., 1984). These results suggest that both the cooling and flavouring effects on voluntary intake are additive. The effects of flavour on voluntary intake have been examined in a group of male children who received plain water, grape-flavoured water and a grape-flavoured sports drink *ad libitum* during cycling exercise in the heat (Wilk and Bar, 1996). Voluntary intake of the flavoured sports drink and flavoured water was significantly greater than voluntary intake of plain water (Wilk and Bar-Or, 1996). The addition of flavour was also found to be important among a group of triathletes and runners with the most liked flavour of sports drink consumed in the greatest quantities throughout exercise and rest (Passe et al., 2000).

In contrast, several studies have found no differences in voluntary intake regardless of CHO content and/or flavour (Wilmore et al., 1998; Wong and Sun, 2014). For instance, there were no significant differences in sensory ratings between a 6% CHO and a 8% CHO sports drink among a group of triathletes and runners despite clear formulation differences between the two (Wilmore et al., 1998). Furthermore, liking of a particular flavour did not affect voluntary intake among a group of exercising male children (Wong and Sun, 2014); in fact, all children consumed sufficient fluid to replace the water that was lost. There are a number of methodological differences which could account for these inconsistencies, however. Firstly, the number, age and training level of participants varied greatly between studies; Passe et al. (2000) tested a total of 49 triathletes and runners, whereas 15 children were assessed in Wilmore et al. (1998) and 14 children in Wong and Sun (2014). Furthermore, while Passe et al. (2000) varied the drink flavour while controlling the CHO and electrolyte content of test drinks, Wilmore et al. (1998) varied the CHO, electrolyte and vitamin content, thereby making it difficult to isolate the impact of flavour liking on voluntary intake.

Table 2.1. Summary of literature investigating the effects of exercise on sensory perception and hedonic response

		Exercise/Dehydration protocol				
Author(s)	**Participants**	**Type**	**Length/ Distance**	**Intensity/ Environment**	**Sensory/hedonic measures**	**Outcome(s)**
Ali et al. (2011)	14 M, recreational runners	Treadmill run	60 min	70% HR_{max}, 18-22°C, 55-70% RH	VAS: intensity of sweetness, saltiness, thirst-quenching ability, and overall liking of 4 CHO-e beverages varying in osmolality, CHO and electrolyte content before, during and after exercise.	BML 0.8-1.1% following exercise. ↑ sweetness intensity for all energy-containing drinks during exercise relative to pre- and post-exercise. ↑ sweetness ratings for the low-CHO high-electrolyte drink but ↔ for other beverages. ↓ saltiness, ↑ sweetness, ↑ thirst-quenching ability and ↑ overall liking with duration of exercise for all drinks.
Appleton (2005)	81 regular exercisers (n=40 achieving high fluid loss – Group H; n=41 achieving low fluid loss – Group L)	Unspecified	60 min	Moderate/high	VAS: sweetness, flavour strength, pleasantness, overall liking of 7 fluids varying in content of energy, electrolytes, and osmolality before and after exercise.	↑ pleasantness of all fluids especially for fluids of lowest osmolality with effects ↑ for Group H compared to L. ↑perceived sweetness intensity of fluids following exercise
Bardis et al. (2017)	10 M, elite heat-acclimatized endurance cyclists	Cycling	30 km	Moderate-high, 31.6°C	VAS: perceived thirst and stomach fullness after each 5-km exercise bout.	↑ BML (-1.8%) after AD trial compared to after PD (-0.5%) trial. ↔ thirst initially ↑ thirst, gastrointestinal, mean skin, and mean body temperature in AD compared to PD trial after final exercise bout.
Crystal et al. (1995)	16 F competitive swimmers (14-17 h exercise/week) 28 F non-athletes (0-7 h exercise/week)				VAS: fattiness, sweetness, overall liking of 16 dairy stimuli varying in fat and sugar content.	↑ perceived intensity of sweetness of high-sucrose dairy beverages in competitive athletes compared to non-exercising controls. Competitive athletes also displayed an apparent aversion for higher sucrose beverages which was particularly pronounced for the ↑ fat beverages.
Figaro and Mack (1997)	6 healthy adults	Cycling	120 min	Moderate, 38°C, <30% RH	VAS: perceived thirst, stomach distension, dryness of mouth before and after exercise.	After exercise: ↑ thirst, ↑ P_{osm}, ↑ $[AVP]_p$, ↓ PV, ↑ BML (2.8-3%).

						During RH: ↓ thirst at 5 min even when fluid was extracted from stomach. ↔ P_{osm}, $[AVP]_p$ or PV until further 25 min.
Horio and Kawamura (1998)	58 healthy, untrained students	Cycling	30 min	50% $\dot{V}O_2$ max, 26°C, 57% RH	9-point hedonic scale: overall liking of pure taste solutions containing sucrose, NaCl, citric acid, caffeine or monosodium glutamate before and after exercise. Triangle Test: detection threshold of 5 basic tastes.	↑ preference scale values for sucrose and citric acid after exercise. ↔ values for NaCl, caffeine and MSG
Horio (2004)	44 healthy, untrained students	Cycling	30 min	50% $\dot{V}O_2$ max, 26°C, 57% RH	7-point hedonic scale: pleasantness of test solutions containing various concentrations of sucrose, glucose, stevioside, sorbitol, erythritol, and saccharin before and after exercise.	↑ preference ratings for sucrose, glucose, stevioside, D-sorbitol, and erythritol after exercise. ↔ ratings for saccharin.
Hubbard et al. (1984)	29 M, healthy adults	Treadmill walk	3 x 30-min	40°C dry bulb/26°C wet bulb	9-point hedonic scale: Liking of 3 beverages (tap water, iodine-treated tap water, iodine-treated cherry-flavoured tap water) at 40°C and 15°C before and after exercise. Voluntary fluid intake during exercise.	↓ intake of warm drinks resulting in BML 2.8 and 3.2%. ↑ liking and intake of cold drinks and ↑ flavoured drinks. ↑↑ intake when drinks were both cooled and flavoured.
Leshem et al. (1999)	21 M, students (n=21 regular "exercisers, n=21 non-exercising "controls")	Routine exercise	60 min	~22°C	Preferred concentration of salt added to tomato soup and sugar added to tea was assessed before, immediately after exercise and the next morning.	Immediately after exercise ↑ NaCl added to soup but ↔ sugar added to tea. Exercisers added ↓ sugar to tea than controls at both baseline and immediately after exercise. The next morning, both preferred NaCl and sugar concentrations ↑ in exercisers.
Narukawa et al. (2009)	35 runners	Half marathon	21 km	~20°C, 40% RH	Triangle Test: detection and difference thresholds of sucrose in solution at 6 different	↑ sweetness perception following exercise coincided with ↑ in degree of physical fatigue.

					concentrations before and after exercise. Degree of physical fatigue on scale 1-10.	
Narukawa et al. (2010)	13 runners	Mountain hike	36 km		VAS: sweetness of sucrose solutions at 2 different concentrations (100 mmol/L and 300 mmol/L) assessed at 4 points (0, 16, 25, and 36km) throughout exercise. 5-point hedonic scale: palatability of sucrose solutions throughout exercise. Degree of physical fatigue on scale 1-10.	↔ taste intensity following exercise. Although the pattern of change in palatability differed between the 2 concentrations – ↑ palatability of the 100 mmol/L solution until the 25 km point, and ↓ back to baseline at 36 km; whereas palatability of the 300 mmol/L ↑ through the final 36 km evaluation with ↑ in degree of physical fatigue.
Passe et al. (2000)	49 triathletes and runners	Aerobic circuit	180 min	65-75% HR$_{max}$, 24-27°C, 26-48% RH	9-point hedonic scale: acceptability of 10 commercially available flavours of a CHO-e drink and water (W) before exercise to determine most-liked (M) and least-liked (L) flavour. VAS: sweetness, off-flavour, and overall flavour after 90 min and 180 min exercise. Voluntary fluid intake every 15 min throughout exercise.	↑ acceptability of M compared to W, and ↑ acceptability of W compared to L at rest. ↑ intake of M compared to L and W throughout exercise. After 90 and 180 min of exercise, ↓ acceptability of W compared to both M and L. ↑ acceptability of L from sedentary to exercise conditions. ↑ perceived intensity of sweetness between sedentary and exercise conditions for both M and L.
Passe et al. (2009)	55 triathletes and runners	Aerobic circuit	120 min	70-75% HR$_{max}$, 21°C, 36% RH	VAS: sweetness, saltiness, bitterness, sourness, and flavour strength of 5 flavoured 6% CHO drinks varying in Na concentration (0, 18, 30, 40, 60 mmol/L) at rest, before, during, and after exercise.	BML 1.14% after exercise. ↔ perception of saltiness intensity with exercise, although ↑ acceptance of highest Na concentration (60 mmol/L) while in exercise context shown by ↑ in overall acceptance and liking of saltiness with exercise compared to sedentary condition.

					9-point hedonic scale: liking of sweetness, saltiness, bitterness, sourness, and overall liking.	Liking of saltiness, but not thirst, was related to fluid intake.
Takamata et al. (1994)	7 healthy adults	Cycling	8 x 30-min	Light, 35°C, 30% RH	VAS: thirst, salt intensity, and palatability to 10 different NaCl solutions (0-1 mol/L NaCl) throughout a 23 h RH period.	After exercise: ↑ P_{osm}, ↑ plasma Na^+ concentration, ↑ $[AVP]_p$, ↓ PV, and ↑ thirst ratings accompanied by ↑ palatability to H_2O. At and after 3 h into RH period: ↑ palatability ratings to 1 mol/L NaCl. At 17 and 23 h into RH period: ↑ palatability to hypertonic NaCl solutions (≥0.3 mol/L), and ↑ thirst ratings.
Wald and Leshem (2003)	80 student exercisers	Aerobics or basketball	90 min	96.1 ± 5.6% HR_{max}	VAS: tastiness, familiarity, flavour intensity of an unfamiliar root beer drink consumed with an untasted salt capsule varying in NaCl level (0, 200, 400, 600 mg) before and after exercise.	↑ conditioned flavour preference for drinks paired with 200 mg and 400 mg of NaCl in those who lost greatest amount of sweat. Flavour aversion observed at higher NaCl level (600 mg) specially in those who lost the least sweat.
Westerp-Platenga et al. (1997)	30 M, adults	Cycling	120 min	60% W_{max},	VAS: taste intensity of 5 concentrations of pure sweet, bitter, salt, and sour solutions, a mixture of these, and a CHO-e solution before and after exercise/sauna.	After both exercise and sauna, ↑BML (3 ± 0.5%), ↑ thirst, ↑ fluid intake and ↑ perception of sweet at relatively low concentrations compared to after rest. Only after exercise, ↑ perception of bitterness at a low concentration compared to after rest.
		Sauna	120 min	80°C		
Wilk and Bar-Or (1996)	12 M, prepubertal exercisers aged 9-12 years	Cycling	4 x 20-min exercise + 25-min rest	50% $\dot{V}O_2$ max, 35°C, 45-50% RH	VAS: sweetness, saltiness, sourness of 3 beverages: unflavoured water (W), grape-flavoured water (FW), and flavoured CHO-e sports drink (CHO-e). VAS: thirst before, during and after exercise.	↑ intake of both FW and CHO-e compared to W. ↑ intake of CHO-e than fluid losses. ↑ BML with W (0.65 % BML). ↔ perception between FW and CHO-e for any taste components. ↔ ratings of saltiness between the 3 drinks.

					5-point intensity scale: stomach fullness before, during and after exercise. Voluntary fluid intake during exercise.	
Wilmore et al. (1988)	15 M, triathletes and runners	Treadmill run	90 min	60% $\dot{V}O_2$ max, 30°C, 50% RH	VAS: sweetness, saltiness, tartness, and overall liking of 2 sports drinks (6% and 8% CHO) and water (W) before and after exercise. Voluntary fluid intake during exercise.	↑ intake of the 2 sports drinks compared to W during recovery. ↔ intake during exercise across the 3 beverages. ↑ intake of most-liked fluid during exercise.
Wong and Sun (2014)	14 prepubertal Chinese boys and girls aged 9-11 years	Walk	4 x 20-min	50% $\dot{V}O_2$ max, 30°C, 70% RH	VAS: sweetness, saltiness, sourness of 4 beverages: plain water (W), grape- flavoured water (GF), orange-flavoured water (OF), and lemon-flavored water (LF) prior to exercise. 5-point hedonic scale: overall liking of the 4 beverages prior to exercise. Voluntary fluid intake during exercise.	↔ intake regardless of drink flavour and ↔ perceived thirst. All children consumed sufficient fluid to replace that lost.

VAS – Visual analogue scale; BML – body mass loss; RH – rehydration; DH – dehydration; AD – *ad libitum* drinking; PD – prescribed/programmed drinking; P_{osm} – plasma osmolality; $[AVP]_p$ – plasma arginine vasopressin; PV – plasma osmolality; W_{max} – maximal work load; M – male; F – female; h – hours; HR – heart rate; HR_{max} – age-predicted maximum heart rate; $\dot{V}O_2$ max – maximal oxygen uptake; CHO – carbohydrate; CHO-e – carbohydrate-electrolyte drink (sports drink); RH – relative humidity; ↑ - increase; ↓ - decrease; ↔ - no change.

2.10 Theory of physiological usefulness

Physical exercise provokes a number of short-term physiological changes including dehydration, electrolyte loss, and depletion of glycogen stores (Maughan et al., 2007). Furthermore, exercise appears to elicit a hedonic shift whereby a beverage that is least liked at rest can progressively increase in pleasantness throughout exercise (Ali et al., 2011; Passe et al., 2000). Although the exact physiological mechanism is unknown, there appears to be a relationship between perceived pleasantness and physiological usefulness (Appleton, 2005; Cabanac, 1971). According to Cabanac (1971) a stimulus “can feel pleasant or unpleasant depending upon its usefulness as determined by internal signals” [p. 1103]. This theory of “physiological usefulness” proposes that sensory perception and hedonic response to food and beverages depend not only on the characteristics of the item, but also on the physiological and psychological state of the consumer (Appleton, 2005). Thus, exercise may be expected to increase overall liking of fluids with a high content of water (low osmolality), electrolytes (e.g. NaCl; saltiness), and/or CHO (sweetness).

2.11 Perception of fluid osmolality

Excessive sweat loss during exercise is accompanied by a substantial increase in water requirements (Appleton, 2005). Thus, in relation to the physiological usefulness theory, food and fluid items with a high-water content (low osmolality) are expected to increase in perceived pleasantness (Appleton, 2005). Sensory perception of fluids varying in osmolality, electrolyte and energy content were evaluated among a group of regular exercisers after exercise-induced fluid loss compared to before fluid loss (Appleton, 2005). Pleasantness ratings were found to increase for all drinks from pre- to post-exercise, especially those with low osmolality and energy content (1.7% and 3.4% CHO; Appleton, 2005). Furthermore, these effects were most marked in participants exhibiting high fluid loss (1% BML) compared to those exhibiting low fluid loss (0.4% BML); thus, confirming a link between fluid requirement and preference for low osmolality fluids (Appleton, 2005). However, the method and intensity of exercise in this study was not controlled, thus, impacting the reliability and validity of these results (Appleton, 2005).

A similar study found no significant differences in overall liking between low- and high-osmolality drinks throughout 60 min of treadmill running, with an increase in overall liking across all beverages during and immediately after exercise (Ali et al., 2011). Perhaps this lack of significance actually indicates the importance of replacing lost fluid over the replenishment of nutrients or electrolytes. One study observed an increase in perceived pleasantness of lower osmolality fluids (<300 mmol/L NaCl) immediately after 8 x 30-min bouts of cycling exercise in the heat (Takamata et al., 1994). In contrast, another study found no significant differences in voluntary intake between a range of flavoured beverages and plain water during cycling exercise in the heat (Wong and Sun, 2014). It was also noted in this study, however, that there were no significant changes in P_{Osm} (Wong and Sun, 2014); thus, it is possible that exercise was of too low an intensity to elicit a compensatory preference for water over a flavoured, higher-osmolality beverage. Exercise duration, intensity and extent of fluid loss seems to influence sensory perception and the overall hedonic response.

2.12 Perception of sweetness

An increase in liking of sweetness has been related to the physiological role of CHO in providing energy and replenishing the body's limited glycogen stores (Horio and Kawamura, 1998; Horio, 2004; Narukawa et al., 2009). Two studies evaluated sensory responses to various test solutions following 30 min of moderate cycling exercise (Horio and Kawamura, 1998; Horio, 2004). One study reported an increase in preference ratings for solutions containing sucrose and citric acid after exercise, while ratings for NaCl, caffeine and monosodium glutamate (MSG) were unchanged (Horio and Kawamura, 1998). The other study observed an increase in preference ratings for solutions containing sucrose, glucose, stevioside, D-sorbitol, and erythritol, but no change for artificial sweetener saccharin (Horio, 2004). Since saccharin provides a sense of sweetness without the energy, it appears that it may be the actual CHO-sweetness that triggers an increase in preference due to its physiological usefulness (Ali et al., 2011; Horio and Kawamura, 1998). Nonetheless, pleasantness ratings for glycosides and sugar alcohols also increased even though they provide little to no metabolic energy (Horio, 2004). Perhaps these natural sweeteners are similar enough in structure to trigger the same receptors on the tongue as sucrose (Horio, 2004). Thus, the lack of effect for saccharin can be explained by its synthetic manufacture and the initial low quality (off-notes or a different sweetness onset) compared to the other sweeteners (Horio, 2004).

An increase in sweetness perception has been observed in a group of runners following a half marathon (Narukawa et al., 2009). A range of 6 different sucrose concentrations were tested before and after exercise. Interestingly, the sucrose detection threshold was significantly lower following exercise, which coincided with an increase in degree of physical fatigue (Narukawa et al., 2009). The authors suggest this behaviour may be related to the need to replenish liver and muscle glycogen stores following exercise (Narukawa et al., 2009). In contrast, no difference in taste intensity was found between a low sucrose (100 mmol/L) and a high sucrose (300 mmol/L) solution following a 36 km mountain hike (Narukawa et al., 2010). It is likely, however, that participants could distinguish between the two concentrations, potentially impacting results. Nonetheless, there was a trend for a change in palatability between the two concentrations – palatability of the 100 mmol/L solution increased steadily until the 25-km point, and decreased back to baseline at 36 km; whereas palatability of the 300 mmol/L solution increased through to the final 36-km evaluation (Narukawa et al., 2010). The authors suggest that, although the lower sucrose concentration was most preferred initially, its CHO content may have become insufficient to meet physiological needs by the time participants reached the 36-km point (Narukawa et al., 2010). Thus, these findings provide further evidence in support of a relationship between exercise duration and/or intensity, CHO requirement, and sweetness perception.

Other studies have also reported an increase in sweetness perception of drinks with increasing exercise duration (Ali et al., 2011; Appleton, 2005; Westerp-Platenga et al., 1997). Throughout 60 min of treadmill running, ratings of sweetness increased for all drinks tested, especially for the low-CHO, high-electrolyte drink (Ali et al., 2011). However, only a weak correlation between sweetness and overall liking was identified (Ali et al., 2011). The authors suggest that perhaps there are other factors contributing to the overall hedonic response; such as saltiness and thirst-quenching ability (Ali et al., 2011), with both of these variables identified as important predictors of overall liking post-exercise (Ali et al., 2011).

2.13 Perception of saltiness

Due to the critical role of Na in fluid balance, nervous signaling and muscle function, the physiological usefulness theory has also been proposed to explain an increase in liking of saltiness throughout exercise (i.e. as the body loses electrolytes in sweat, preference for saltiness in drinks increases; Ali et al., 2011). Sodium is lost in variable amounts through sweat leading to an increase in plasma osmolality; in turn, the hormones of Na conservation including aldosterone and renin increase (Johnson, 2007). It has been speculated that the spontaneous avidity for salt, often referred to as ‘sodium appetite’, is related to changes in the activity of these hormones (Leshem et al., 1999). Indeed, Na depletion in humans following exercise has been shown to produce moderate sensory changes and increases in preferences for salty foods and fluids (Ali et al., 2011).

In order to determine whether salt perception is affected by exercise, a variety of sports drink formulations with NaCl content ranging from 140-421 mg/L (2.4-7.2 mmol/L), were tested among a group of healthy, trained males before, during and after 60 min of treadmill running (Ali et al., 2011). Ratings for saltiness were significantly lower during exercise compared to before exercise, suggesting a reduced oral sensitivity to Na in an exercise context (Ali et al., 2011). In contrast, 120 min of aerobic circuit exercise had no effect on perception of saltiness of solutions varying in Na content (0, 18, 30, 40, and 60 mmol/L) among a group of triathletes and runners (Passe et al., 2009). Participants did however, become more accepting of the highest Na concentration (60 mmol/L; 1.4 g/L) while in an exercise context as reflected by significant increases in overall acceptance and liking of saltiness throughout exercise relative to pre-exercise sedentary conditions (Passe et al., 2009). Furthermore, liking of saltiness, but not thirst, was related to fluid intake, thus providing further support for a role of beverage palatability in driving fluid intake during exercise (Passe et al., 2009).

In contrast, no changes in salt perception threshold or liking of saltiness were found among a group of healthy, untrained students after 30 min of moderate exercise (Horio and Kawamura, 1998). However, overall liking of the lower NaCl concentrations (0.0109 and 0.038 mol/L; 0.64 and 2.22 g/L, respectively) were directionally lower following exercise, and liking of the higher concentrations (0.152 and 0.304 mol/L; 8.88 and 17.8 g/L NaCl, respectively) were directionally higher (Horio and Kawamura, 1998). The shorter exercise duration may explain the lack of statistical significance; longer duration may be necessary to bring on the salt appetite in a statistically significant way (Passe et al., 2009). Nonetheless, the directional results of this study suggest that untrained subjects may also prefer saltier beverages following acute exercise, provided the exercise is long and intense enough to significantly increase Na requirements.

In order to elicit significant increases in fluid and Na requirements, seven healthy volunteers completed 8 x 30-min bouts of cycling exercise in the heat during 7 h of restricted fluid intake and Na deprivation (Takamata et al., 1994). At regular intervals throughout the exercise session and the rehydration protocol, subjective ratings of thirst and palatability to various NaCl solutions were evaluated (Takamata et al., 1994). Plasma osmolality and Na concentration were significantly higher immediately after exercise accompanied by an increase in thirst and palatability ratings for the more hypotonic solutions (<0.3 mol/L NaCl; <17.5 g/L; Takamata et al., 1994). Meanwhile, palatability ratings of the hypertonic (0.3 and 1.0 mol/L NaCl; 17.5 and 58.4 g/L, respectively) solutions immediately after dehydration were unchanged from control levels at rest (Takamata et al., 1994). Nonetheless, 17-23 h into the Na-free rehydration period an increase in palatability of the hypertonic NaCl solutions was observed (Takamata et al., 1994). The authors attribute this increased preference for Na, but not water, to the regulation of fluid balance and osmolality (Takamata et al., 1994). Conversely, others may attribute this delayed increase in palatability to increased appetite after exercise (Lluch et al., 1998; Thompson et al., 1998; Verger et al., 1992) or to beliefs about the need to replace salt, especially since there were no control groups and the sample size was small (n=7; Leshem et al., 1999; Takamata et al., 1994). Furthermore, it is unclear if the delayed effect is Na-specific since there was no control for taste (Leshem et al., 1999; Takamata et al., 1994).

Perception of saltiness has also been investigated following 1 h of routine exercise among healthy, untrained students (Leshem et al., 1999). The preferred amount of NaCl added to tomato soup and sugar added to tea was compared pre- and post-exercise and between a group of student “exercisers” and a group of non-exercising controls (Leshem et al., 1999). Immediately after exercise, the amount of NaCl added to flavour soup increased by approximately 50% compared to pre-exercise baseline NaCl preference and to non-exercising controls (Leshem et al., 1999). Meanwhile, the amount of sugar to flavour tea was unaltered (Leshem et al., 1999). The next morning, concentrations of both salt in soup and sugar in tea were elevated for the “exercisers”. The authors attributed the delayed and non-specific changes in preference to hunger, while the immediate and specific increase in NaCl preference was attributed to the need to replace Na lost through sweat (Leshem et al., 1999). These findings reinforce previous indications that the taste of salt becomes more attractive to humans as physiological requirements increase.

In order to further explore the effects of Na on voluntary fluid intake, various NaCl concentrations were delivered in an untasted capsule form and paired with 100 ml of a novel-tasting drink after 90 min of moderate-high intensity exercise (Wald and Leshem, 2003). While baseline preference was not significantly different across NaCl dose groups, preference change was greatest for participants exhibiting high sweat loss (11.51-19.11 g/kg), followed by medium (9.01-11.50 g/kg) and low (4.57-9.0 g/kg) sweat loss during the 90-min sessions (Wald and Leshem, 2003). In other words, moderate amounts of NaCl (400 mg) may trigger an increase in drink palatability when sweat losses are high because subjects are conditioned to replace water and salt lost through exercise. At higher NaCl levels (600 mg NaCl), however, flavour aversion was observed especially amongst those who sweated little (Wald and Leshem, 2003). Perhaps this is because the amount of Na lost through sweat was not sufficient to greatly reduce plasma Na concentration and elicit a sodium appetite. In summary, these findings suggest that palatability of higher NaCl concentrations will increase in proportion to the amount of Na lost through sweat.

2.14 Psychological factors

Aside from physiological differences in taste perception and acceptance, a major component of the human response after exercise is psychological (Passe et al., 2000). Increases in perceived pleasantness have been shown to correlate highly with beliefs and experience of subsequent beneficial effects (Rogers, 1999; Yeomans et al., 2000). For instance, the “exercisers” in the study by Leshem et al. (1999) (compared to the control group) restricted the amount of sugar added to their tea as they believed that sweet items were ‘fattening’. There were, however, no differences in salt preference among the groups and none of the participants reported avoiding salt (Leshem et al., 1999). Even though this suggests that cognitive beliefs about salt intake do not explain the differences found immediately after exercise, it is possible that beliefs about the importance of salt replacement following severe Na loss through sweat may contribute to the increase in liking of saltiness after exercise (Takamata et al., 1994). In other words, an association between beliefs about the restorative benefit of higher electrolyte beverages and the taste of salt, may influence salt preference in an exercise context.

The overall hedonic response is largely influenced by familiarity which tends to coincide with subject characteristics such as level of athletic training and exposure to sports beverages. For example, Passe et al. (2009) investigated changes in sensory perception in a group of highly trained triathletes and runners who were undoubtably familiar with sports beverages. It is possible that expectation impacted pre-exercise hedonic judgements especially because perception of saltiness remained consistent throughout the study (Passe et al., 2009). Indeed, participants may perceive high-salt beverages as more pleasant pre-exercise because they expect from their past training that this type of solution will aid in their performance and recovery (Passe et al., 2009). Nonetheless, similar changes in sensory hedonics have been observed following exercise in untrained subjects as well, thus reinforcing the role of physiological drivers on perception and voluntary intake (Horio, 2004; Horio and Kawamura, 1998; Leshem et al., 1999; Wald and Leshem, 2003; Wilk and Bar-Or, 1996).

Finally, while the aforementioned studies examined the effect of an acute bout of exercise, it appears that chronic exercise and training status may also affect sensory perception and hedonic response (Crystal et al., 1995; Merry et al. 2010). One study compared taste preference among a group of female competitive swimmers (undertaking 14-17 h exercise per week) with a group of female non-athletes (undertaking 0-7 h exercise per week; Crystal et al., 1995). The competitive athletes perceived high-sucrose dairy beverages as significantly sweeter than non-exercising controls and also displayed an apparent aversion for higher sucrose beverages, which was particularly pronounced for the higher fat beverages (Crystal et al., 1995). Therefore, it seems that chronic exercise may induce greater sensitivity to sweetness and lower liking of high sweetness. Alternatively, it could also be that competitive swimmers’ diets are generally lower in sugar and hence are not familiar with sugary beverages. These findings highlight potential differences associated with training status (i.e. recreational participants versus elite athletes), such as tolerance to hypohydration, dietary and lifestyle factors, as well as potential psychological effects related to the dietary restraint of competitive athletes. All of these factors could impact expectations and hedonic responses which may contribute to the inconsistent results reported across the literature.

2.15 Summary

A range of sports drinks have been designed to promote hydration throughout exercise, however, there is ongoing concern around the adequacy of their nutrient and electrolyte concentrations for prolonged exercise, especially in the heat. The composition of ORS appears to be more suitable for exercise accompanied by excessive sweat losses, although it is unknown whether the palatability of these beverages is sufficient to promote voluntary fluid intake. Results from published studies suggest that acute exercise of a certain threshold intensity affects consistent perceptual and hedonic changes across the population: osmoregulatory thirst, increased palatability of salt, and increased perception and palatability of sweetness. These sensory changes are in line with the theory of physiological usefulness i.e. replacing water, electrolytes and glycogen. However, individual metabolic and psychological variation modulate these effects. Furthermore, it is difficult to compare the results from published studies to make firm conclusions due to differences in subject characteristics (gender, age, athletic ability) as well as the method and extent of dehydration achieved (i.e. exercise type, duration, body mass loss). Nonetheless, this review of literature provides evidence for sensory and hedonic changes during exercise which may be related to physiological usefulness.

CHAPTER 3: Sensory perception of an oral rehydration solution throughout exercise: a randomised, double-blind, placebo-controlled study

This chapter has been formatted for the Journal of Physiology and Behaviour

3.1 Abstract

Aim To assess the palatability of an oral rehydration solution (ORS) at rest and throughout 60 min of moderate intensity exercise in the heat.

Methods Twenty-seven recreationally active participants (n=13 males; n=14 females) completed sensory analysis of an ORS, a traditional sports drink (TS) and a flavoured water placebo (PL) at rest and throughout 60 min (3 x 20-min bouts) of cycling exercise at 70% age-predicted maximum heart rate (HR_{max}) at 30-35°C. Before and every 20 min after exercise, drinks were rated based on liking of sweetness, liking of saltiness, thirst-quenching ability, and overall liking on a 9-point hedonic scale. Hydration status was assessed by changes in semi-nude body mass, urine osmolality (U_{Osm}), urine specific gravity (U_{SG}), urine colour (U_{Col}), saliva osmolality (S_{Osm}), and saliva total protein concentration (S_{PC}).

Results After 60 min of exercise, participants had lost an average of 1.36 ± 0.39 % of body mass and there was an increase in S_{Osm}, S_{PC}, U_{SG} and U_{Col} ($p<0.05$) but no change in U_{Osm} ($p>0.05$). At all time points, liking of sweetness, saltiness, thirst-quenching ability and overall liking was higher for the TS and PL compared to the ORS ($p<0.05$). However, the saltiness liking and thirst-quenching ability of the ORS increased significantly after 60 min of exercise compared to before exercise ($p<0.05$). There was also a significant change in predictors of overall liking with pre-exercise ratings mostly determined by liking of sweetness, saltiness and thirst-quenching ability ($p<0.001$); whereas only liking of saltiness predicted overall liking post-exercise ($R^2=0.751$; $p<0.001$).

Conclusions There appears to be a hedonic shift during exercise in which the perception of saltiness becomes the most important predictor of overall liking. This finding supports the potential use of an ORS as a valuable means of hydration during the latter stages of prolonged and/or intense exercise in the heat.

Keywords: dehydration, electrolytes, palatability, saltiness, sports drinks, thirst

3.2 Introduction

Voluntary dehydration occurs when *ad libitum* fluid intake is insufficient to match sweat losses, leading to a cumulative loss of body water (Baker et al., 2016). Threshold decreases in blood volume (hypovolemia) and increases in plasma osmolality (hyperosmolality) usually stimulate thirst and subsequent fluid intake (Hughes et al., 2018; James et al., 2019). During exercise however, thirst becomes an unreliable stimulus to drinking and is often alleviated before complete rehydration is achieved (Figaro and Mack, 1997; Kenefick, 2018). Indeed, athletes typically lose 1-2% of their body mass during exercise despite fluids being freely available (Baker et al., 2016; Passe et al., 2007). This is problematic as a loss of body mass ≥2% is associated with severe impairments in thermoregulatory, metabolic and cardiovascular functions, often leading to adverse effects on performance and health (Casa et al., 2012; Cheuvront and Kenefick, 2014; Sawka et al., 2015).

Sports drinks are designed to promote hydration before, during and after exercise; they exist in a variety of formulations with water, carbohydrate (CHO; 6-9%) and electrolytes, particularly sodium (Na) being the main ingredients (Rodriguez et al., 2009; Sawka et al., 2007). Carbohydrate is added to provide sweetness and energy; however, the higher the level of CHO, the slower the rate of gastric emptying and higher risk of gastrointestinal distress (Desbrow et al., 2014). In addition, the electrolyte content of traditional sports drinks (22-25 mmol/L Na) appears to be insufficient to match Na losses through sweat for many endurance athletes (Baker et al., 2016). Sodium is an essential electrolyte which functions to maintain plasma osmolality and stimulate thirst (Evans et al., 2017). For these reasons, oral rehydration solutions (ORS) with relatively higher Na (30-90 mmol/L) and lower CHO concentrations (2-3% CHO) may be more effective for promoting hydration, particularly when sweat losses are large (Murray and Stofan, 2000). Due to poor palatability ratings, however, ORS are generally not used in an exercise context (Evans et al., 2017).

Beverage palatability has been identified as a key determinant of voluntary fluid intake with food and fluid items being much more likely to be consumed if they are perceived as being pleasant (Passe et al., 2000; Wilmore et al., 1988). Sensory characteristics such as beverage temperature, carbonation level, and flavour all contribute to the overall hedonic experience, with cool (<15°C; Hubbard et al., 1984), flavoured (Wilk and Bar-Or, 1996), non-carbonated (Passe et al., 1997) beverages found to be most palatable during exercise and thus, consumed in the largest quantities (Passe et al., 2000). However, there appears to be a hedonic shift during exercise in which food and fluid items which are least liked at rest can progressively increase in perceived pleasantness (Passe et al., 2000). Indeed, a number of researchers have related changes in sensory perception to the theory of physiological usefulness (Ali et al., 2011; Cabanac, 1971; Horio and Kawamura, 1998; Leshem, 1999; Takamata et al., 1994).

Prolonged exercise elicits an increase in requirements for water, CHO and electrolytes, thus an increase in pleasantness of various food and fluid items has been attributed to their physiological usefulness (Cabanac, 1971; Passe et al., 2000). For example, an increase in liking of low osmolality fluids immediately after exercise has been attributed to the importance of maintaining body fluid balance (Appleton, 2005). Alternatively, increases in perception and liking of sweetness have been attributed to the role of CHO in energy provision during exercise (Horio, 2004; Horio and Kawamura, 1998). Most importantly, the physiological usefulness theory has been proposed to explain an increase in salt preference or 'Na appetite' with exercise, especially in those exhibiting large sweat losses (Leshem et al., 1999; Passe et al., 2009; Takamata et al., 1994). Compared to traditional sports drinks, ORS have a relatively high intensity of saltiness contributing to poor palatability ratings at rest. However, to date there have been no studies testing the palatability of an ORS throughout exercise in the heat. Sweat and salt losses are exacerbated during exercise in the heat; thus, the aim of this study was to test the palatability of an ORS at rest and at various stages of exercise-induced fluid loss.

3.3 Methods

3.3.1 Participants

Twenty-seven recreationally active participants (n=13 males; n=14 females; mean ± SD; age 25 ± 8.0 years, height 172.2 ± 8.5 cm, body mass 69.8 ± 8.9 kg) volunteered to take part in this study. All participants were recruited on the basis that they were capable of performing 60 min of cycling exercise (3 x 20-min bouts) in a warm room (30-35°C) at a moderate intensity (70-80% HR_{max}) whilst wearing a sweat suit to promote fluid loss. Participants were provided with an information sheet (see Appendix A), and following completion of a health screening questionnaire (see Appendix B), written informed consent was obtained (see Appendix C). Participants were excluded if they had a heart condition, took prescription drugs, experienced chest pain while exercising or any other issues that may prevent them from completing 60 min of moderate intensity exercise in the heat. All protocols and procedures had prior approval by the local human ethics committee (see Appendix D).

3.3.2 Study design

Sensory evaluation was conducted in a single trial, of an ORS, a traditional sports drink, and flavoured water placebo at rest and after each of 3 x 20-min bouts of exercise in a randomised, double blind design. Urine samples were collected before and after the 60 min of exercise, saliva samples, aural temperature and semi-nude body mass were assessed before and after each 20-min exercise bout as outlined in Figure 3.1.

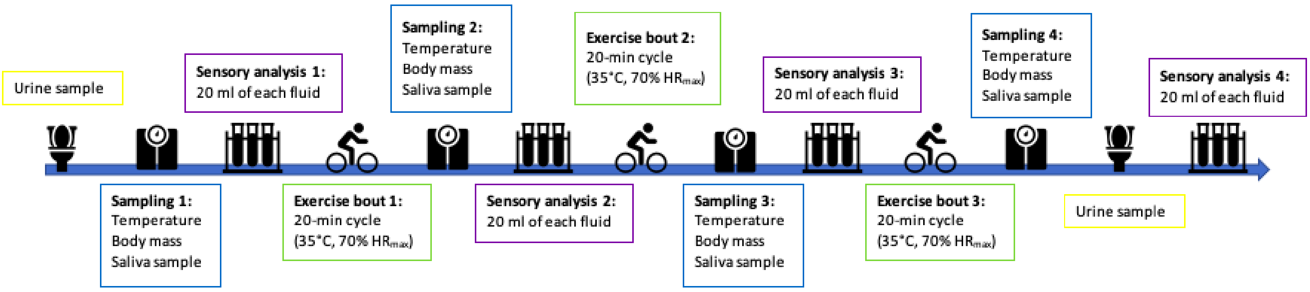


Figure 3.1. Overview of the exercise and sensory protocol.

3.3.3 Drinks

Three drinks of varying osmolality, electrolyte content, and energy content per litre (Table 3.1) were used in this study: an oral rehydration solution (ORS), a beverage formulation based on a traditional sports drink (TS), and a flavoured water placebo (PL). Drinks were prepared in the Food Technology Laboratory, pasteurised and hot filled, then stored in a chiller at 4°C. All drinks were colourless, non-carbonated, with the same concentration of mixed berry flavouring (Symrise Pty, Ltd, Australia) and served as 20-ml samples in clear, plastic containers at room temperature.

Table 3.1. Composition of the drinks used for this study

Ingredients (g/L)		ORS	TS	PL
Carbohydrate	Glucose	13 (72 mmol/L)	0	0
	Sucrose	8 (23.4 mmol/L)	59 (172.4 mmol/L)	0
	Fructose	3 (16.6 mmol/L)	0	0
	Maltodextrin	0	16 (31.7 mmol/L)	0
	Total	24 (2.4%)	75 (7.5%)	0
Electrolytes	Sodium chloride	2.6 (44.5 mmol/L)	0.28 (4.79 mmol/L)	0
	Potassium chloride	2 (26.8 mmol/L)	0	0
	Sodium citrate	2 (7.75 mmol/L)	0	0
	Potassium citrate	1 (3.26 mmol/L)	0.141 (0.46 mmol/L)	0
	Total	7.6	0.421	0
Citric acid		0	0.191	0
Berry flavour		0.8	0.8	0.8

Carbohydrate content (g/L and mmol/L), electrolyte content (g/L and mmol/L), and flavouring (g/L) for each drink: Oral rehydration solution (ORS), placebo (PL), traditional sports drink (TS) as made up for this study.

3.3.4 Familiarisation procedures

At least 24 h prior to the main trial, participants were required to attend the laboratory for a familiarisation session. Height (stadiometer; Surgical and Medical Products, NZ) and body composition (lean body mass and percentage body fat) using bioelectrical impedance analysis (BIA; InBody230 BIA, Biospace Co., Ltd, Korea) were measured. Participants were familiarised with the sensory protocol and methods for collecting urine and saliva samples (see Appendix E). Participants tried on the sweat suit and then performed a short (5 min) incremental cycle to determine the appropriate load equivalent to 70-80% age-predicted HR_{max}.

3.3.5 Main experimental procedures

Participants were required to record a food diary and refrain from caffeine, alcohol and physical activity for at least 24 h prior to the main trial. Upon arrival to the laboratory, samples of urine (midstream) and saliva (using the bud method) were collected (see Appendix E for detailed procedures). Semi-nude body mass and aural temperature were measured, and an initial sensory analysis was performed. Participants completed 3 x 20-min cycling bouts at the predetermined load and speed (70-80% of age-predicted HR max) in a warm room (temperature 35.3 ± 1.4°C, humidity 41 ± 6%) while wearing a sweat suit to promote fluid loss. During exercise, heart rate was recorded every 5 min via short-range telemetry (Polar Eletro S610i, Polar, Kempele, Finland). After each 20-min exercise bout, aural temperature and perceptual scales (ratings of perceived exertion (RPE; Borg, 1982), felt arousal scale (FAS; Svebak and Murgatroyd, 1985), feeling scale (FS; Hardy and Rejeski, 1989), thermal comfort; see Appendix E) were assessed. After removing the sweat suit and towel drying, semi-nude body mass was measured in a private room, a saliva sample was collected, and a sensory analysis was performed. After the final exercise bout, a urine sample was collected. Participants were then able to take a shower and rehydrate with refreshments provided. Urine samples were used to determine osmolality using an osmometer (Astori Technica- Osmotouch 1 osmometer, Poncarale, Italy), urine specific gravity using a refractometer and colour using a colour scale (see Appendix E for detailed procedures). Saliva samples were used to determine osmolality using an osmometer and protein content using the Bradford method (Bradford, 1976; see Appendix E for detailed procedures).

3.3.4 Sensory analysis

Liking of sweetness, saltiness, thirst-quenching ability as well as overall liking for each of the three drinks was evaluated before and after each 20-min exercise bout on a 9-point hedonic scale labelled with anchors appropriate for the sensory property being tested (e.g. “*not at all sweet*” and "*very sweet*" for sweetness; "*not at all salty*" and "*very salty*" for saltiness; "*not at all thirst quenching*" and "*thirst quenching*" for thirst-quenching ability and "*dislike extremely*" to "*like extremely*" for overall liking). In a private room, participants were presented with 20 ml of each fluid in clear, plastic containers and tested in a randomized order according to the software application (iPad, Apple Inc, Cupertino, California; see Appendix E). Participants were required to consume the entire bolus and expectorate using filtered water between each sample. These were weighed afterwards to ensure participants did not also consume the filtered water.

3.3.7 Statistical analyses

Statistical analysis was conducted using IBM SPSS Statistics (Version 24.0. Armonk, NY: IBM Corp). Sensory data was analysed using a two-way analysis of variance (ANOVA) with repeated measures to compare each treatment (ORS, PL, TS) across each time point (0, 20, 40, 60 min of exercise). Mauchly's test of sphericity was used to determine whether the assumption of sphericity was being violated by the data. Where this did occur, the Huynh-Feldt correction was applied. Physiological responses were analysed using a one-way ANOVA for parametric data and Wilcoxon signed rank-tests were performed for non-parametric data. Where a main effect or interaction effect was observed, a pairwise post-hoc analysis using the Holm-Bonferroni adjustment was performed to determine specific differences. Levene's test was used to test the assumption for homogeneity of variance. Relationships between variables were tested using the Pearson's correlation coefficient for parametric data and Spearman's correlation for non-parametric data and interpreted as "weak, r=0.1", "moderate, r=0.3", and "strong, r=0.7" (Mukaka, 2012). Multiple linear regression analyses were conducted to determine the key predictors of overall liking. Cohen's *d* was calculated to measure the magnitude of the findings and interpreted using Cohen's (1988) guidelines for effect sizes as "small, *d*=0.2," "medium, *d*=0.5," and "large, *d*=0.8". Parametric data are presented as mean ± standard deviation (SD), log-transformed data as geometric mean (95% confidence intervals; CI) and non-parametric data as median (25, 75 percentiles). Significance level was p<0.05 for all tests.

3.4 Results

3.4.1 Physiological and perceptual responses to exercise

Physiological responses throughout stages of exercise are reported in Table 3.2. After 60 min of exercise participants achieved a mean (± SD) body mass loss (BML) of 0.94 ± 0.30 kg (1.36 ± 0.39 %). From 0 min to 60 min of exercise, there was an increase in mean (95% CI) saliva osmolality (S_{Osm}) from 84.71 (78.28, 91.66) mOsmol/kg to 112.76 (102.47, 124.10) mOsmol/kg (p<0.001) and saliva total protein concentration (S_{PC}) from 1.27 (1.06, 1.49) mg/ml to 3.17 (2.51, 3.96) mg/ml (p<0.001). There was no change in urine osmolality (U_{Osm}; p=0.102) but urine colour (U_{Col}) and urine specific gravity (U_{SG}) increased from a median (25, 75 percentiles) of 3.0 (1.0, 4.0) to 4.0 (3.0, 7.0; p<0.001) and from 1.014 (1.01, 1.02) to 1.017 (1.014, 1.025), respectively (p=0.001). Heart rate (p<0.001), aural temperature (p=0.008), ratings of perceived exertion (p<0.001), feeling scale (p<0.001), and thermal comfort (p<0.001) all increased throughout the 60 min of exercise. Percent BML showed a moderate positive correlation with S_{PC} (r=0.353, p=0.001) and S_{Osm} (r=0.375, p=0.001). There was also a moderate relationship between percent BML and U_{SG} (r=0.287, p=0.035) and a weak but insignificant relationship between BML and U_{Osm} (r=0.220, p=0.116). Percent BML showed a moderate negative correlation with ratings of saltiness liking (r=-0.316, p=0.004), thirst-quenching ability (r=-0.323, p=0.003) and overall liking of fluids (r=-0.234, p=0.036), but no relationship with sweetness liking (r=-0.183, p=0.102).

Table 3.2. Physiological and perceptual responses throughout exercise

Exercise sample time	0 min	20 min	40 min	60 min
Body mass (kg)†	69.01 ± 8.63	68.81 ± 8.59	68.51 ± 8.54	68.07 ± 8.49
Body mass loss (kg)†	-	0.20 ± 0.11	0.50 ± 0.18	0.94 ± 0.30**
Body mass loss (%)†	-	0.29 ± 0.16	0.72 ± 0.24	1.36 ± 0.39**
Saliva osmolality (mOsmol/kg)⊥	84.71 (78.28, 91.66)	91.15 (85.13, 97.59)	101.35 (94.02, 109.25)	112.76 (102.47, 124.10)**
Saliva proteins (mg/ml)⊥	1.27 (1.06, 1.49)	1.59 (1.27, 1.94)	2.33 (1.90, 2.83)	3.17 (2.51, 3.96)**
Urine specific gravity‡	1.014 (1.01, 1.02)	-	-	1.017 (1.014, 1.025)*
Urine osmolality (mOsmol/kg)‡	516 (227, 876)	-	-	586 (476, 796)
Urine colour‡	3.0 (1.0, 4.0)	-	-	4.0 (3.0, 7.0)**
Aural temperature (°C)†	36.1 ± 0.6	37.4 ± 2.0	38.3 ± 0.4	38.4 ± 0.6*
Heart rate (bpm)†	82.9 ± 19.0	152.4 ± 19.8	160.7 ± 22.8	169.7 ± 20.2**
Ratings of perceived exertion†	-	13.2 ± 1.5	15.6 ± 1.3	17.2 ± 1.3**
Felt arousal scale†	-	3.5 ± 1.0	3.8 ± 1.0	3.7 ± 1.2
Feeling Scale†	-	1.3 ± 1.9	-0.6 ± 1.9	-1.4 ± 1.8**
Thermal comfort†	-	5.1 ± 1.3	6.7 ± 1.1	7.5 ± 1.4**
Work rate (W)†	-	98.2 ± 22.7	97.7 ± 23.7	95.7 ± 23.9

†Values are means ± SD. ⊥Values are geometric means (95% CI). ‡Values are medians (25, 75 percentiles). *Significant change at 60 min (p<0.05). **Significant change at 60 min (p<0.001).

3.4.2 Sensory analyses

Liking of saltiness

Figure 3.2 shows the mean (± SD) ratings of saltiness liking for each drink after each exercise time. At all time points, liking of saltiness differed between drinks (main effect of treatment, p<0.001); liking of saltiness was higher for PL (5.48 ± 1.58, p=0.012, *d*=0.84) and TS (5.91 ± 1.49, p<0.001 *d*=1.09) than ORS (3.93 ± 2.09), while there were no differences between PL and TS (p<0.05, *d*=0.28). There was no main effect of exercise time (p=0.109) and no interaction between drink type and exercise time (p=0.199). There was a significant difference in percent change in saltiness ratings between drinks throughout exercise ($\chi^2(2) = 7.043$, *p*=0.030); median (25, 75 percentiles) percent change in saltiness ratings for the ORS, PL and TS were 20% (0, 100%), 0% (-16.7, 20%), and 0% (-22.2, 20%), respectively. There was a significant increase in liking of saltiness for ORS vs. PL (Z = -2.341, *p*=0.019) and for ORS vs. TS (Z = -2.386, *p*=0.017) but there was no difference between PL and TS (Z = -0.382, *p*=0.702; see Appendix F for supplementary results).

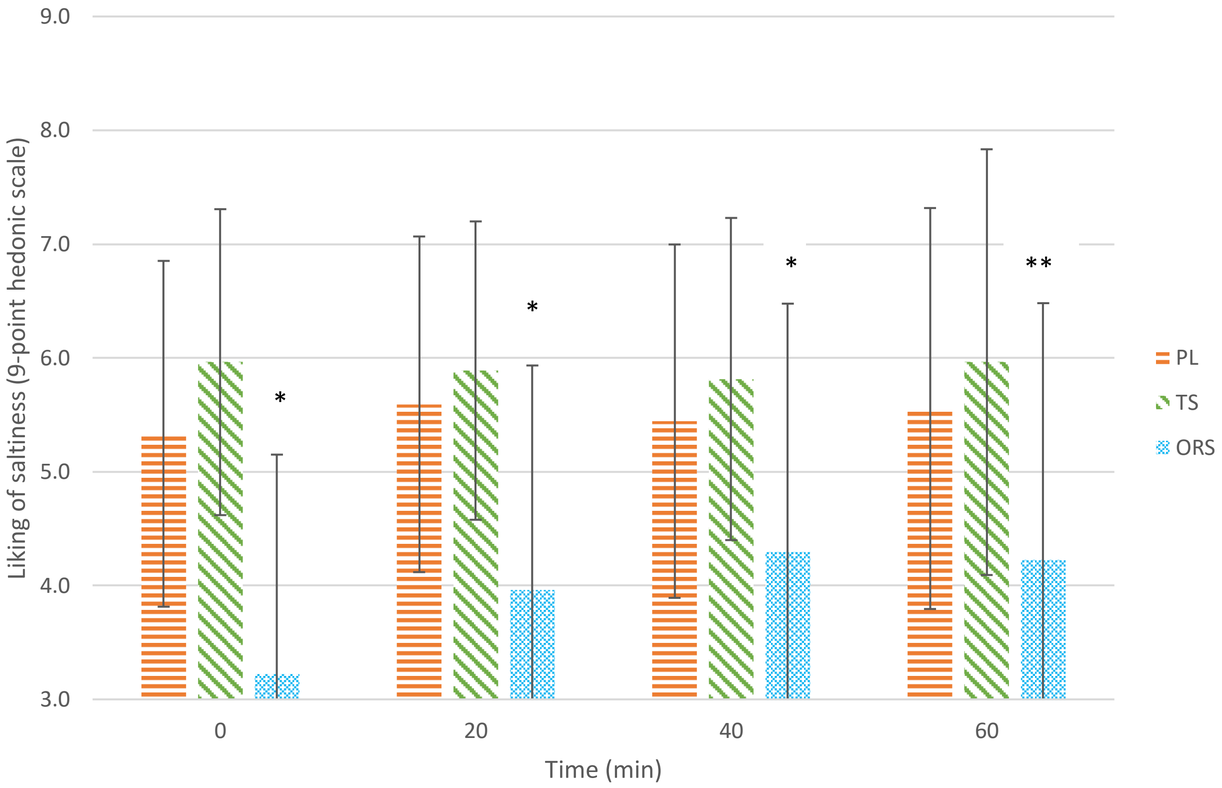


Figure 3.2. Liking of saltiness of drinks: ORS – oral rehydration solution, PL – placebo, TS – traditional sports drink, on a 9-point hedonic scale throughout 60 min of exercise. Values are mean ± SD. *Significant effect of the treatment vs. the placebo (p<0.05). **Significant delta change in ratings compared to 0 min before exercise (p<0.05).

Liking of sweetness

Figure 3.3 shows the mean (± SD) ratings of sweetness liking for each drink after each exercise time. At all time points, liking of sweetness differed between drinks (main effect of treatment, p=0.003); sweetness liking was higher for PL (5.75 ± 1.57, p=0.09, *d*=0.76) and TS (5.96 ± 2.11, p=0.015, *d*=0.76) than ORS (4.53 ± 1.65), while there were no differences between PL and TS (p<0.05, *d*=0.11). There was no main effect of exercise time (p=0.985) and no interaction between drink type and exercise time (p=0.732). There was no difference in percent change in sweetness ratings between drinks throughout exercise ($\chi^2(2) = 3.293$, *p*=0.193); median (25, 75 percentiles) perceived sweetness for the ORS, PL and TS were 0% (-20, 25%), 0% (0, 33.3%), and 0% (-16.7, 28.6%), respectively (see Appendix F for supplementary results).

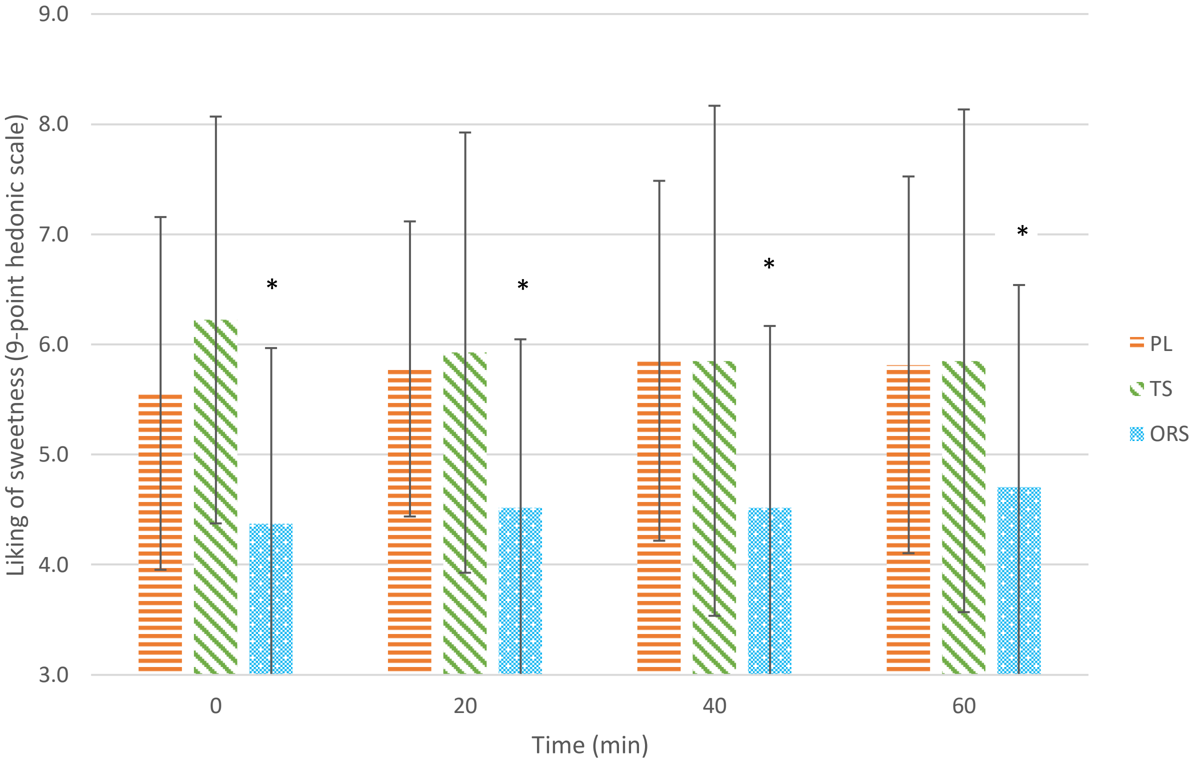


Figure 3.3. Liking of sweetness of drinks: ORS – oral rehydration solution, PL – placebo, TS – traditional sports drink, on a 9-point hedonic scale throughout 60 min of exercise. Values are mean ± SD. *Significant effect of the treatment vs. the placebo (p<0.05).

Thirst-quenching ability

Figure 3.4 shows the mean (± SD) ratings of thirst-quenching ability for each drink after each exercise time. At all time points, thirst-quenching ability differed between drinks (main effect of treatment, p=0.001); ratings of thirst-quenching ability were higher for PL (6.16 ± 1.65, p=0.002, *d*=0.98) and TS (5.72 ± 1.94, p=0.025, *d*=0.67) than ORS (4.45 ± 1.85), while there were no differences between PL and TS (p>0.05, *d*=0.24). There was no main effect of exercise time (p=0.336) and no interaction between drink type and exercise time (p=0.151). There was a significant difference in percent change in thirst-quenching ability ratings between drinks throughout exercise ($\chi^2(2) = 7.327$, *p*=0.026); median (25, 75 percentiles) percent change in thirst-quenching ability ratings for the ORS, PL and TS were 50% (0, 66.7%), 0% (-14.3, 28.6%), and 0% (-25, 14.3%), respectively. There was a significant increase in percent thirst-quenching ability for ORS vs. PL (Z = -2.046, *p*=0.041) and for ORS vs. TS (Z = -2.681, *p*=0.007). There was also a trend for a difference between PL and TS (Z = -1.925, *p*=0.054; see Appendix F for supplementary results).

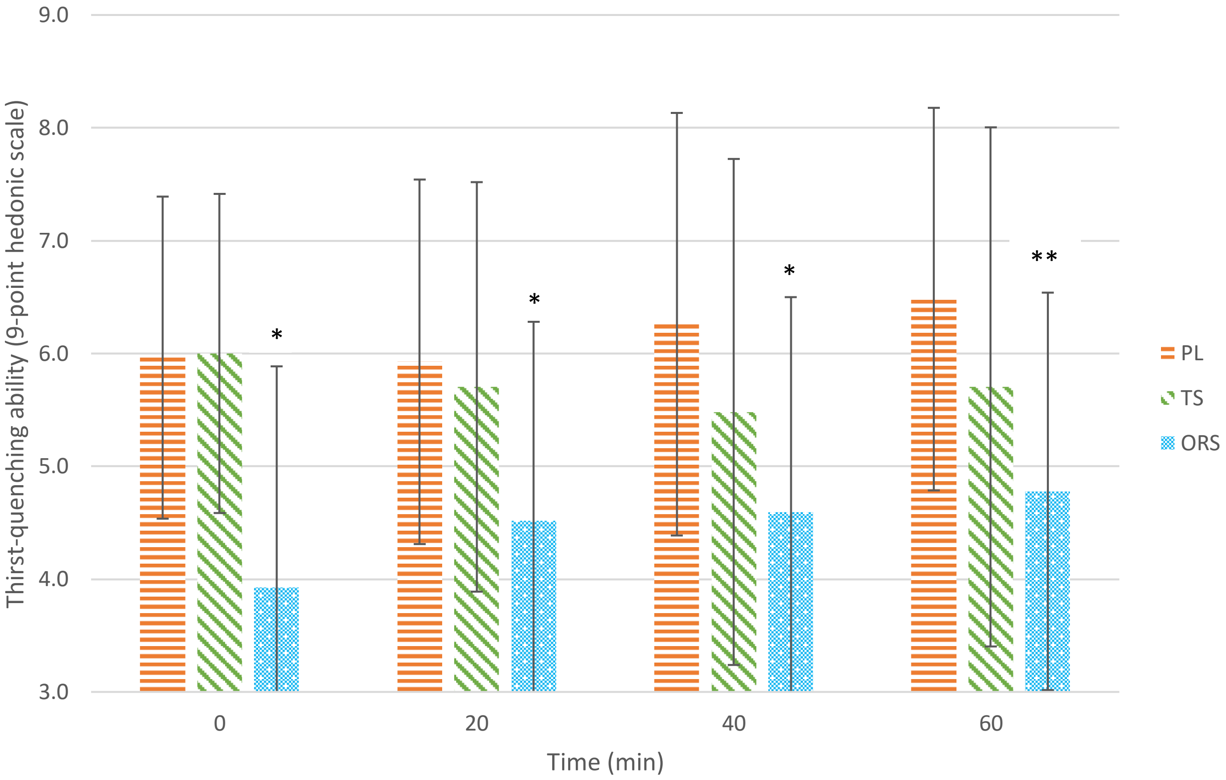


Figure 3.4. Thirst-quenching ability of drinks: ORS – oral rehydration solution, PL – placebo, TS – traditional sports drink, on a 9-point hedonic scale throughout 60 min of exercise. Values are mean ± SD. *Significant effect of the treatment vs. the placebo (p<0.05). **Significant delta change in ratings compared to 0 min before exercise (p<0.05).

Overall liking

Figure 3.5. shows the mean (± SD) ratings of overall liking for each drink after each exercise time. At all time points, ratings of overall liking differed between drinks (main effect of treatment, $p<0.001$); overall liking was higher for PL (6.00 ± 1.49, $p=0.001$, *d*=1.1) and TS (6.07 ± 1.82, $p<0.001$, *d*=1.06) than ORS (4.01 ± 2.06), while there were no differences between PL and TS ($p>0.05$, *d*=0.04). There was no main effect of exercise time ($p=0.353$) and no interaction between drink type and exercise time ($p=0.251$). There was no significant difference in percent change in overall liking ratings between drinks throughout exercise ($\chi^2(2) = 4.989$, *p*=0.083). Median (25, 75 percentiles) percent change in ratings of overall liking for the ORS, PL and TS were 0% (0, 100%), 0% (-12.5, 40%), and 0% (-20, 0%), respectively (supplementary results are given in Appendix F).

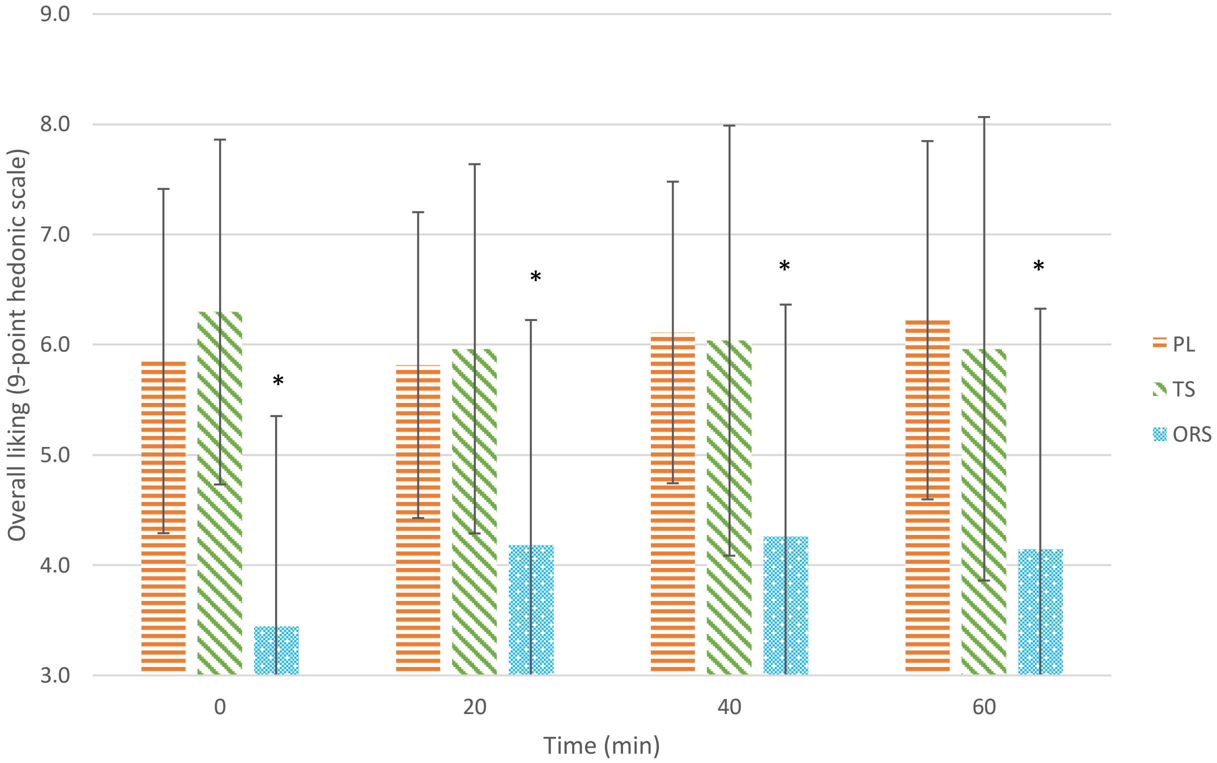


Figure 3.5. Overall liking of drinks: ORS – oral rehydration solution, PL – placebo, TS – traditional sports drink, on a 9-point hedonic scale throughout 60 min of exercise. Values are mean ± SD. *Significant effect of the treatment vs. the placebo ($p<0.05$).

Predictors of overall liking

Liking of sweetness, saltiness and thirst-quenching ability were identified as the main predictors of overall liking for all drinks pre-exercise ($R^2=0.850$; $p<0.001$). However, only liking of saltiness was identified as a predictor of overall liking post-exercise ($R^2=0.751$; $p<0.001$). For the ORS, overall liking was mainly determined by liking of sweetness and saltiness pre-exercise ($R^2=0.837$; $p<0.001$), whereas liking of saltiness and thirst-quenching ability were the most important predictors post-exercise ($R^2=0.938$, $p<0.001$).

3.5 Discussion

The aim of this study was to investigate the palatability of an ORS at various stages of exercise in the heat and at rest. The main sensory finding was the change in predictors of overall liking as a function of exercise, with liking of saltiness becoming the most important factor for overall liking of all drinks post-exercise.

3.5.1 Liking of saltiness

The ORS used in the current study had the highest NaCl concentration (2.6 g/L; 44.5 mmol/L) and not surprisingly, received the lowest liking of saltiness of the fluids tested at all time points. Liking of saltiness did not change at any time point throughout exercise for any of the fluids; although after 60 min of exercise, liking of saltiness for the ORS increased by a median (25, 75 percentiles) of 20% (0.0, 100%; $p<0.05$), perhaps indicating an increase in palatability to the high salt concentration. These findings are consistent with a study investigating the dose-response effects of drink Na content (0, 18, 30, 40, and 60 mmol/L) on sensory perception and palatability in a group of trained athletes (Passe et al., 2009). Although no differences in salt perception were observed as a function of exercise, participants became more accepting of the drink containing the highest Na concentration (60 mmol/L; 1.4 g/L) while in an exercise context as reflected by significant increases in overall acceptance and liking of saltiness compared to the sedentary condition (Passe et al., 2009). Similarly, throughout 60 min of moderate-high intensity exercise, a decrease in perceived salt intensity was observed for a variety of sports drinks with NaCl concentrations of 140-421 mg/L (2.4-7.2 mmol/L) among a group of runners (Ali et al., 2011). Furthermore, after 60 min of routine exercise, the amount of salt added to flavour soup by a group of regular exercisers significantly increased ($p<0.05$; Leshem et al., 1999). In contrast, after only 30 min of cycling at 50% $\dot{V}O_2$ max, there were no differences in salt thresholds compared to before exercise (Horio and Kawamura, 1998). Thus, the duration and intensity of exercise, and extent of fluid loss appears to determine salt preference and liking (Takamata et al., 1994). For instance, after a 7 h water and Na-depletion period involving 8 x 30-min bouts of cycling exercise in the heat (35°C), a significant increase in palatability to hypertonic NaCl (≥300 mmol/L; 17.5 g/L) solutions was observed in a group of healthy volunteers (Takamata et al., 1994). Interestingly, a similar effect between salt dose and sweat loss was observed when untasted salt capsules were paired with a novel-tasting drink (Wald and Leshem, 2003). After 90 min of moderate exercise, beverage palatability ratings increased when paired with 400 mg NaCl in an untasted capsule form, especially in participants who lost the greatest amount of sweat (Wald and Leshem). At higher NaCl concentrations (600 mg NaCl) however, flavour aversion was observed, especially in participants with low sweat loss (Wald and Leshem, 2003). Even though these observations appear to be unrelated to liking of the saltiness taste, they provide further evidence for a relationship between salt preference and its physiological usefulness.

3.5.2 Liking of sweetness

While the TS and PL had a higher liking of sweetness than the ORS at all time points, sweetness liking did not change for any of the drinks throughout exercise in the current study. Similarly, another study found no changes in liking of sweetness for a range of fluids varying in energy, electrolyte content and osmolality throughout 60 min of exercise (Appleton 2005); there was however, an increase in sweetness ratings of all drinks from pre- to post-exercise. In contrast, an increase in liking of sweetness has been reported following 30 min of moderate exercise in a group of students ($p<0.05$; Horio and Kawamura, 1998; Horio, 2004). Interestingly, this increase was not observed for fluids sweetened with the artificial sweetener saccharin, perhaps due to its synthetic nature and inability to provide energy (Horio, 2004). Following a half marathon, an increase in liking of sweetness was observed accompanied by an increase in the degree of physical fatigue (Narukawa et al., 2009). The authors attributed this CHO-seeking behaviour to the need to replenish glycogen stores with exercise, thus supporting the theory of physiological usefulness (Narukawa et al., 2009). However, this was not the case following a 12 h mountain hike with no significant change in liking of sweetness of a low (100 mmol/L) and high (300 mmol/L) sucrose-containing solution (Narukawa et al., 2010). It is likely, however, that exercisers could easily distinguish between the two distinct sucrose concentrations, thus potentially impacting results (Narukawa et al., 2010). Furthermore, another study identified only a weak correlation between sweetness and overall liking which increased throughout 60 min of moderate-high intensity exercise for all CHO-containing drinks (Ali et al., 2011). These discrepancies may indicate that other factors are contributing to the overall hedonic response; such as liking of saltiness and thirst-quenching ability. In the current study both of these variables (saltiness liking and thirst quenching ability) were identified as important predictors of overall liking of the ORS post-exercise ($R^2=0.938$, $p<0.001$).

3.5.3 Fluid osmolality

In the current study, there were no significant changes in sensory variables for the PL which had the lowest osmolality and received similar sensory ratings from rest and throughout 60 min of exercise. Furthermore, while there was a significant increase in saliva osmolality, saliva total protein concentration, urine osmolality and urine colour, this study only reached a BML of 1.36 ± 0.39 % after 60 min of exercise, thus did not achieve the threshold for clinical “dehydration” (≥2% BML;

Sawka et al., 2007; Thomas et al., 2008). Perhaps sensory changes may have occurred following fluid loss to this extent (i.e. >2.0% BML). A similar study observed an increase in pleasantness ratings for the fluids with the lowest osmolality (1.7 and 3.4% CHO) immediately after exercise (Appleton, 2005). Furthermore, this effect was most pronounced in participants who lost the greatest amount of sweat (1% BML vs. 0.4% BML), thus suggesting that water balance is more important than electrolyte or energy intake – especially in those who sweat more during exercise (Appleton, 2005). However, in the Appleton (2005) study the exercise protocol was not controlled (i.e. the method and intensity), thus, impacting the reliability and validity of these results.

In contrast, other studies have shown no significant differences in overall liking between a variety of sports drinks regardless of their osmolality throughout 60 min of moderate-high intensity treadmill exercise (Ali et al., 2011). However, ratings of overall liking did increase similarly across all beverages during and immediately after exercise (Ali et al., 2011). Perhaps this lack of significance indicates the importance of replacing lost fluid over the replenishment of nutrients or electrolytes immediately after exercise. Furthermore, the exercise duration, intensity, and extent of fluid loss appears to influence sensory perception and the overall hedonic response. Indeed, immediately after 8 x 30-min cycling bouts in the heat, an increase in perceived pleasantness of lower osmolality fluids (<300 mmol/L; 17.5 g/L NaCl) was observed (Takamata et al., 1994). In contrast, no significant changes in voluntary intake were observed between a range of flavoured beverages and plain water throughout 4 x 20-min bouts of exercise (at 50% $\dot{V}O_2$ max) in the heat (Wong and Sun, 2014). Wong and Sun (2014) also reported no significant changes in P_{Osm}, considered the gold standard for assessing hydration status (Thomas et al., 2008); thus, perhaps the exercise was too light or of insufficient duration to elicit significant water requirements (Wong and Sun, 2014).

3.5.4 Thirst-quenching ability

While the current study did not find any changes in thirst-quenching ability for any of the drinks, there was an increase in percent thirst-quenching ability ratings for the ORS after 60 min of exercise ($p<0.05$). Furthermore, thirst-quenching ability, together with liking of saltiness, was identified as a key predictor of overall liking of the ORS post-exercise ($R^2=0.938$, $p<0.001$). Similarly, another study found an increase in thirst-quenching ability and overall liking for sports drinks varying in osmolality, CHO and electrolyte content throughout exercise (Ali et al., 2011).

3.5.5 Predictors of overall liking

In the current study, the TS was the most-preferred and the ORS was the least-liked beverage at all time points; nonetheless, research points to a hedonic shift during exercise in which the hedonic value of a beverage can change dramatically from sedentary to exercise conditions (Passe et al., 2000). Substantial increases in acceptability of the least-liked beverage have been observed over 180 min of exercise at 70-75% HR_{max} (Passe et al., 2000). Similarly, palatability ratings of hypertonic NaCl solutions ($\geq$300 mmol; 17.5 g/L NaCl) have been shown to increase throughout thermal and exercise-induced fluid loss (Takamata et al., 1994). Ali et al. (2011) found overall liking to increase with exercise for all drinks tested (including water), suggesting that replenishment of lost fluids during exercise is the most important factor in determining overall liking. In contrast, there were no changes in overall liking for any of the drinks in the current study; instead, liking of saltiness and thirst-quenching ability became the most important predictors of overall liking following 60 min of exercise (R^2=0.938, $p<0.001$). Furthermore, after 60 min of exercise the ORS showed a significant increase in percent liking of saltiness and thirst-quenching ability ratings, perhaps indicating an enhanced palatability to saltiness. These findings are consistent with the theory of physiological usefulness in which exercise elicits an increase in liking of saltiness as salt is progressively lost through sweat. Therefore, it seems reasonable to expect a further improvement in overall liking of the ORS among highly trained athletes (relative to recreational exercisers) who are likely to exercise for a longer duration, at a higher intensity, and with larger sweat losses.

3.5.6 Limitations and strengths

The main limitation of this study is the extent of fluid loss and dehydration achieved. Research suggests the critical value for exercise-induced dehydration as $\geq$2% body mass loss (Sawka et al., 2007; Thomas et al., 2008), however, in this study, only 1.36 ± 0.39% body mass loss was achieved after 60 min of exercise. Although significant increases in $U_{SG,}$ $U_{Col,}$ S_{Osm} and, S_{PC} were observed, there was no significant change in U_{Osm} ($p>0.05$). Furthermore, the American College of Sports Medicine define dehydration as a U_{SG} 1.020 and/or U_{Osm} 700 mOsmol/kg (Sawka et al., 2007), and participants in the current study only achieved a median (25, 75 percentiles) U_{SG} of 1.017 (1.014, 1.025) and U_{Osm} of 586 mOsmol/kg (476, 796) after 60 min of exercise. Thus, according to these threshold values, participants in this study were not clinically dehydrated. Acute progressive dehydration to 3% BML has previously shown to increase S_{Osm} and S_{PC} to 105 ± 41 mOsmol/kg and 1.80 ± 0.82 mg/ml, respectively (Walsh et al., 2004b). At 1.36% BML, participants in the current study achieved a mean (95% CI) S_{Osm} and S_{PC} of 112.8 (102, 124.1) mOsmol/kg and 3.17 (3.51, 3.96) mg/ml, respectively, thus indicating signs of dehydration based on these values. However, this study did not measure

P_{Osm}, considered the gold standard for assessing hydration status (Thomas et al., 2008). This technique is invasive and risks compromising athlete participation, however, it would have been useful to help quantify the extent of dehydration achieved, especially because S_{PC} has shown to correlate strongly with P_{Osm} during progressive acute dehydration (Wald et al., 2004a). Moreover, exercise consisted of only 3 x 20-min bouts at a moderate work rate. It would be interesting to see if longer ($\geq$ 2 h) and/or more intense exercise that achieves a higher fluid loss (i.e. $\geq$2% body mass loss), would influence sensory ratings. Another potential limitation was that participants ingested a total of 60 ml of fluids (3x 20-ml samples) throughout the sensory and exercise protocol. Perhaps this amount of fluid intake may have affected the dehydration process; nonetheless, one of the study's key strengths was the experimental design which enabled all three treatments to be tested in the same visit. This allowed for consistent sample collection practices especially as urine and saliva sampling protocols are subject to timing and uniformity and can also be confounded by diet and lifestyle factors. This may have helped participant recruitment and contribution as participants were only required for one main trial; indeed, it is likely participants may not have wanted to repeat the exercise and testing protocol possibly leading to participant dropout if there was more than one trial. Another key strength was the use of an iPad application for sensory testing to ensure double blinding and randomisation of samples. A potential limitation, however, was that participants in this study were only recreationally active. Future research should aim to test a variety of subject groups since training status and psychological factors such as familiarity and previous beliefs have been shown to influence sensory perception and behaviour (Crystal et al., 1995; Passe et al., 2009; Yeomans et al., 2000). Nonetheless, this was the first study to compare palatability ratings of an ORS with a traditional sports drink and placebo before and throughout exercise in the heat.

3.6 Conclusion

In summary, the electrolyte content of traditional sports drinks is insufficient for most athletes undertaking prolonged exercise in the heat. Moreover, the same beverage is often considered suitable for hydration at all stages of exercise despite a number of physiological and perceptual changes including fluid and electrolyte loss, glycogen depletion, and increased thirst, fatigue, and increased liking of saltiness occurring in the later stages or prolonged or intense exercise. Even though the ORS was identified as the least-liked beverage at rest, participants' liking of its saltiness and thirst-quenching ability significantly increased from pre- to post-exercise. Furthermore, liking of saltiness and thirst-quenching ability became the most important predictors of overall liking post-exercise. This may be an important step for the formulation of sports beverages that meet both physiological and perceptual requirements for athletes undertaking prolonged exercise in the heat.

CHAPTER 4: CONCLUSIONS

4.1 Overview and achievement of study aims and objectives

The purpose of this study was to investigate sensory perception of an ORS at rest and throughout exercise in the heat. While the importance of promoting hydration during exercise is well recognised, the electrolyte content of traditional sports drinks (20-25 mmol/L NaCl) appears to be insufficient to replace sweat losses for athletes undertaking prolonged exercise in the heat. In order to promote more effective hydration, enhance performance, and reduce risk of heat illness, an oral rehydration solution (ORS) has been formulated with a relatively higher electrolyte (30-90 mmol/L) and lower CHO concentration (2-3% CHO). Consequently, the intensity of saltiness may be perceived as unpleasant and compromise voluntary intake. However, there is growing evidence for an increase in liking of saltiness with exercise, particularly when sweat losses are high. Therefore the overall liking of an ORS may improve as the physiological requirements for electrolytes increase. This was the first study, to the authors' knowledge, to compare changes in sensory perception of an ORS with a traditional sports drink (TS) and flavoured water placebo (PL) at rest and throughout exercise in the heat.

The first objective of this study was to assess changes in hydration status throughout exercise. This was achieved by taking measures of semi-nude body mass, urine and saliva samples, pre- and post-exercise, as well as after each 20-min exercise bout. Although there was a significant increase in percent body mass loss (BML), saliva osmolality, saliva total protein concentration, urine osmolality and urine colour following 60 min of exercise, this study did not achieve the threshold for clinical "dehydration" (≥2% BML; Sawka et al., 2007). Furthermore, there was no significant change in urine osmolality perhaps indicating that the exercise-induced fluid loss was insufficient for significant effects to occur (Sawka et al., 2007). The second objective of this study was to examine whether exercise and/or exercise-induced fluid loss has an effect on sensory perception and overall liking of the ORS. Although clinical dehydration was not achieved, there was a significant change in predictors of overall liking, with liking of saltiness becoming the most important predictor following exercise.

4.2 Findings and concluding remarks

Findings from this study indicate a potential role for ORS as a suitable hydration drink particularly during the latter stages of exercise that is prolonged and/or in the heat. Although it was the least-liked beverage at rest, a trend for an increase in liking of saltiness and overall liking of the ORS is in line with previous research and in support of the theory of physiological usefulness (Cabanac, 1971; Passe et al., 2000). However, this study did not achieve dehydration (≥2% body mass loss; Sawka et al., 2007) and did not measure P_{Osm}, considered the gold standard for the assessment of hydration status (Thomas et al., 2008). It would be interesting to see if this trend reaches significance at higher levels of fluid and Na loss with either longer exercise duration and/or higher intensity.

4.3 Research contribution

This study has made a valuable contribution to our understanding of the relationship between exercise, fluid loss and hedonic response. These findings lead to implications for sports beverage development, as well as sensory and behavioural sciences. At the concept development and formulation stages, developers of sports beverages would greatly benefit from understanding the specific taste sensitivities and preferences of exercised subjects, compared to rested subjects. Furthermore, a more in-depth understanding of the key drivers of voluntary fluid intake will help to guide hydration practices in order to promote hydration, enhance performance, and reduce the risk of heat illness throughout prolonged, intense exercise.

4.4 Strengths and limitations

There are a number of challenges involved with sensory testing particularly due to the large psychological component involving beliefs and familiarity. Participants in this study were only recreationally active and likely held very different expectations for how sports drinks should taste. A key strength of this study, however, is that it is the first to formulate and assess the palatability of an ORS throughout exercise in a group of recreationally active participants. Furthermore, the relatively large sample size and the use of an iPad application for sensory evaluation reduced the study's main limitations of bias, subjective analysis, and restricted generalisability.

4.4.1 Choice of aims and objectives

The aims and objectives were strong because they were well defined, simple, measurable, achievable and timely. They were supported by an in-depth literature search that highlighted the need for studies investigating the effect of exercise on palatability, one of the key determinants of voluntary fluid intake. A follow-on study that achieves higher levels of fluid and Na loss (≥2% loss of body mass) should supplement our findings and provide further support for ORS as an effective hydration beverage during prolonged exercise in the heat.

4.4.2 Exercise protocol

A key strength of this study was that the entire exercise protocol could be completed in one visit, which may have helped participant recruitment and retention. Furthermore, this reduced bias and allowed for consistent sample collection practices especially as urine and saliva sampling protocols are subject to timing and uniformity and can also be confounded by diet and rate and/or speed of breathing. A limitation, however, was that this exercise protocol, did not achieve sufficient fluid loss for participants to classify as clinically dehydrated (≥2% loss of body mass; Sawka et al., 2007). The literature indicated that exercise must be of a certain intensity and duration (≥ 2 h) to elicit significant physiological and sensory effects (Sawka et al., 1885; 2007; Takamata et al., 1994; Walsh et al., 2004a; 2004b). Therefore, the exercise protocol in future studies should be long and intense enough (≥ 2 h in the heat) to elicit a loss of body mass of at least 2%.

4.4.3 Sensory protocol

Another key strength of this study was that sensory evaluations were conducted using an iPad application to ensure the study was double-blinded. Furthermore, a 9-point hedonic scale was used to evaluate each sensory property which is consistent with other studies, thus enabling findings to be compared (Hubbard et al., 1984; Passe et al., 2000; Yeomans, 1998). A potential limitation was that participants ingested a total of 60 ml of fluids (3x 20-ml samples) throughout the sensory and exercise protocol. Perhaps this amount of fluid intake may have affected the dehydration process; nonetheless, this study design allowed for all three treatments to be tested at the same time, potentially reducing bias related to different physiological states and lifestyle factors (e.g. diet and exercise).

4.4.4 Recruitment and data collection

A strength of this study was that several methods were used to recruit participants, which likely improved the study’s research and sample size. Furthermore, participants were only required for one main trial as opposed to four; they were also reimbursed with fuel vouchers and were not required to provide any invasive blood samples as this often affects participant recruitment.

4.4.5 Sample size

Based on significant findings reported from a similar study that examined sensory perception of sports drinks throughout exercise in 14 recreationally active exercisers (Ali et al., 2011), we aimed to recruit 30 participants. Based on G*Power (sample size estimation app), for effect size of 0.25, power of 0.80, number of interventions (3), number of measurements (4), and alpha value of $p<0.05$, the sample size estimate was 24. The current study used a sample size of 27 recreationally active males and females, thus, serving as a study strength as larger sample sizes can generate more powerful and reliable results (Field, 2009).

4.4. Statistical analysis

Another strength of this study was the range of statistical analysis procedures used. Descriptive statistics allowed the study to investigate physiological and sensory changes throughout different stages of exercise. Correlation helped to identify any relationships between exercise and sensory variables and regression analyses identified the key predictors of overall liking; this information might be useful for guiding the development of hydration beverages suitable for different stages of exercise.

4.5 Directions for future research

Given that this study has provided evidence suggesting there is an increase in palatability of an ORS throughout exercise in a group of recreationally active individuals, future research should test palatability among a range of athletic abilities. In addition, the exercise duration and intensity needs to be sufficient to elicit a body mass loss of ≥2% (Sawka, 2007). Additional markers such as P_{Osm}, S_{Osm}, S_{PC}, and/or urinary markers should also be used to ensure dehydration (Cheuvront et al., 2014). Further research among clinically dehydrated individuals of a range of athletic abilities should provide greater insight on this topic and direct future research. In addition, the timing of sensory testing should be considered (i.e. at rest, during exercise, immediately after, or hours after) as both perception and hedonic response seem to vary with changing physiological states.

Future studies should aim to quantify voluntary fluid intake during prolonged, intense exercise and recovery when all three beverages are provided *ad libitum*. Moreover, it would be interesting to examine and compare the effects of ORS, TS, and PL on cognitive and physical performance.

4.6 Final recommendations

In order to further assess the efficacy of an ORS as a hydration beverage during prolonged exercise, selective participant recruitment and effective hydration status monitoring are required. From our research, recommendations for future studies could include the following:

- Selective participant recruitment to ensure findings are representative of target consumer population (i.e. competitive endurance athletes vs. recreationally active individuals).
- Include an initial questionnaire to identify previous exposure and familiarity with sports beverages and/or ORS.
- Ensure exercise protocol is sufficient to elicit $\geq$2% BML and significant changes in plasma and/or urinary markers.
- Include serial measures of hydration status throughout rest and exercise (i.e. changes in semi-nude body mass with a urinary marker, saliva sample or plasma osmolality).
- Consider the impact of the ingested volume of sample fluids on the dehydration process.
- Follow-on studies that quantify voluntary fluid intake of each beverage when participants have *ad libitum* access to all three beverages throughout exercise and rest.
- Further studies investigating the effects of beverage type and total intake on cognitive and physical performance.

REFERENCES

Adams, W. M., Vandermark, L. W., Belval, L. N., & Casa, D. J. (2019). The Utility of Thirst as a Measure of Hydration Status Following Exercise-Induced Dehydration. Nutrients, 11(11). doi:10.3390/nu11112689

Adan, A. (2012). Cognitive performance and dehydration. J Am Coll Nutr, 31(2), 71-78. doi:10.1080/07315724.2012.10720011

Adolph, E. F., Rochester, N. Y. U. R. D. U., United, S., Office of Scientific, R., Development, & Committee on Medical, R. (1947). Physiology of man in the desert E.F. Adolph and associates. New York: Interscience Publishers.

Ali, A., Duizer, L., Foster, K., Grigor, J., & Wei, W. (2011). Changes in sensory perception of sports drinks when consumed pre, during and post exercise. Physiol Behav, 102(5), 437-443. doi:10.1016/j.physbeh.2010.12.013

American College of Sports, M. (2009). American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. Med Sci Sports Exerc, 41(3), 687-708. doi:10.1249/MSS.0b013e3181915670

American College of Sports, M., Armstrong, L. E., Casa, D. J., Millard-Stafford, M., Moran, D. S., Pyne, S. W., & Roberts, W. O. (2007). American College of Sports Medicine position stand. Exertional heat illness during training and competition. Med Sci Sports Exerc, 39(3), 556-572. doi:10.1249/MSS.0b013e31802fa199

American College of Sports, M., Sawka, M. N., Burke, L. M., Eichner, E. R., Maughan, R. J., Montain, S. J., & Stachenfeld, N. S. (2007). American College of Sports Medicine position stand. Exercise and fluid replacement. Med Sci Sports Exerc, 39(2), 377-390. doi:10.1249/mss.0b013e31802ca597

Appleton, K. M. (2005). Changes in the perceived pleasantness of fluids before and after fluid loss through exercise: a demonstration of the association between perceived pleasantness and physiological usefulness in everyday life. Physiol Behav, 83(5), 813-819. doi:10.1016/j.physbeh.2004.10.001

Armstrong, L. E., Costill, D. L., & Fink, W. J. (1985). Influence of diuretic-induced dehydration on competitive running performance. Med Sci Sports Exerc, 17(4), 456-461. doi:10.1249/00005768-198508000-00009

Armstrong, L. E., Kavouras, S. A., Walsh, N. P., & Roberts, W. O. (2016). Diagnosing dehydration? Blend evidence with clinical observations. Curr Opin Clin Nutr Metab Care, 19(6), 434-438. doi:10.1097/MCO.0000000000000320

Arnaoutis, G., Verginadis, P., Seal, A. D., Vogiatzis, I., Sidossis, L. S., & Kavouras, S. A. (2018). Progressive Dehydration in Junior Laser Class Sailors During World Championship. Int J Sport Nutr Exerc Metab, 28(1), 75-81. doi:10.1123/ijsnem.2017-0142

Atlas, S. A. (2007). The renin-angiotensin aldosterone system: pathophysiological role and pharmacologic inhibition. J Manag Care Pharm, 13(8 Suppl B), 9-20. http://doi:10.18553/jmcp.2007.13.s8-b.9. PMID: 17970613.

Ayotte, D., Jr., & Corcoran, M. P. (2018). Individualized hydration plans improve performance outcomes for collegiate athletes engaging in in-season training. J Int Soc Sports Nutr, 15(1), 27. doi:10.1186/s12970-018-0230-2

Baker, L. B., Barnes, K. A., Anderson, M. L., Passe, D. H., & Stofan, J. R. (2016). Normative data for regional sweat sodium concentration and whole-body sweating rate in athletes. J Sports Sci, 34(4), 358-368. doi:10.1080/02640414.2015.1055291

Balment, R. J. (2002). Control of water balance in mammals. Symp Soc Exp Biol(54), 153-168. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/14992150

Bardis, C. N., Kavouras, S. A., Adams, J. D., Geladas, N. D., Panagiotakos, D. B., & Sidossis, L. S. (2017). Prescribed Drinking Leads to Better Cycling Performance than Ad Libitum Drinking. Med Sci Sports Exerc, 49(6), 1244-1251. doi:10.1249/MSS.0000000000001202

Bates, G. P., & Miller, V. S. (2008). Sweat rate and sodium loss during work in the heat. J Occup Med Toxicol, 3, 4. doi:10.1186/1745-6673-3-4

Binder, H. J., Brown, I., Ramakrishna, B. S., & Young, G. P. (2014). Oral rehydration therapy in the second decade of the twenty-first century. Curr Gastroenterol Rep, 16(3), 376. doi:10.1007/s11894-014-0376-2

Bishop, N. C., Blannin, A. K., Robson, P. J., Walsh, N. P., & Gleeson, M. (1999). The effects of carbohydrate supplementation on immune responses to a soccer-specific exercise protocol. J Sports Sci, 17(10), 787-796. doi:10.1080/026404199365506

Borg, G. A. (1982). Psychophysical bases of perceived exertion. Med Sci Sports Exerc, 14, 377–381. doi.org/10.1249/00005768-198205000-00012

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 72, 248-254. doi:10.1006/abio.1976.9999

Brunstrom, J. M., Tribbeck, P. M., & MacRae, A. W. (2000). The role of mouth state in the termination of drinking behavior in humans. Physiol Behav, 68(4), 579-583. doi:10.1016/s0031-9384(99)00210-3

Burdon, C. A., Johnson, N. A., Chapman, P. G., & O'Connor, H. T. (2012). Influence of Beverage Temperature on Palatability and Fluid Ingestion During Endurance Exercise: a systematic review. Int J Sport Nutr Exerc Metab. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22710391

Cabanac, M. (1971). Physiological role of pleasure. Science, 173(4002), 1103-1107. doi:10.1126/science.173.4002.1103

Cabanac, M. (1992). Pleasure: the common currency. J Theor Biol, 155(2), 173-200. doi:10.1016/s0022-5193(05)80594-6

Campa, F., Piras, A., Raffi, M., Trofè, A., Perazzolo, M., Mascherini, G., & Toselli, S. (2020). The Effects of Dehydration on Metabolic and Neuromuscular Functionality during Cycling. Int. J. Environ. Res. Public Health, 17, 1161. https://doi.org/10.3390/ijerph17041161

Carter, J., Jeukendrup, A. E., & Jones, D. A. (2005). The effect of sweetness on the efficacy of carbohydrate supplementation during exercise in the heat. Can J Appl Physiol, 30(4), 379-391. doi:10.1139/h05-128

Casa, D. J., Armstrong, L. E., Kenny, G. P., O'Connor, F. G., & Huggins, R. A. (2012). Exertional heat stroke: new concepts regarding cause and care. Curr Sports Med Rep, 11(3), 115-123. doi:10.1249/JSR.0b013e31825615cc

Cheuvront, S. N., & Kenefick, R. W. (2014). Dehydration: physiology, assessment, and performance effects. Compr Physiol, 4(1), 257-285. doi:10.1002/cphy.c130017

Cheuvront, S. N., Kenefick, R. W., Charkoudian, N., & Sawka, M. N. (2013). Physiologic basis for understanding quantitative dehydration assessment. Am J Clin Nutr, 97(3), 455-462. doi:10.3945/ajcn.112.044172

Cheuvront, S. N., Montain, S. J., & Sawka, M. N. (2007). Fluid replacement and performance during the marathon. Sports Med, 37(4-5), 353-357. doi:10.2165/00007256-200737040-00020

Cheuvront, S. N., Kenefick, R. W., Montain, S. J., & Sawka, M. N. (2010). Mechanisms of aerobic performance impairment with heat stress and dehydration. Journal of Applied Physiology, 109(6), 1989-1995. https://doiorg.ezproxy.massey.ac.nz/10.1152/japplphysiol.00367.2010

Cohen, J. (1988). Statistical power analysis for the behavioral sciences. Hillsdale, N.J.: L. Erlbaum Associates.

Cole, M., Hopker, J. G., Wiles, J. D., & Coleman, D. A. (2018). The effects of acute carbohydrate and caffeine feeding strategies on cycling efficiency. Journal of Sports Sciences, 36(7), 817–823. https://doi.org/10.1080/02640414.2017.1343956

Cramer, M. N., & Jay, O. (2016). Biophysical aspects of human thermoregulation during heat stress. Auton Neurosci, 196, 3-13. doi:10.1016/j.autneu.2016.03.001

Crystal, S., Frye, C. A., & Kanarek, R. B. (1995). Taste preferences and sensory perceptions in female varsity swimmers. Appetite, 24(1), 25-36. doi:10.1016/s0195-6663(95)80003-4

Desbrow, B., Jansen, S., Barrett, A., Leveritt, M. D., & Irwin, C. (2014). Comparing the rehydration potential of different milk-based drinks to a carbohydrate-electrolyte beverage. Appl Physiol Nutr Metab, 39(12), 1366-1372. doi:10.1139/apnm-2014-0174

Dumke, C. L., McBride, J. M., Nieman, D. C., Gowin, W. D., Utter, A. C., & McAnulty, S. R. (2007). Effect of duration and exogenous carbohydrate on gross efficiency during cycling. Journal of Strength and Conditioning Research, 21(4), 1214–1219. https://doi.org/10.1519/R22396.1

El-Sharkawy, A. M., Sahota, O., & Lobo, D. N. (2015). Acute and chronic effects of hydration status on health. Nutr Rev, 73 Suppl 2, 97-109. doi:10.1093/nutrit/nuv038

Ely, B. R., Cheuvront, S. N., Kenefick, R. W., & Sawka, M. N. (2011). Limitations of salivary osmolality as a marker of hydration status. Med Sci Sports Exerc, 43(6), 1080-1084. doi:10.1249/MSS.0b013e3182058643

Evans, G. H., James, L. J., Shirreffs, S. M., & Maughan, R. J. (2017). Optimizing the restoration and maintenance of fluid balance after exercise-induced dehydration. J Appl Physiol (1985), 122(4), 945-951. doi:10.1152/japplphysiol.00745.2016

Evans, G. H., Shirreffs, S. M., & Maughan, R. J. (2009). Postexercise rehydration in man: the effects of carbohydrate content and osmolality of drinks ingested ad libitum. Appl Physiol Nutr Metab, 34(4), 785-793. doi:10.1139/H09-065

Field, A. P. (2009). Discovering statistics using SPSS : (and sex and drugs and rock 'n' roll). Los Angeles [i.e. Thousand Oaks, Calif.]; London: SAGE Publications.

Figaro, M. K., & Mack, G. W. (1997). Regulation of fluid intake in dehydrated humans: role of oropharyngeal stimulation. Am J Physiol, 272(6 Pt 2), R1740-1746. doi:10.1152/ajpregu.1997.272.6.R1740

Fortes, M. B., Diment, B. C., Di Felice, U., Gunn, A. E., Kendall, J. L., Esmaeelpour, M., & Walsh, N. P. (2011). Tear fluid osmolarity as a potential marker of hydration status. Med Sci Sports Exerc, 43(8), 1590-1597. doi:10.1249/MSS.0b013e31820e7cb6

Gagge, A. P., Stolwijk, J. A., & Hardy, J. D. (1967). Comfort and thermal sensations and associated physiological responses at various ambient temperatures. Environ Res, 1(1), 1-20. doi:10.1016/0013-9351(67)90002-3

Ganio, M. S., Armstrong, L. E., Casa, D. J., McDermott, B. P., Lee, E. C., Yamamoto, L. M., . . . Lieberman, H. R. (2011). Mild dehydration impairs cognitive performance and mood of men. Br J Nutr, 106(10), 1535-1543. doi:10.1017/S0007114511002005

Goldman, M. B., Robertson, G. L., & Hedeker, D. (1996). Oropharyngeal regulation of water balance in polydipsic schizophrenics. Clin Endocrinol (Oxf), 44(1), 31-37. doi:10.1046/j.1365-2265.1996.641463.x

Gonzalez-Alonso, J., Calbet, J. A., & Nielsen, B. (1998). Muscle blood flow is reduced with dehydration during prolonged exercise in humans. J Physiol, 513 (Pt 3), 895-905. doi:10.1111/j.1469-7793.1998.895ba.x

Gonzalez-Alonso, J., Mora-Rodriguez, R., & Coyle, E. F. (2000). Stroke volume during exercise: interaction of environment and hydration. Am J Physiol Heart Circ Physiol, 278(2), H321-330. doi:10.1152/ajpheart.2000.278.2.H321

Greenleaf, J. E. (1992). Problem: thirst, drinking behavior, and involuntary dehydration. Med Sci Sports Exerc, 24(6), 645-656. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1602937

Greenleaf, J. E., & Sargent, F., 2nd. (1965). Voluntary dehydration in man. J Appl Physiol, 20(4), 719-724. doi:10.1152/jappl.1965.20.4.719

Hamouti, N., Del Coso, J., Avila, A., & Mora-Rodriguez, R. (2010). Effects of athletes' muscle mass on urinary markers of hydration status. Eur J Appl Physiol, 109(2), 213-219. doi:10.1007/s00421-009-1333-x

Hardy, C. J., & Rejeski, W. J. (1989). Not what, but how one feels: The measurement of affect during exercise. Journal of Sport & Exercise Psychology, 11(3), 304–317. doi.org/10.1123/jsep.11.3.304

Hew-Butler, T., Loi, V., Pani, A., & Rosner, M. H. (2017). Exercise-Associated Hyponatremia: 2017 Update. Front Med (Lausanne), 4, 21. doi:10.3389/fmed.2017.00021

Hew-Butler, T., Rosner, M. H., Fowkes-Godek, S., Dugas, J. P., Hoffman, M. D., Lewis, D. P., . . . Verbalis, J. G. (2015). Statement of the 3rd International Exercise-Associated Hyponatremia Consensus Development Conference, Carlsbad, California, 2015. Br J Sports Med, 49(22), 1432-1446. doi:10.1136/bjsports-2015-095004

Hoffman, M. D., Goulet, E. D. B., & Maughan, R. J. (2018). Considerations in the Use of Body Mass Change to Estimate Change in Hydration Status During a 161-Kilometer Ultramarathon Running Competition. Sports Med, 48(2), 243-250. doi:10.1007/s40279-017-0782-3

Holland, J. J., Skinner, T. L., Irwin, C. G., Leveritt, M. D., & Goulet, E. D. B. (2017). The Influence of Drinking Fluid on Endurance Cycling Performance: A Meta-Analysis. Sports Med, 47(11), 2269-2284. doi:10.1007/s40279-017-0739-6

Horio, T. (2004). Effect of physical exercise on human preference for solutions of various sweet substances. Percept Mot Skills, 99(3 Pt 1), 1061-1070. doi:10.2466/pms.99.3.1061-1070

Horio, T., & Kawamura, Y. (1998). Influence of physical exercise on human preferences for various taste solutions. Chem Senses, 23(4), 417-421. doi:10.1093/chemse/23.4.417

Howe, A. S., & Boden, B. P. (2007). Heat-related illness in athletes. Am J Sports Med, 35(8), 1384-1395. doi:10.1177/0363546507305013

Hubbard, R. W., Sandick, B. L., Matthew, W. T., Francesconi, R. P., Sampson, J. B., Durkot, M. J., . . . Engell, D. B. (1984). Voluntary dehydration and alliesthesia for water. J Appl Physiol Respir Environ Exerc Physiol, 57(3), 868-873. doi:10.1152/jappl.1984.57.3.868

Hughes, F., Mythen, M., & Montgomery, H. (2018). The sensitivity of the human thirst response to changes in plasma osmolality: a systematic review. Perioper Med (Lond), 7, 1. doi:10.1186/s13741-017-0081-4

James, L. J., Funnell, M. P., James, R. M., & Mears, S. A. (2019). Does Hypohydration Really Impair Endurance Performance? Methodological Considerations for Interpreting Hydration Research. Sports Med, 49(Suppl 2), 103-114. doi:10.1007/s40279-019-01188-5

Johnson, A. K. (2007). The sensory psychobiology of thirst and salt appetite. Med Sci Sports Exerc, 39(8), 1388-1400. doi:10.1249/mss.0b013e3180686de8

Kenefick, R. W. (2018). Drinking Strategies: Planned Drinking Versus Drinking to Thirst. Sports Med, 48(Suppl 1), 31-37. doi:10.1007/s40279-017-0844-6

Koeppen, B. M., & Stanton, B. A. (2013). Physiology of Body Fluids. In B. Koeppen & B. Stanton (Eds), Renal Physiology (5th ed., pp. 1-14). Mosby. doi.org/10.1016/B978-0-323-08691-2.00001-6

Kovacs, E. M., Senden, J. M., & Brouns, F. (1999). Urine color, osmolality and specific electrical conductance are not accurate measures of hydration status during postexercise rehydration. J Sports Med Phys Fitness, 39(1), 47-53. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10230169

Kwiatek, M. A., Menne, D., Steingoetter, A., Goetze, O., Forras-Kaufman, Z., Kaufman, E., . . . Fox, M. R. (2009). Effect of meal volume and calorie load on postprandial gastric function and emptying: studies under physiological conditions by combined fiber-optic pressure measurement and MRI. Am J Physiol Gastrointest Liver Physiol, 297(5), G894-901. doi:10.1152/ajpgi.00117.2009

Leshem, M., Abutbul, A., & Eilon, R. (1999). Exercise increases the preference for salt in humans. Appetite, 32(2), 251-260. doi:10.1006/appe.1999.0228

Lluch, A., King, N. A., & Blundell, J. E. (1998). Exercise in dietary restrained women: no effect on energy intake but change in hedonic ratings. Eur J Clin Nutr, 52(4), 300-307. doi:10.1038/sj.ejcn.1600555

Logan-Sprenger, H. M. (2019). Fluid balance and thermoregulatory responses of competitive triathletes. J Therm Biol, 79, 69-72. doi:10.1016/j.jtherbio.2018.12.003

Lopez, R. M., Casa, D. J., Jensen, K. A., Stearns, R. L., DeMartini, J. K., Pagnotta, K. D., . . . Maresh, C. M. (2016). Comparison of Two Fluid Replacement Protocols During a 20-km Trail Running Race in the Heat. J Strength Cond Res, 30(9), 2609-2616. doi:10.1519/JSC.0000000000001359

Martinez, J. R. (1990). Cellular mechanisms underlying the production of primary secretory fluid in salivary glands. Crit Rev Oral Biol Med, 1(1), 67-78. doi:10.1177/10454411900010010601

Maughan, R. J. (1998). The sports drink as a functional food: formulations for successful performance. Proc Nutr Soc, 57(1), 15-23. doi:10.1079/pns19980005

Maughan, R. J., & Leiper, J. B. (1995). Sodium intake and post-exercise rehydration in man. Eur J Appl Physiol Occup Physiol, 71(4), 311-319. doi:10.1007/BF00240410

Maughan, R. J., Leiper, J. B., & Shirreffs, S. M. (1996). Restoration of fluid balance after exercise-induced dehydration: effects of food and fluid intake. Eur J Appl Physiol Occup Physiol, 73(3-4), 317-325. doi:10.1007/BF02425493

Maughan, R. J., & Shirreffs, S. M. (1997). Recovery from prolonged exercise: restoration of water and electrolyte balance. J Sports Sci, 15(3), 297-303. doi:10.1080/026404197367308

Maughan, R. J., Shirreffs, S. M., & Leiper, J. B. (2007). Errors in the estimation of hydration status from changes in body mass. J Sports Sci, 25(7), 797-804. doi:10.1080/02640410600875143

Maughan, R. J., Shirreffs, S. M., & Watson, P. (2007). Exercise, heat, hydration and the brain. J Am Coll Nutr, 26(5 Suppl), 604S-612S. doi:10.1080/07315724.2007.10719666

McDermott, B. P., Anderson, S. A., Armstrong, L. E., Casa, D. J., Cheuvront, S. N., Cooper, L., . . . Roberts, W. O. (2017). National Athletic Trainers' Association Position Statement: Fluid Replacement for the Physically Active. J Athl Train, 52(9), 877-895. doi:10.4085/1062-6050-52.9.02

McKinley, M. J., & Johnson, A. K. (2004). The physiological regulation of thirst and fluid intake. News Physiol Sci, 19, 1-6. doi:10.1152/nips.01470.2003

Merry, T. L., Ainslie, P. N., & Cotter, J. D. (2010). Effects of aerobic fitness on hypohydration-induced physiological strain and exercise impairment. Acta Physiol (Oxf), 198(2), 179-190. doi:10.1111/j.1748-1716.2009.02051.x

Merson, S. J., Maughan, R. J., & Shirreffs, S. M. (2008). Rehydration with drinks differing in sodium concentration and recovery from moderate exercise-induced hypohydration in man. Eur J Appl Physiol, 103(5), 585-594. doi:10.1007/s00421-008-0748-0

Meyer, F., Bar-Or, O., MacDougall, D., & Heigenhauser, G. J. (1992). Sweat electrolyte loss during exercise in the heat: effects of gender and maturation. Med Sci Sports Exerc, 24(7), 776-781. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1501562

Meyer, F., Bar-Or, O., MacDougall, D., & Heigenhauser, G. J. (1992). Sweat electrolyte loss during exercise in the heat: effects of gender and maturation. Med Sci Sports Exerc, 24(7), 776-781. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1501562

Montain, S. J., & Coyle, E. F. (1992). Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. J Appl Physiol (1985), 73(4), 1340-1350. doi:10.1152/jappl.1992.73.4.1340

Montain, S. J., & Coyle, E. F. (1992). Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. J Appl Physiol (1985), 73(4), 1340-1350. doi:10.1152/jappl.1992.73.4.1340

Montain, S. J., Sawka, M. N., & Wenger, C. B. (2001). Hyponatremia associated with exercise: risk factors and pathogenesis. Exerc Sport Sci Rev, 29(3), 113-117. doi:10.1097/00003677-200107000-00005

Mukaka, M. M. (2012). Statistics corner: A guide to appropriate use of correlation coefficient in medical research. Malawi Med J, 24(3), 69-71. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23638278

Murray, R., & Stofan, J. (2000). Formulating Carbohydrate-Electrolyte Drinks for Optimal Efficacy. Sports Drinks: Basic Science and Practical Aspects, 197-224. doi:10.1201/9781420037180.ch8

Murray, R., & Stofan, J. (2000). Formulating Carbohydrate-Electrolyte Drinks for Optimal Efficacy. Sports Drinks: Basic Science and Practical Aspects, 197-224. doi:10.1201/9781420037180.ch8

Narukawa, M., Ue, H., Morita, K., Kuga, S., Isaka, T., & Hayashi, Y. (2009). Change in Taste Sensitivity to Sucrose Due to Physical Fatigue. Food Science and Technology Research - FOOD SCI TECHNOL RES, 15, 195-198. doi:10.3136/fstr.15.195

Narukawa, M., Ue, H., Uemura, M., Morita, K., Kuga, S., Isaka, T., & Hayashi, Y. (2010). Influence of Prolonged Exercise on Sweet Taste Perception. Food Science and Technology Research, 16, 513-516. doi:10.3136/fstr.16.513

Noakes, T. D., Goodwin, N., Rayner, B. L., Branken, T., & Taylor, R. K. (1985). Water intoxication: a possible complication during endurance exercise. Med Sci Sports Exerc, 17(3), 370-375. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/4021781

Noakes, T. D., Sharwood, K., Speedy, D., Hew, T., Reid, S., Dugas, J., . . . Weschler, L. (2005). Three independent biological mechanisms cause exercise-associated hyponatremia: evidence from 2,135 weighed competitive athletic performances. Proc Natl Acad Sci U S A, 102(51), 18550-18555. doi:10.1073/pnas.0509096102

Nuccio, R. P., Barnes, K. A., Carter, J. M., & Baker, L. B. (2017). Fluid Balance in Team Sport Athletes and the Effect of Hypohydration on Cognitive, Technical, and Physical Performance. Sports Med, 47(10), 1951-1982. doi:10.1007/s40279-017-0738-7

Oppliger, R. A., Magnes, S. A., Popowski, L. A., & Gisolfi, C. V. (2005). Accuracy of urine specific gravity and osmolality as indicators of hydration status. Int J Sport Nutr Exerc Metab, 15(3), 236-251. doi:10.1123/ijsnem.15.3.236

Passe, D., Horn, M., Stofan, J., Horswill, C., & Murray, R. (2007). Voluntary dehydration in runners despite favorable conditions for fluid intake. Int J Sport Nutr Exerc Metab, 17(3), 284-295. doi:10.1123/ijsnem.17.3.284

Passe, D. H., Horn, M., & Murray, R. (1997). The effects of beverage carbonation on sensory responses and voluntary fluid intake following exercise. Int J Sport Nutr, 7(4), 286-297. doi:10.1123/ijsn.7.4.286

Passe, D. H., Horn, M., & Murray, R. (2000). Impact of beverage acceptability on fluid intake during exercise. Appetite, 35(3), 219-229. doi:10.1006/appe.2000.0352

Passe, D. H., Stofan, J. R., Rowe, C. L., Horswill, C. A., & Murray, R. (2009). Exercise condition affects hedonic responses to sodium in a sport drink. Appetite, 52(3), 561-567. doi:10.1016/j.appet.2009.01.008

Perrier, E. T., Buendia-Jimenez, I., Vecchio, M., Armstrong, L. E., Tack, I., & Klein, A. (2015). Twenty-four-hour urine osmolality as a physiological index of adequate water intake. Dis Markers, 2015, 231063. doi:10.1155/2015/231063

Pliner, P. (1982). The effects of mere exposure on liking for edible substances. Appetite, 3(3), 283-290. doi:10.1016/s0195-6663(82)80026-3

Popowski, L. A., Oppliger, R. A., Patrick Lambert, G., Johnson, R. F., Kim Johnson, A., & Gisolf, C. V. (2001). Blood and urinary measures of hydration status during progressive acute dehydration. Med Sci Sports Exerc, 33(5), 747-753. doi:10.1097/00005768-200105000-00011

Rodriguez, N. R., DiMarco, N. M., Langley, S., American Dietetic, A., Dietitians of, C., American College of Sports Medicine, N., & Athletic, P. (2009). Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. J Am Diet Assoc, 109(3), 509-527. doi:10.1016/j.jada.2009.01.005

Rogers, P. J. (1999). Eating habits and appetite control: a psychobiological perspective. Proc Nutr Soc, 58(1), 59-67. doi:10.1079/pns19990009

Rolston, D. D., Hunt, J. B., Fairclough, P. D., Wilks, M., Levison, D. A., Clark, M. L., & Farthing, M. J. (1990). Jejunal water and sodium secretion occurs in chronic idiopathic intestinal pseudo-obstruction. J Clin Gastroenterol, 12(2), 153-156. doi:10.1097/00004836-199004000-00008

Romijn, J. A., Coyle, E. F., Sidossis, L. S., Gastaldelli, A., Horowitz, J. F., Endert, E., & Wolfe, R. R. (1993). Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. Am J Physiol, 265(3 Pt 1), E380-391. doi:10.1152/ajpendo.1993.265.3.E380

Sawka, M. N. (1992). Physiological consequences of hypohydration: exercise performance and thermoregulation. Med Sci Sports Exerc, 24(6), 657-670. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1602938

Sawka, M. N., Cheuvront, S. N., & Kenefick, R. W. (2015). Hypohydration and Human Performance: Impact of Environment and Physiological Mechanisms. Sports Med, 45 Suppl 1, S51-60. doi:10.1007/s40279-015-0395-7

Schleh, M. W., & Dumke, C. L. (2018). Comparison of Sports Drink Versus Oral Rehydration Solution During Exercise in the Heat. Wilderness Environ Med, 29(2), 185-193. doi:10.1016/j.wem.2018.01.005

Ship, J. A., & Fischer, D. J. (1999). Metabolic indicators of hydration status in the prediction of parotid salivary-gland function. Arch Oral Biol, 44(4), 343-350. doi:10.1016/s0003-9969(99)00004-7

Shirreffs, S. M. (2000). Markers of hydration status. J Sports Med Phys Fitness, 40(1), 80-84. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10822913

Shirreffs, S. M. (2009a). Conference on "Multidisciplinary approaches to nutritional problems". Symposium on "Performance, exercise and health". Hydration, fluids and performance. Proc Nutr Soc, 68(1), 17-22. doi:10.1017/S002966510800877X

Shirreffs, S. M. (2009b). Hydration in sport and exercise: water, sports drinks and other drinks. Nutrition Bulletin, 34(4), 374-379. doi:10.1111/j.1467-3010.2009.01790.x

Shirreffs, S. M., & Maughan, R. J. (1998). Volume repletion after exercise-induced volume depletion in humans: replacement of water and sodium losses. Am J Physiol, 274(5), F868-875. doi:10.1152/ajprenal.1998.274.5.F868

Smith, D. L., Shalmiyeva, I., Deblois, J., & Winke, M. (2012). Use of salivary osmolality to assess dehydration. Prehosp Emerg Care, 16(1), 128-135. doi:10.3109/10903127.2011.614044

Sommerfield, L. M., McAnulty, S. R., McBride, J. M., Zwetsloot, J. J., Austin, M. D., Mehlhorn, J. D., . . . Utter, A. C. (2016). Validity of Urine Specific Gravity When Compared With Plasma Osmolality as a Measure of Hydration Status in Male and Female NCAA Collegiate Athletes. J Strength Cond Res, 30(8), 2219-2225. doi:10.1519/JSC.0000000000001313

Svebak, S., & Murgatroyd, S. (1985). Metamotivational dominance: A multimethod validation of reversal theory constructs. Journal of Personality and Social Psychology, 48(1), 107–116. doi.org/10.1037/0022-3514.48.1.107

Szlyk, P. C., Hubbard, R. W., Matthew, W. T., Armstrong, L. E., & Kerstein, M. D. (1987). Mechanisms of voluntary dehydration among troops in the field. Mil Med, 152(8), 405-407. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3116459

Szlyk, P. C., Sils, I. V., Francesconi, R. P., Hubbard, R. W., & Armstrong, L. E. (1989). Effects of water temperature and flavoring on voluntary dehydration in men. Physiol Behav, 45(3), 639-647. doi:10.1016/0031-9384(89)90085-1

Takamata, A., Mack, G. W., Gillen, C. M., & Nadel, E. R. (1994). Sodium appetite, thirst, and body fluid regulation in humans during rehydration without sodium replacement. Am J Physiol, 266(5 Pt 2), R1493-1502. doi:10.1152/ajpregu.1994.266.5.R1493

Temesi, J., Johnson, N. A., Raymond, J., Burdon, C. A., & O'Connor, H. T. (2011). Carbohydrate ingestion during endurance exercise improves performance in adults. J Nutr, 141(5), 890-897. doi:10.3945/jn.110.137075

Thomas, D. R., Cote, T. R., Lawhorne, L., Levenson, S. A., Rubenstein, L. Z., Smith, D. A., . . . Dehydration, C. (2008). Understanding clinical dehydration and its treatment. J Am Med Dir Assoc, 9(5), 292-301. doi:10.1016/j.jamda.2008.03.006

Thompson, J. L., Butterfield, G. E., Gylfadottir, U. K., Yesavage, J., Marcus, R., Hintz, R. L., . . . Hoffman, A. R. (1998). Effects of human growth hormone, insulin-like growth factor I, and diet and exercise on body composition of obese postmenopausal women. J Clin Endocrinol Metab, 83(5), 1477-1484. doi:10.1210/jcem.83.5.4826

Thornton, S. N. (2010). Thirst and hydration: Physiology and consequences of dysfunction. Physiology and Behaviour, 100(1), 15-21. https://doi.org/10.1016/j.physbeh.2010.02.026

Tsintzas, O. K., Williams, C., Singh, R., Wilson, W., & Burrin, J. (1995). Influence of carbohydrate-electrolyte drinks on marathon running performance. Eur J Appl Physiol Occup Physiol, 70(2), 154-160. doi:10.1007/BF00361543

Verger, P., Lanteaume, M. T., & Louis-Sylvestre, J. (1992). Human intake and choice of foods at intervals after exercise. Appetite, 18(2), 93-99. doi:10.1016/0195-6663(92)90186-a

Villiger, M., Stoop, R., Vetsch, T., Hohenauer, E., Pini, M., Clarys, P., . . . Clijsen, R. (2018). Evaluation and review of body fluids saliva, sweat and tear compared to biochemical hydration assessment markers within blood and urine. Eur J Clin Nutr, 72(1), 69-76. doi:10.1038/ejcn.2017.136

Wald, N., & Leshem, M. (2003). Salt conditions a flavor preference or aversion after exercise depending on NaCl dose and sweat loss. Appetite, 40(3), 277-284. doi:10.1016/s0195-6663(03)00013-8

Walsh, N. P., Blannin, A. K., Clark, A. M., Cook, L., Robson, P. J., & Gleeson, M. (1999). The effects of high-intensity intermittent exercise on saliva IgA, total protein and alpha-amylase. J Sports Sci, 17(2), 129-134. doi:10.1080/026404199366226

Walsh, N. P., Laing, S. J., Oliver, S. J., Montague, J. C., Walters, R., & Bilzon, J. L. (2004a). Saliva parameters as potential indices of hydration status during acute dehydration. Med Sci Sports Exerc, 36(9), 1535-1542. doi:10.1249/01.mss.0000139797.26760.06

Walsh, N. P., Montague, J. C., Callow, N., & Rowlands, A. V. (2004b). Saliva flow rate, total protein concentration and osmolality as potential markers of whole body hydration status during progressive acute dehydration in humans. Arch Oral Biol, 49(2), 149-154. doi:10.1016/j.archoralbio.2003.08.001

Westerterp-Plantenga, M. S., Verwegen, C. R., Ijedema, M. J., Wijckmans, N. E., & Saris, W. H. (1997). Acute effects of exercise or sauna on appetite in obese and nonobese men. Physiol Behav, 62(6), 1345-1354. doi:10.1016/s0031-9384(97)00353-3

Wilk, B., & Bar-Or, O. (1996). Effect of drink flavor and NaCL on voluntary drinking and hydration in boys exercising in the heat. J Appl Physiol (1985), 80(4), 1112-1117. doi:10.1152/jappl.1996.80.4.1112

Wilmore, J. H., Morton, A. R., Gilbey, H. J., & Wood, R. J. (1998). Role of taste preference on fluid intake during and after 90 min of running at 60% of VO2max in the heat. Med Sci Sports Exerc, 30(4), 587-595. doi:10.1097/00005768-199804000-00018

Wong, S. H., & Sun, F. H. (2014). Effect of beverage flavor on body hydration in Hong Kong Chinese children exercising in a hot environment. Pediatr Exerc Sci, 26(2), 177-186. doi:10.1123/pes.2013-0080

Yeomans, M. R. (1998). Taste, palatability and the control of appetite. Proc Nutr Soc, 57(4), 609-615. doi:10.1079/pns19980089

Yeomans, M. R., Jackson, A., Lee, M. D., Steer, B., Tinley, E., Durlach, P., & Rogers, P. J. (2000). Acquisition and extinction of flavour preferences conditioned by caffeine in humans. Appetite, 35(2), 131-141. doi:10.1006/appe.2000.0339

Zubac, D., Reale, R., Karnincic, H., Sivric, A., & Jelaska, I. (2018). Urine specific gravity as an indicator of dehydration in Olympic combat sport athletes; considerations for research and practice. Eur J Sport Sci, 18(7), 920-929. doi:10.1080/17461391.2018.1468483

APPENDICES

Appendix A. Participant Information Sheet

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Sensory perception of oral rehydration solution at different levels of exercise-induced dehydration

PARTICIPANT INFORMATION SHEET

Researcher Introduction

We are physical activity, human health and food technology researchers at Massey University. We would like to invite you to take part in a study investigating sensory properties of three drinks before, during and after exercise-induced dehydration.

Invitation to Participate in Research Study

Sports drinks have become very popular aids for athletes and exercisers all over the world. Nevertheless, because they are often perceived as being too high in sugar content, having too many artificial ingredients and being too sweet, some athletes will choose not to consume the most popular brands and find alternative rehydration solutions. Moreover, some athletes who become dehydrated following their event may seek products that were originally developed for individuals suffering from diarrhoea i.e. oral rehydration solutions (ORS) – especially those who may sweat more than others and/or are 'salty' sweaters. Existing rehydration products in the sports drink market contain relatively low levels of sodium (225-450 mg/L) and higher levels of carbohydrates (7-8%). We have developed an ORS that shows good acceptability with consumers. However, sensory testing was conducted in a resting, well-hydrated state and we need to assess the acceptability of the product when participants are dehydrated as, previously we have shown that exercise and dehydration affects ratings of sweetness, saltiness and liking of a sports drink. Therefore, the aim of this study is to undertake sensory analysis and consumer acceptability of an ORS at rest and at varying levels of dehydration.

Participant Recruitment

Men and women (aged 18-50 years) are invited to participate in this study. Individuals with any of the conditions listed on the "health checklist" should not volunteer to participate. If you are unsure about any of the listed conditions, then you should consult with the researchers. Upon completion of the study, each participant will receive a $20 MTA voucher (per visit) to cover travel expenses for participation.

Project Procedures and Participant Involvement

If you agree to participate, you will be asked to come to the Sport and Exercise Laboratory on two occasions (Building 60 Massey University Oteha Rohe Campus, Albany Highway, Albany). You will be asked to wear appropriate comfortable clothing. The first visit (60 min) will be for a familiarisation of the procedures and equipment to be used for the main trial, as well as completing a short exercise test (to find out a moderate intensity cycling intensity; 70% heart rate max). For the main trial, we will initially obtain a urine sample (to estimate hydration status and used for electrolyte analysis) and saliva sample (using chewing saliva method, for electrolyte and protein analysis), as well as aural (inner ear) temperature and then (in a private room) measure body mass. After donning the 'sweat suit' (waterproof rain jacket and pants), you will perform moderate intensity cycling exercise, for 3 x 20-min bouts, in a warm room (35°C). You will complete a sensory evaluation at 0, 20, 40, and 60 min of exercise with three different drinks. Therefore, four sensory evaluation tests will be performed for each solution. We will provide 3 x 20-ml sample of each of the three solutions at each 20-min interval to undertake sensory analysis (assessment of sweetness, saltiness, thirst-quenching ability, and liking of the beverage). Saliva samples will be collected at the end of each 20-min bout of exercise. Aural temperature and body mass (after towel drying) will be measured after each 20-min bout of exercise. You will be asked to refrain from consuming caffeine and alcohol, and not to exercise for 24 hours prior to the main trials. You will also need to record your diet for 24 h prior to the main trial.

Participant's Rights

You are under no obligation to accept this invitation. Should you choose to participate, you have the right to: • decline to answer any particular question
• withdraw from the study at any time, even after signing a consent form (if you choose to withdraw you cannot withdraw your data from the analysis after the data collection has been completed)

• ask any questions about the study at any time during participation
• provide information on the understanding that your name will not be used unless you give permission to the researcher
• be given access to a summary of the project findings when it is concluded

Good Practice and Cultural Safety for Massey University Research

We have discussed this type of study with Messina Shaw (Student Recruitment Adviser - Māori Academic Support). We have considered the inclusion of Māori and indigenous values and concepts, allowing for the use of whānau support and appropriate Māori protocols. We acknowledge the concept of manaakitanga, respecting the participant's inherent dignity and acting in a caring manner towards them by way of:

- Taking full responsibility to perform research in a safe and ethical manner (aroha)
- Providing the participant with all of the critical information regarding the study in a clear way, so they can make informed decisions (tūmanako and whakapono)
- An awareness of the cultural significance and sensitivity for a culturally safe implementation of the study (māhaki)
- Respect for the privacy and confidentiality of Māori participants
- Acknowledging the tapu (sacred) nature of blood/human tissue by offering remaining samples (if appropriate) back to the donor and keeping human samples secured and separated from other biological material, to ensure that the tapu māheuheu is not mixed with or contaminated by other tapu or noa (profane) substances.

Confidentiality

All data collected will be used solely for research purposes and has the possibility of being presented at an international journal. All personal information will be kept confidential by assigning numbers to each participant. No names will be visible on any papers on which you provide information. All data/information will be dealt with confidentiality and will be stored in a secure location for five years on the Massey University Albany Campus. After this time, it will be disposed of by an appropriate staff member from the School of Sport, Exercise and Nutrition.

Project Contacts

If you have any questions regarding this study, please do not hesitate to contact either of the following people for assistance:

A/Prof Ajmol Ali (School of Sport, Exercise and Nutrition, Massey University) a.ali@massey.ac.nz (09) 213 6414

Ms Mathilde Cancalon (School of Sport, Exercise and Nutrition, Massey University) Mathilde.Cancalon@enscbp.fr (09) 213 6494

Dr Charles Diako (School of Food and Advanced Technology, Massey University) C.Diako@massey.ac.nz (09) 213 6637

A/Prof Andrew Foskett (School of Sport, Exercise and Nutrition, Massey University) a.foskett@massey.ac.nz (09) 213 6412

Dr Wendy O'Brien (School of Sport, Exercise and Nutrition, Massey University) W.J.OBrien@massey.ac.nz (09) 213 6494

A/Prof Kay Rutherfurd-Markwick (School of Health Sciences, Massey University) k.j.rutherfurd@massey.ac.nz (09) 213 6646

A/Prof Marie Wong (School of Food and Advanced Technology, Massey University) M.Wong@massey.ac.nz (09) 213 6656

Committee Approval Statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application xx/xx. If you have any concerns about the conduct of this research, please contact Dr Lesley Batten, Chair, Massey University Human Ethics Committee: Southern A, telephone 06 356 9099 x 85094, email humanethicsoutha@massey.ac.nz .

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted, you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Appendix B. Health screening Questionnaire

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Sensory perception of oral rehydration solution at different levels of exercise-induced dehydration

Health Screening Questionnaire

Name:____

Address: ____

Phone: ________________________________

Age: ________________

Gender:_______________

Please read the following questions carefully. If you have any difficulty, please advise the medical practitioner, nurse or exercise specialist who is conducting the exercise test.

Please answer all of the following questions by ticking only one box for each question:

The questions are based upon the Physical Activity Readiness Questionnaire (PAR-Q), originally devised by the British Columbia Dept of Health (Canada), as revised by [1]Thomas *et al.* (1992) and [2]Cardinal *et al.* (1996), and with added requirements of the Massey University Human Ethics Committee. The information provided by you on this form will be treated with the strictest confidentiality.

Qu 1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

Yes ☐ No ☐

Qu 2. Do you feel a pain in your chest when you do physical activity?

Yes ☐ No ☐

Qu 3. In the past month have you had chest pain when you were not doing physical activity?

Yes ☐ No ☐

Qu 4. Do you lose your balance because of dizziness or do you ever lose consciousness?

Yes ☐ No ☐

Qu 5. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?

Yes ☐ No ☐

Qu 6. Have you been hospitalised recently?

Yes ☐ No ☐

Qu 7. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?

Yes ☐ No ☐

Qu 8. Have any immediate family had heart problems prior to the age of 60?

Yes ☐ No ☐

Qu 9. Are there any issues that may prevent you from completing an approximately 45 min moderate intensity effort cycle (adjusted for your fitness level) in warm room (30°C) while wearing a sweat suit? If yes, please explain.

Yes ☐ No ☐

____

____

____

I have read, understood and completed this questionnaire.

Signature ***(Participant):*** ____ Date: ___________________

References

1. Thomas S, Reading J and Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can J Sport Sci* 17(4): 338-345.
2. Cardinal BJ, Esters J and Cardinal MK. Evaluation of the revised physical activity readiness questionnaire in older adults. *Med Sci Sports Exerc* 28(4): 468-472

Appendix C. Participant Consent Form

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Sensory perception of oral rehydration solution at different levels of exercise-induced dehydration

CONSENT FORM FOR STUDY VOLUNTEERS

This consent form will be held for a minimum period of five (5) years

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I understand that I have the right to withdraw from the study at any time and to decline to answer any particular questions (if I choose to withdraw I cannot withdraw my data from the analysis after the data collection has been completed).

I agree to provide information to the researcher on the understanding that my name will not be used without my permission. (The information will be used only for this research and publications arising from this research project.)

I agree to participate in this study under the conditions set out in the Information Sheet.

Signature: ____
Date____________________
Full Name (printed) ____
Phone Number_______________________ **Age** _______________ **Date of Birth** _________

Are you willing to be contacted regarding future research projects within the School of Sport, Exercise and Nutrition? Your name and email address will be saved in a secure location. You will be sent periodic newsletters regarding research studies within the School. You can opt out of this newsletter at any time.

Tick here if you accept. ☐

Appendix D. Letter of Ethics Approval from Massey University Human Ethics Committee

MASSEY
UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Date: 05 August 2019

Dear A/Pro Aj Ali
Re: Ethics Notification - SOA 19/38 - Sensory perception of oral rehydration solution (ORS) at

different levels of exercise-induced dehydration

Thank you for the above application that was considered by the Massey University Human Ethics Committee: Human Ethics Southern A Committee at their meeting held on Monday, 5 August, 2019.

Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

Professor Craig Johnson
Chair, Human Ethics Chairs' Committee and Director (Research Ethics)

Research Ethics Office, Research and Enterprise
Massey University, Private Bag 11 222, Palmerston North, 4442, New Zealand T 06 350 5573; 06 350 5575 F 06 355 7973 E humanethics@massey.ac.nz W http://humanethics.massey.ac.nz

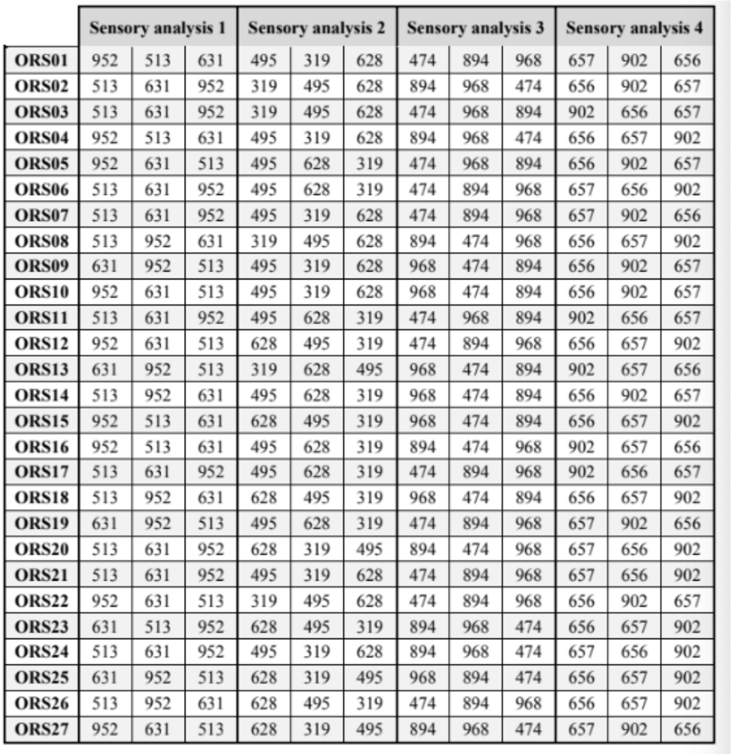
Appendix E. Supplementary Methods

Sensory protocol

To ensure the sensory protocol was double-blinded, samples were identified by a random three-digit code using a label (Table 6.1). Samples were presented in the order of the test matrix (Figure 6.1) according to instructions given by an iPad application (iPad, Apple Inc, Cupertino, California). For each sensory property (saltiness, sweetness, thirst-quenching ability, overall liking), participants were asked to rate their liking on a 9-point hedonic scale (Figure 6.2).

Table 6.1. Sample codes

	Sensory analysis 1	**Sensory analysis 2**	**Sensory analysis 3**	**Sensory analysis 4**
ORS	631	628	894	657
PL	513	319	968	902
TS	952	495	474	656



	Sensory analysis 1			**Sensory analysis 2**			**Sensory analysis 3**			**Sensory analysis 4**		
ORS01	952	513	631	495	319	628	474	894	968	657	902	656
ORS02	513	631	952	319	495	628	894	968	474	656	902	657
ORS03	513	631	952	319	495	628	474	968	894	902	656	657
ORS04	952	513	631	495	319	628	894	968	474	656	657	902
ORS05	952	631	513	495	628	319	474	968	894	656	902	657
ORS06	513	631	952	495	628	319	474	894	968	657	656	902
ORS07	513	631	952	495	319	628	474	894	968	657	902	656
ORS08	513	952	631	319	495	628	894	474	968	656	657	902
ORS09	631	952	513	495	319	628	968	474	894	656	902	657
ORS10	952	631	513	495	319	628	968	474	894	656	902	657
ORS11	513	631	952	495	628	319	474	968	894	902	656	657
ORS12	952	631	513	628	495	319	474	894	968	656	657	902
ORS13	631	952	513	319	628	495	968	474	894	902	657	656
ORS14	513	952	631	495	628	319	968	474	894	656	902	657
ORS15	952	513	631	628	495	319	968	474	894	656	657	902
ORS16	952	513	631	495	628	319	894	474	968	902	657	656
ORS17	513	631	952	495	628	319	474	894	968	902	656	657
ORS18	513	952	631	628	495	319	968	474	894	656	657	902
ORS19	631	952	513	495	628	319	474	894	968	657	902	656
ORS20	513	631	952	628	319	495	894	474	968	657	656	902
ORS21	513	631	952	495	319	628	474	894	968	657	656	902
ORS22	952	631	513	319	495	628	474	894	968	656	902	657
ORS23	631	513	952	628	495	319	894	968	474	656	657	902
ORS24	513	631	952	495	319	628	894	968	474	657	656	902
ORS25	631	952	513	628	319	495	968	894	474	656	657	902
ORS26	513	952	631	628	495	319	474	894	968	656	657	902
ORS27	952	631	513	628	319	495	894	968	474	657	902	656

Figure 6.1. Sample presentation order matrix

Figure 6.2. Sensory 9-point hedonic scale

Collection of a urine specimen for refractometry and osmolality

Equipment

Refractometer and deionised water for calibration
Osmometer (Astori Technica- Osmotouch 1 osmometer, Poncarale, Italy).
Colour scale (Figure 6.3.)
Transfer pipettes
Urine collection tray
Barrier gloves

Collection:

1. Provide participant with the instructions below, a urine collection tray and barrier gloves.
2. In the privacy of a toilet the participant passes initial urine into the toilet**.**
3. From now on collect **all** urine passed into the urine collection tray
   If bowels need to be evacuated, collect urine so that no urine is lost.
4. The participant passes the urine collection tray to the researcher.

Measurement:

- Ensure the refractometer provides a zero reading with deionised water. Calibrate with a flathead screwdriver if necessary. Use a transfer pipette to asprire ~1 mL of urine onto the measurement surface of the refractometer. The lid must be closed gently otherwise urine may splatter. Take the reading. Clean the refractometer with tap water and disposable paper towels.
- Assess osmolality using the osmometer.
- Assess urine colour using the urine colour scale.
- Discard the urine contaminated collection tray and transfer pipette into a biohazard bag. Finally dispose of the barrier gloves into a biohazard bag.

Health and Safety:

Staff:

1) Apply standard precautions when dealing with biological samples. See College of Health Safety Manual.

2) This is an aseptic procedure, and aseptic technique should be followed.

3) Disposable latex gloves should be worn. Before using these gloves, check whether the trial participant is allergic to latex and where necessary use non-latex gloves (e.g. nitrile)

Figure 6.3. Urine colour scale

Collection of a saliva specimen for osmolality and protein content

Equipment

Scales

A seat for the participant

Sample collection pot

Barrier gloves

Sterile Q-tip cotton bud

Transfer pipettes

Sterile paraffin wax

Scales accurate to 1 mg

Osmometer (Astori Technica- Osmotouch 1 osmometer, Poncarale, Italy)

Spectrophotometer (Tecan)

Whatman #1 paper (Whatman)

Collection:

1. Weigh the saliva collection pot to an accuracy of 1 mg, record this value.
2. Provide the participant with the instructions below, barrier gloves and a sample collection pot. Ensure it has been at least 5 mins since their last drink or meal.
3. The participant should sit, leaning forwards with their head tilted downwards. The participant should swallow before any sample is collected. 1 mL of saliva is needed for meaningful analyses.

   For the **Novel Bud Method** participants are provided a sterile Q-tip to place into their mouth between the cheek and molars on whichever side feels comfortable. During sampling it is important that participants perform minimal orofacial movement – no laughing, smiling, talking etcetera. Saliva can collect under the tongue, participants are able to push the saliva towards the bud with their tongue if necessary. After two minutes the participant places the bud into the sample collection pot. The bud should look very moist with a saturated appearance. If the bud appears dry ask the participant if they would place it back into their mouth again for a further minute.

4. Record the total time taken for sample collection. The researcher should don barrier gloves and measure the weight of the sample collection pot including saliva.
5. Saliva specimens are stored upright at < -20°C. All gloves may be discarded into a biohazardous waste receptacle.

Measurement:

- Measure saliva osmolality using the osmometer.
- Assess saliva protein concentration using the **Bradford Method** (Bradford, 1976).

For the **Bradford reagent**, dissolve 50 mg of Coomassie Brilliant Blue G-250 (Sigma-Aldrich, catalog number: 27815) in 50 ml of methanol and add 100 ml 85% (w/v) phosphoric acid (H3PO4). Add the acid solution mixture slowly into 850 ml of H2O and let the dye dissolve completely (*note: Do not add H2O into the acid solution*). Filter using Whatman #1 paper to remove the precipitates just before use. Store in a dark bottle at 4 °C.

For the **Standard assay procedure** (for samples with 5-100 µg ml^{-1} protein), prepare five to eight dilutions of a protein (usually Bovine Serum Albumin; BSA; Sigma-Aldrich) standard with a range of 5 to 100 ug protein. Dilute unknown protein samples to obtain 5-100 ug protein/30 ul. Add 30 ul each of standard solution and unknown protein sample to an appropriately labelled test tube. Set two blank tubes. For the standard curve, add 30 ul H_2O instead of the standard solution. For the unknown protein samples, add 30 ul protein preparation buffer instead. Protein solutions are normally assayed in duplicate or triplicate. Add 1.5 ml of Bradford reagent to each tube and mix well. Incubate at room temperature for at least 5 min and no more than 1 h. Measure absorbance at 595 nm.

For the **Microassay procedure** (<50 µg ml^{-1} protein), prepare five standard solutions (1 ml each) containing 0, 10, 20, 30, 40 and 50 ug ml^{-1} BSA. Pipette 800 ul of each standard and sample solution (containing <50 ug ml^{-1} protein) into a clean, dry test tube. Add 200 ul of dye reagent to each tube and vortex. Follow the procedure described above for the standard assay procedure.

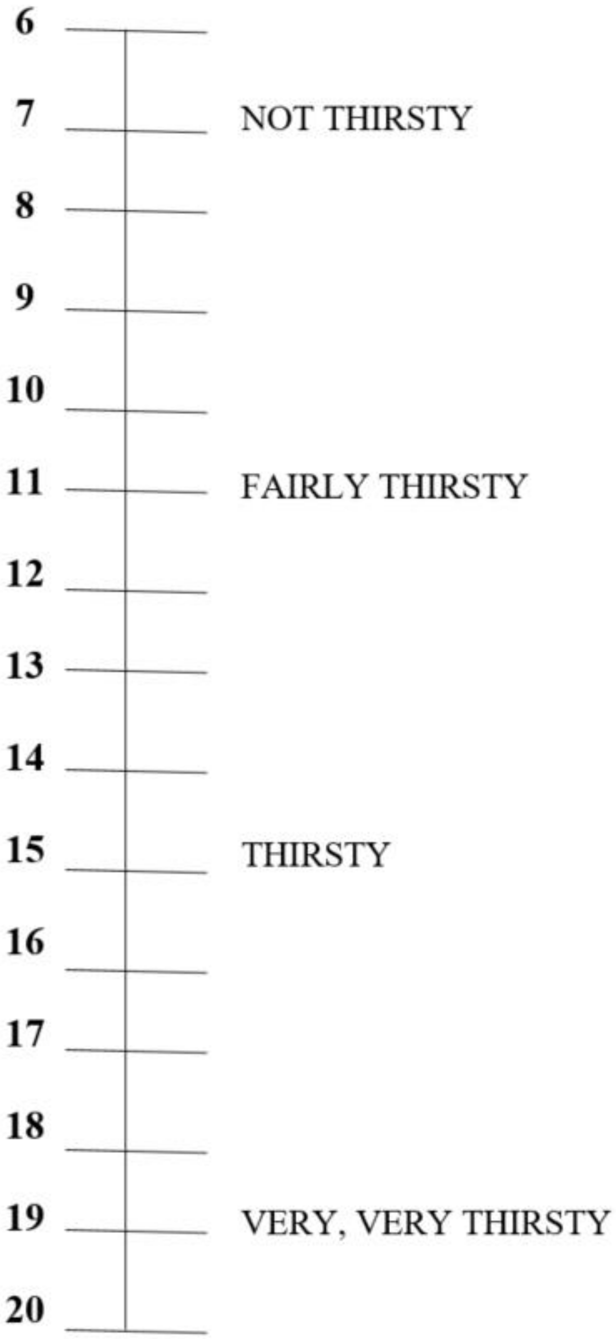
Health and Safety:

Staff:

1) Apply standard precautions when dealing with biological samples. See IFNHH Safety Manual.
2) This is an aseptic procedure, and aseptic technique should be followed.
3) Disposable latex gloves should be worn. Before using these gloves, check whether the trial participant is allergic to latex and where necessary use non-latex gloves (e.g. nitrile).

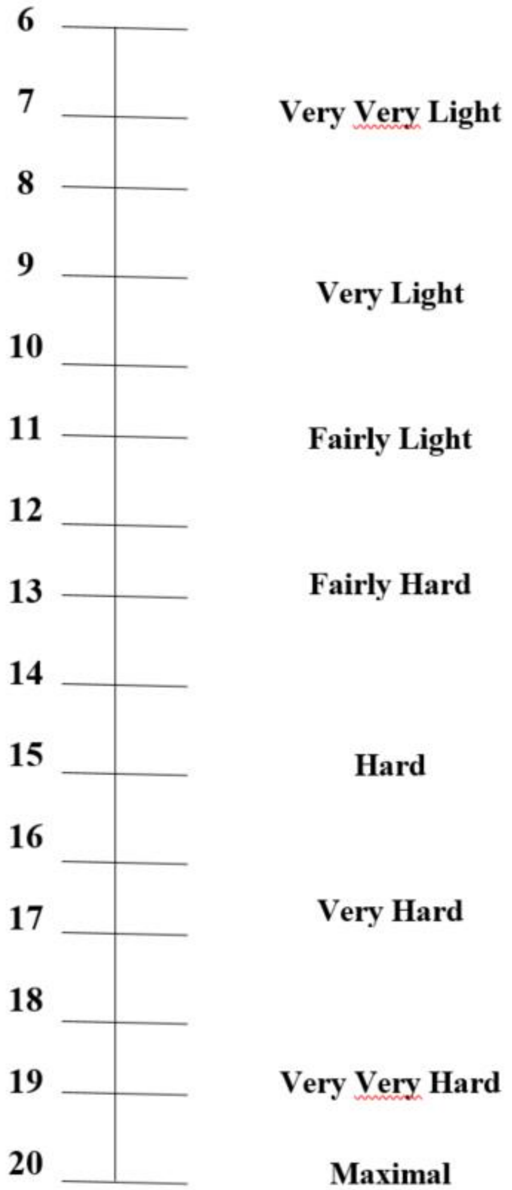
Perceptual scales

Thirst Scale



Feelings of thirst indicate that you may be dehydrated. Please use this scale to indicate your level of thirst at this moment in time.

Rating of Perceived Exertion



6	
7	Very Very Light
8	
9	Very Light
10	
11	Fairly Light
12	
13	Fairly Hard
14	
15	Hard
16	
17	Very Hard
18	
19	Very Very Hard
20	Maximal

During the exercise bout, we want you to pay close attention to how hard you feel the exercise work rate is. This feeling should reflect your total amount of exertion and fatigue,

combining all sensations and feelings of physical stress, effort, and fatigue.

Try not to underestimate or overestimate your feeling of exertion; be as accurate as you can.

FEELING SCALE

+5	Very good
+4	
+3	Good
+2	
+1	Fairly good
0	Neutral
-1	Fairly bad
-2	
-3	Bad
-4	
-5	Very bad

While participating in exercise, it is common to experience changes in mood. Some individuals find exercise pleasurable, whereas others find it to be unpleasurable. Additionally, feeling may fluctuate across time. That is, one might feel good and bad a number of times during exercise. Scientists have developed this scale to measure such responses.

FELT AROUSAL SCALE

1 Low arousal

2

3

4

5

6 High arousal

By “arousal” we mean how “worked-up” you feel. You might experience high arousal in one of a variety of ways, for example as excitement or anxiety or anger. Low arousal might also be experienced by you in one of a number of different ways, for example as relaxation or boredom or calmness.

Appendix F. Supplementary Results

Table 6.2. Sensory ratings for each fluid at each time point throughout exercise

	Liking of sweetness			**Liking of saltiness**			**Thirst-quenching ability**			**Overall liking**		
Time	ORS	PL	TS	ORS	PL	TS	ORS	PL	TS	ORS	PL	TS
0-min†	4.4 ± 1.6	5.6 ± 1.6	6.2 ± 1.8	3.2 ± 1.9	5.3 ± 1.5	6.0 ± 1.3	3.9 ± 2.0	6.0 ± 1.4	6.0 ± 1.4	3.4 ± 1.9	5.9 ± 1.6	6.3 ± 1.6
20-min†	4.5 ± 1.5	5.8 ± 1.3	5.9 ± 2.0	4.0 ± 2.0	5.6 ± 1.5	5.9 ± 1.3	4.5 ± 1.8	5.9 ± 1.6	5.7 ± 1.8	4.2 ± 2.0	5.8 ± 1.4	6.0 ± 1.7
40-min†	4.5 ± 1.6	5.9 ± 1.6	5.9 ± 2.3	4.3 ± 2.2	5.4 ± 1.6	5.8 ± 1.4	4.6 ± 1.9	6.3 ± 1.9	5.5 ± 2.2	4.3 ± 2.1	6.1 ± 1.4	6.0 ± 2.0
60-min†	4.7 ± 1.8	5.8 ± 1.7	5.9 ± 2.3	4.2 ± 2.3	5.5 ± 1.8	6.0 ± 1.9	4.8 ± 1.8	6.5 ± 1.7	5.7 ± 2.3	4.1 ± 2.2	6.2 ± 1.6	6.0 ± 2.1
Δchange (%)‡	0.0 (-20, 25)	0.0 (0.0, 33.3)	0.0 (-16.7, 28.6)	20.0 (0.0, 100)*	0.0 (-16.7, 20)	0.0 (-27.2, 20)	50.0 (0.0, 66.7)*	0.0 (-14.3, 28.6)	0.0 (-25, 14.3)	0.0 (0.0, 100)	0.0 (-12.5, 40)	0.0 (-20, 0.0)

Sensory ratings (9-point hedonic scale) throughout 60-min of exercise for each fluid: ORS - oral rehydration solution, PL – placebo, TS - traditional sports drink.

†Values are means ± SD.

‡Values are medians (25, 75 percentiles).

*Significant difference in percent change after 60 min of exercise compared to 0 min before exercise (p<0.05).