



MONASH University

**Exertional-heat stress induced gastrointestinal perturbations:
Prevention and management strategies**

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A thesis submitted for the degree of Doctor of Philosophy at

Monash University in 2017

Department of Nutrition, Dietetics and Food

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Abstract

Prolonged physical exertion in the heat substantially challenges thermoregulation and cardiovascular function, resulting in an exaggerated reduction in blood flow to the gastrointestinal tract.(1) This reduction in blood flow and hyperthermia during prolonged exertional-heat stress may compromise the integrity of the gastrointestinal tract which can lead to gastrointestinal symptoms, systemic endotoxaemia and cytokinaemia, and may have acute and chronic health implications, such as fatal septic shock, ischemic colitis and inflammatory bowel disease.(2)

Therefore, the purpose of this thesis was to firstly, investigate the effect of exposure to 35°C ambient temperature (T_{amb}) and 30°C T_{amb} during prolonged physical exertion on gastrointestinal integrity, symptoms, systemic endotoxin and cytokine profile, and explore relationships between changes in core body temperature and gastrointestinal perturbations. Secondly, this thesis aimed to investigate the effect of novel nutrition prevention and management strategies, including carbohydrate and protein intake, and the temperature of ingested water, on exertional-heat stress induced perturbations to gastrointestinal integrity, symptoms and systemic responses.

Findings from this thesis indicate that exposure to 30°C and 35°C during 2 h running at 60% maximal oxygen uptake ($\dot{V}O_{2max}$) increases intestinal epithelial injury, gastrointestinal symptoms and cytokinaemia compared to running in temperate (22°C) conditions. However, heat exposure did not affect small intestine permeability or intestinal inflammation above equivalent exercise in temperate conditions. Further, exposure to 35°C during prolonged physical exertion resulted in more substantial and prolonged gastrointestinal perturbations than observed on 30°C, including perturbations to endotoxin profile and a greater compensatory anti-inflammatory cytokine profile. Moreover, it was observed that increases in rectal temperature during physical exertion were positively associated with intestinal epithelial injury, symptoms of nausea and urge to regurgitate, and the compensatory anti-inflammatory cytokine response.

Frequent carbohydrate (i.e., glucose) and protein (i.e., hydrolysed whey) ingestion before and during exertional-heat stress (35°C T_{amb}) abolished intestinal epithelial injury and reduced small intestine permeability compared to when only water was ingested. Carbohydrate also reduced post-exercise plasma interleukin (IL)-6 and increased anti-endotoxin antibodies compared to water intake. Whereas, protein increased the incidence and severity of gastrointestinal symptoms compared to both carbohydrate and water intake. Consumption of cold (0°C) and cool (7°C) water before and during exertional-heat stress showed trends for modest reductions in intestinal epithelial injury and upper-gastrointestinal symptoms compared to temperate (22°C) water intake. However, the temperature of consumed water had no effect on systemic cytokine profile.

Collectively, the studies within this thesis demonstrate that heat exposure during prolonged physical exertion substantially injures the intestinal epithelium and contributes to the development of gastrointestinal symptoms, systemic cytokinaemia and perturbs systemic endotoxin profile. The studies are the first to clearly establish a link between exertional-heat stress and gastrointestinal symptoms. The prevention and management studies in this thesis present novel and practical nutrition intervention strategies that ameliorate gastrointestinal perturbations during exertional-heat stress. The most effective strategy to support gastrointestinal health during exertional-heat stress appears to be regular carbohydrate consumption. In conjunction with carbohydrates, regular cold or cool fluid intake may be a beneficial strategy to attenuate thermoregulatory strain, support gastrointestinal health and reduce upper-gastrointestinal symptoms in some individuals.

Thesis including published works declaration

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

This thesis includes one original paper published in a peer reviewed journal, one paper accepted for publication and two submitted publications. The core themes of the thesis are sports medicine, applied exercise physiology and sports nutrition. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the Department of Nutrition, Dietetics and Food under the primary supervision of Dr. Ricardo Costa. The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research. In the case of chapters 4-7, my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status	Nature and % student contribution	Co-author name(s) Nature and % of Co-author's contribution	Co-author(s), Monash student
4	The impact of exertional-heat stress on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profile	Submitted	60% Concept, data and sample collection, data and sample analysis, drafting manuscript	1) Anthony Khoo, assisted with data collection, final manuscript review 2% 2) Cecilia Kitic, data analysis, input in to manuscript 2% 3) Peter Gibson, concept and input in to manuscript 1% 4) Ricardo Costa, concept, assistance with data and sample collection, data and sample analysis, input in to manuscript, 35%	1) Yes 2) No 3) No 4) No
5	The impact of mild heat stress during prolonged running on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profile.	Accepted	60% Concept, data and sample collection, data and sample analysis, drafting manuscript	1) Anthony Khoo, assisted with data collection, final manuscript review 2% 2) Cecilia Kitic, data analysis, input in to manuscript 2% 3) Peter Gibson, concept and input in to manuscript 1% 4) Ricardo Costa, concept, assistance with data and sample collection, data and sample analysis, input in to manuscript, 35%	1) Yes 2) No 3) No 4) No

6	Carbohydrate and protein intake during exertional-heat stress ameliorates intestinal epithelial injury and small intestine permeability.	Published	60% Concept, data and sample collection, data and sample analysis, drafting manuscript	1) Anthony Khoo, assisted with data collection, final manuscript review 2% 2) Cecilia Kitic, data analysis, input in to manuscript 2% 3) Peter Gibson, concept and input in to manuscript 1% 4) Ricardo Costa, concept, assistance with data and sample collection, data and sample analysis, input in to manuscript, 35%	1) Yes 2) No 3) No 4) No
7	Does the temperature of water ingested during exertional-heat stress influence gastrointestinal injury, symptoms and inflammatory cytokine profile?	Submitted	75% Concept, data and sample collection, data and sample analysis, drafting manuscript	1) Ricardo Costa, concept, data and sample analysis, input in to manuscript, 25%	1) No

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

Student signature:



Date: 23rd October 2017

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

Main Supervisor signature:



Date: 25th October 2017

Publications

The following two publications are included as **Chapter 5** and **6**, respectively within this thesis with the contribution to publications previously outlined in the 'thesis including published works declaration'.

Snipe RMJ, Kitic C, Khoo A, Gibson P, Costa RJS. The impact of mild heat stress during prolonged running on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profiles. *Int J Sports Med.*2017[In Press].

Snipe RMJ, Kitic C, Khoo A, Gibson P, Costa RJS. Carbohydrate and protein intake during exertional heat stress ameliorates intestinal epithelial injury and small intestine permeability. *Appl Physiol Nutr Metab.* 2017;DOI:10.1139/apnm-2017-0361.

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

Costa RJS, Snipe RMJ, Kitic C, Gibson P. low-FODMAP diet for exercise-induced gastrointestinal syndrome - authors' reply. *Aliment Pharmacol Ther.* 2017;DOI: 10.1111/apt.14358.

Costa RJS, Snipe RMJ, Kitic C, Gibson P. Systematic review: Exercise-induced gastrointestinal syndrome - Implications for health and intestinal disease. *Aliment Pharmacol Ther.* 2017;46:246–265.

Miall A, Khoo A, Rauch C, Snipe RMJ, Camões-Costa V, Gibson P et al. Two weeks of repetitive gut-challenge reduces exercise associated gastrointestinal symptoms and malabsorption. *Scand J Med Sci Sports*. 2017;DOI:10.1111/sms.12912.

Costa RJS, Miall A, Khoo A, Rauch C, Snipe RMJ, Camões-Costa V et al. Gut-training: The impact of two weeks repetitive gut-challenge during exercise on gastrointestinal status, glucose availability, fuel kinetics, and running performance. *Appl Physiol Nutr Metab*. 2017;42(5):547-557.

Costa RJS, Snipe RMJ, Camões-Costa V, Scheer BV, Murray A. The impact of gastrointestinal symptoms and dermatological injuries on nutritional intake and hydration status during ultramarathon events. *Sports Medicine – Open*. 2016;2(16):1-14.DOI: 10.1186/s40798-015-0041-9.

Submitted publications

The following two publications are included as **Chapter 4** and **7**, respectively within this thesis. The contribution to these publications has previously been outlined in the ‘thesis including published works declaration’.

Snipe RMJ, Kitic C, Khoo A, Gibson P, Costa RJS. The impact of exertional-heat stress on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profile. *Eur J Appl Physiol*. In review.

Snipe RMJ, Costa RJS. Does the temperature of consumed water during exertional-heat stress influence gastrointestinal injury, symptoms and cytokinaemia? *J Sci Med Sport*. In review.

Conference abstracts

Snipe R, Costa RJS. Does the temperature of consumed water during exertional-heat stress influence gastrointestinal injury and symptoms? In: Sports Dietitians Australia, editor. Propelling your knowledge and practice: Proceedings of Sports Dietitians Australia Conference; 20-21 Oct 2017; Melbourne Australia. p. 24.

Camões-Costa V, Snipe R, Dixon D, Costa RJS. Dairy milk beverage enhances neutrophil function after immunodepressive exercise in response to a bacterial challenge. Paper presented at: Sports Dietitians Australia Conference: Propelling your knowledge and practice; 20-21 Oct 2017; Melbourne Australia.

Snipe R, Kitic C, Gibson P, Costa RJS. Can carbohydrate and protein intake prevent gut-immune perturbations induced by exertional-heat stress? In: The International Society of Exercise and Immunology, editor. 13th ISEI Symposium: Training our immune system for health and performance; 11-14 Jul 2017; Coimbra Portugal.

Snipe R, Kitic C, Gibson P, Costa RJS. Carbohydrate and protein intake during exertional-heat stress ameliorates intestinal epithelial damage. *Nutrition & Dietetics*. 2016;73(Suppl. 1):10–50.

Snipe R, Kitic C, Gibson P, Costa RJS. Heat stress during prolonged running results in exacerbated intestinal epithelial injury and gastrointestinal symptoms. In: Exercise and Sports Science Australia, editor. Research to practice 2016: Conference proceedings (e-book). 14-16 Apr 2016; Melbourne Australia. p. 168.

Snipe R, Dixon D, Gibson P, Costa RJS. The effects of exercise-induced dehydration on gastrointestinal integrity and symptoms. In: Sports Dietitians Australia, editor. Leadership and innovation for practice success: Proceedings of Sports Dietitians Australia Conference; 23-24 Oct 2015; Melbourne Australia. p. 27.

Costa RJS, Snipe R, Dixon, D. Maintenance of euhydration during endurance exercise prevents exercise-induced depressions in neutrophil function. In: Sports Dietitians Australia, editor. Leadership and innovation for practice success: Proceedings of Sports Dietitians Australia Conference; 23-24 Oct 2015; Melbourne Australia. p. 13.

Miall A, Khoo A, Rauch C, Snipe R, Costa RJS. Does gut-training reduce gastrointestinal symptom and carbohydrate malabsorption during endurance running? In: Sports Dietitians Australia, editor. Leadership and innovation for practice success: Proceedings of Sports Dietitians Australia Conference; 23-24 Oct 2015; Melbourne Australia. p. 28.

Khoo A, Miall, A, Rauch C, Snipe R, Costa RJS. Gut training exhibits no change in total carbohydrate or fat oxidation rates during endurance running. In: Sports Dietitians Australia, editor. Leadership and innovation for practice success: Proceedings of Sports Dietitians Australia Conference; 23-24 Oct 2015; Melbourne Australia. p. 12.

Research grants and awards

2017 Sports Dietitians Australia “emerging sports nutrition researcher” award.

2017 International Society of Exercise and Immunology (ISEI) symposium travel award sponsored by the University of Newcastle (\$2000.00).

2015 Sports Medicine Australia (SMA) Research Grant, Sports Medicine Australia Research Foundation. Proposal title: “The effects of internal cooling during exertional heat stress on the prevention of gut injury and gastrointestinal-induced heat stroke” (\$2000.00).

Acknowledgements

First and foremost, I gratefully acknowledge and thank my supervisor Dr. Ricardo Costa for his guidance, research training and mentorship throughout my PhD. His enthusiasm, work-ethic, friendly and supportive nature have been instrumental in my continuation and progression throughout this PhD. I would also like to gratefully acknowledge and thank my associate supervisor Prof. Helen Truby for her guidance, advice and feedback which have progressed my research knowledge and skills. A big thank you and acknowledgement to Dr. Cecilia Kitic and Prof. Peter Gibson for their contributions to various aspects of study design, data analysis and contributions to manuscripts. A special thank you to Anthony Khoo who was always reliable, willing to assist with data collection and made the time in the laboratory fun and entertaining. A huge thank you to all the study participants who contributed time, effort and their bodies so that I could conduct this research. Thank you to my fellow PhD students in the Department of Nutrition, Dietetics and Food for making this journey so much more enjoyable. Last but not least (by any means), I thank my husband Wes who has been a constant source of support, both emotionally and financially, throughout this PhD and has always encouraged me to follow my dreams. Thanks for all that you have done, from helping me during the first weeks of undergraduate chemistry to listening to me talk about the same research for the past three and a half years and everything in between. Without you this PhD would not have been possible. A big thank you to my parents who have always stretched above and beyond to be there when I need them, and thank you Mum for all the childcare and hours of travelling that you had to undergo. A final and special thank you to my son Winston who endured many hours in the laboratory throughout pregnancy, and many more in childcare so that I could finish this PhD. You have been a constant source of love and happiness and are undoubtedly my greatest achievement to date. This research was supported by an Australian Government Research Training Program (RTP) Scholarship; a Monash University, Faculty of Medicine, Nursing and Health Sciences, Faculty Strategic Grant Scheme, SGS15-0128; and a 2015 Sports Medicine Australia Research Foundation grant.

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Thesis Format

The general introduction (**Chapter 1**) and literature review (**Chapter 2**) provides background, rationale and justification for the aims of the research presented in this thesis. This is followed by a description of the general methods (**Chapter 3**), which provides additional detail of the methods that are not listed within the publication presented in each chapter. This thesis consists of four independent experimental studies which build on the findings of the previous study and are presented in the format of the manuscript submitted or accepted for publication, but have been re-numbered and referenced to provide consistency. This includes two studies that explore the effects of exertional-heat stress on gastrointestinal perturbations and systemic responses, followed by two studies exploring the effectiveness of different prevention and management strategies. The first study investigated the effects of prolonged (2 h) submaximal (60% maximal oxygen uptake ($\dot{V}O_{2max}$)) running in 35°C ambient temperature (T_{amb}), compared with temperate (22°C T_{amb}) conditions on gastrointestinal integrity and gastrointestinal symptoms as primary outcomes, and the associated systemic endotoxin and cytokine profiles as secondary outcomes (**Chapter 4**). Additionally, this study explored relationships between exercise-induced increases in core body temperature with gastrointestinal perturbations and systemic responses. The second study is a complementary study investigating the effects of mild exertional-heat stress (30°C T_{amb}), which is more-commonly experienced by athletes and active occupational workers, compared with temperate conditions on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profiles (**Chapter 5**). The third study investigates the frequent ingestion of carbohydrate and protein during exertional-heat stress on the prevention and management of perturbations to gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profile (**Chapter 6**). The fourth study investigates the impact of frequent water ingestion at different temperatures during exertional-heat stress on the prevention and management of perturbations to gastrointestinal integrity, gastrointestinal symptoms and systemic cytokine responses (**Chapter 7**). These experimental studies are followed by a general discussion

chapter (**Chapter 8**) that provides a summary and critical analysis of the overarching findings of this thesis, including limitations and directions for future research, and is followed by the thesis conclusions.

Throughout the thesis, abbreviations are defined at first use, excluding the list of tables and figures. A full list of abbreviations, tables and figures is provided prior to **Chapter 1**. An overlap in content inevitably occurs between chapters due to the complementary nature of the studies and existing links between chapters. Bold type is used to reference other sections within this thesis.

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List of Abbreviations

ANOVA	analysis of variance
bpm	beats per minute
CV	coefficient of variation
Δ	change
CI	confidence interval
COLD	cold water (0°C)
COOL	cool water (7°C)
ELISA	enzyme linked immunosorbent assay
\geq	equal to or greater than
\leq	equal to or less than
EHS	exertional-heat stress
FODMAP	fermentable oligosaccharides, disaccharides, monosaccharides and polyols
g	gram
GLUC	glucose
h	hour
HOT	hot ambient conditions (35°C)
IL-1 β	interleukin 1 beta
IL-1ra	interleukin 1 receptor antagonist
IL-6	interleukin 6
IL-8	interleukin 8
IL-10	interleukin 10
IBS	irritable bowel syndrome
IU	international units
I-FABP	intestinal fatty acid binding protein

kg	kilogram
L	litre
μg	microgram
μL	microlitre
MJ	megajoule
m	metre
mmol	millimole
mOsmol	milliosmole
mL	millilitre
min	minute
nmol	nanomole
pg	picogram
RPE	rating of perceived exertion
T _{amb}	ambient temperature
TEMP	temperate ambient conditions or water at temperate conditions (22°C)
TNF-α	tumour necrosis factor alpha
T _{re}	rectal temperature
$\dot{V}O_{2max}$	maximal oxygen uptake
vs	versus
WARM	warm ambient conditions (30°C)
WPH	whey protein hydrolysate
w/v	water volume
y	year

CHAPTER ONE

General Introduction

Regular physical exertion of a moderate to vigorous intensity is widely advocated in global public health policy to reduce the risk of chronic disease and improve overall health and well-being.(3, 4) Indeed, current evidence suggests that regular physical activity reduces the risk of cardiovascular disease, stroke, type 2 diabetes and some forms of cancer (e.g., colon and breast cancer).(3) Such health benefits have been reported to occur in a dose-dependent manner, whereby higher levels of physical activity are associated with greater health benefits.(3) Although high levels of physical activity or exercise are beneficial in preventing chronic disease, excessive exercise stress may damage the gastrointestinal tract.(2) The gastrointestinal tract is well-equipped to repair the transient damage induced by short duration exercise.(5) However, prolonged strenuous exercise and/or repetitive exercise stress with insufficient recovery time may create greater damage to the gastrointestinal tract that can potentially lead to systemic endotoxaemia, cytokinaemia and a range of acute health conditions including septic shock, gastritis, colitis, paralytic ileus and ischaemic bowel.(2, 6-13) Recent anecdotal evidence also suggests that prolonged strenuous exercise (e.g. ultramarathon running) may increase the risk for development and/or the progression of chronic gastrointestinal conditions such as inflammatory bowel disease and functional gastrointestinal disorders (e.g. irritable bowel syndrome) however, the predisposition for the development of these gastrointestinal conditions in the absence of strenuous exercise is currently unknown.(14) Gastrointestinal disturbances appear to increase with the magnitude of exercise stress and may affect up to 96% of athletes competing in endurance running events.(2, 15-18) While severe health outcomes have been infrequently reported following strenuous exercise, the growing popularity of endurance-based sports has the potential to increase the incidence of exercise-induced gastrointestinal conditions providing a medical burden to event organisers, sports medics and gastroenterologists.(7, 8, 19) Indeed, the number of runners

completing a marathon in Australia has more than tripled over the ten-year period from 2006 to 2016.(20)

The term “exercise induced gastrointestinal syndrome” has recently been used to describe the cluster of gastrointestinal and associated systemic perturbations arising from exercise stress.(2) The physiological changes associated with exercise stress alter circulatory and neuroendocrine responses (**Figure 2.1**), subsequently impacting gastrointestinal motility and transit, nutrient absorption, intestinal epithelial integrity, gastrointestinal permeability, intestinal bacterial translocation and associated systemic responses (e.g., endotoxaemia and cytokinaemia). Such perturbations have been implicated in the development of gastrointestinal symptoms and the aforementioned health conditions.(2) A recent systematic review by Costa et al.(2) has highlighted that changes to gastrointestinal integrity and function are augmented with increasing exercise duration (≥ 2 h) and intensity ($\geq 60\% \dot{V}O_{2\max}$), and appear to be further exacerbated by running exercise, heat exposure and/or high core body temperatures. Indeed, physical exertion in the heat creates a major physiological challenge due to changes in cardiovascular, thermoregulatory, metabolic and neuromuscular function.(21) Such physiological changes are therefore, likely to exacerbate the circulatory and neuroendocrine responses contributing to “exercise-induced gastrointestinal syndrome”.(1) However, to date no studies have comprehensively investigated the effects of prolonged submaximal exertional-heat stress on gastrointestinal integrity, symptoms and systemic endotoxin and cytokine profile.

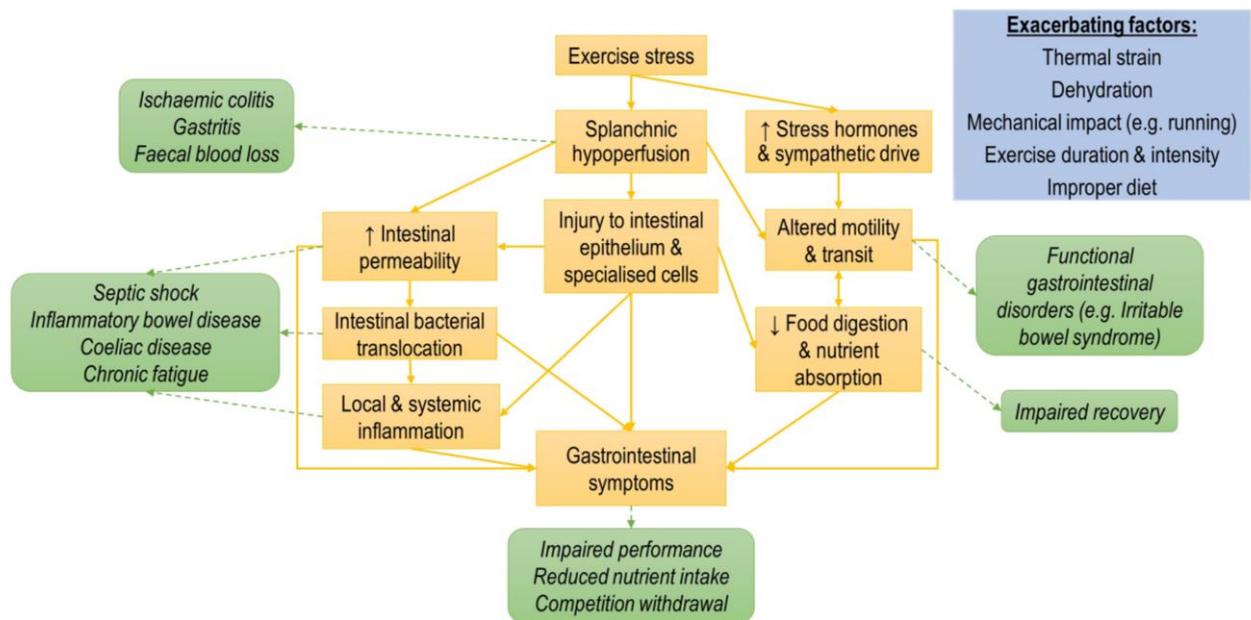


Figure 2.1 Exercise-induced gastrointestinal syndrome, exacerbating factors and health and performance implications. Created by thesis author, adapted from Costa et al.(2)

The most commonly reported gastrointestinal perturbation in response to exercise is the presence of gastrointestinal symptoms such as nausea, stomach pain, vomiting and diarrhoea, which consistently affect >60% of athletes during competitive endurance events.(15, 16, 22, 23) The presence of gastrointestinal symptoms has been shown to adversely affect exercise performance, reduce nutrient intake during and after exercise, and in severe cases, cause withdrawal from competitive events.(22-24) Such high reports of gastrointestinal symptoms are therefore a major medical issue in endurance running competition. The aetiology of exercise-associated gastrointestinal symptoms is multifactorial, with nutrition (e.g., pre-exercise and during exercise nutrition intake), mechanical (e.g., vibration, jostling and jarring of gastrointestinal contents) and physiological (e.g., splanchnic hypoperfusion, motility) factors believed to be major contributors.(2, 25-28) The highest rates of gastrointestinal symptoms (85-96%) have been consistently reported during prolonged running exercise with heat exposure.(15, 22, 23) Considering these observations, it is plausible that factors associated with heat exposure, such as increased sweat rates, body mass loss, heart rate, core body temperature and stress hormones may directly and/or indirectly contribute to the development of

gastrointestinal symptoms. However, to date the influence of heat exposure on the development of gastrointestinal symptoms has not yet been explored.

The focus of this thesis was therefore, to firstly determine the effects of prolonged submaximal exertional-heat stress in the form of running exercise on gastrointestinal integrity, including intestinal epithelial injury, small intestine permeability and intestinal inflammation, and gastrointestinal symptoms as primary outcomes. In addition, systemic endotoxin (i.e., circulatory endotoxin and anti-endotoxin antibody concentrations) and cytokine profile (i.e., interleukin (IL)-6, IL-1 β , tumour necrosis factor (TNF)- α , IL-8, IL-10, and IL-1 receptor antagonist (ra)) responses were determined as secondary outcomes. Further, this thesis explores relationships between exercise-induced changes in core body temperature and gastrointestinal perturbations, and investigated the application of practical sports nutrition strategies for the prevention and management of exertional-heat stress induced gastrointestinal perturbations. The findings from the novel research within this thesis have the potential to enhance knowledge and understanding on the effects of heat exposure during prolonged submaximal running on gastrointestinal perturbations, including various elements of exercise-induced gastrointestinal syndrome. Moreover, findings from the prevention and management studies have the potential to influence health and performance outcomes for endurance athletes and active occupational workers (i.e., military, firefighters, miners, farmers) who undertake prolonged submaximal workloads during exposure to warm or hot ambient conditions, often with consecutive days of physical exertion in the heat.

CHAPTER TWO

Literature Review

Exposure to warm and hot ambient conditions during physical exertion is commonly experienced by active occupational workers, recreational and elite athletes competing in team and endurance-based sports, and by individuals undertaking recreational or leisure activities. High ambient temperatures during physical exertion increases skin blood flow and may impair the dissipation of metabolic heat production, creating a thermoregulatory strain (e.g. elevated core body temperature and heart rate) that subsequently exacerbates splanchnic hypoperfusion and stress hormone responses.(21, 29, 30) This elevation in stress hormone responses and splanchnic hypoperfusion appear to be key contributing factors to perturbations to gastrointestinal integrity and various elements of exercise-induced gastrointestinal syndrome.(2)

The following literature review will synthesise the current evidence regarding the effects of exercise, in particular exertional-heat stress, on gastrointestinal integrity, symptoms and associated systemic endotoxin and cytokine responses. Additionally, the role of acute (i.e., before and during) nutrition strategies in preventing exercise and exertional-heat stress associated gastrointestinal perturbations and systemic responses will be reviewed. In **section 2.1** the effects of exercise and exertional-heat stress on gastrointestinal integrity, symptoms and systemic endotoxin and cytokine profile will be reviewed along with relevant markers of each gastrointestinal and systemic perturbation. **Section 2.2** and **2.3** will review the effects of nutrition intake, focusing on carbohydrates, protein and the temperature of fluids consumed before and during exercise and exertional-heat stress on gastrointestinal integrity, symptoms and systemic endotoxin and cytokine profile.

2.1 Gastrointestinal perturbations in response to exercise and heat stress

2.1.1 Gastrointestinal integrity in response to exercise and heat stress

The gastrointestinal tract has two very important roles during prolonged exercise. Firstly, the gastrointestinal tract functions as a physical barrier that separates the bacteria and potentially pathogenic contents of the intestinal lumen from systemic circulation. Secondly, this barrier provides a large surface area for the absorption of nutrients, including water and exogenous fuel sources, which are critical for maintaining hydration and supporting workload during prolonged physical exertion, particularly in the heat. It is therefore not surprising that damage to the integrity of the gastrointestinal tract during prolonged exercise has been linked to symptomatic episodes and may contribute to the development of several acute health conditions including, gastritis, ischaemic colitis, faecal blood loss and septic shock, which in severe cases may require hospitalisation and/or surgical intervention.(6, 8, 11, 31, 32)

Splanchnic hypoperfusion is believed to be a major contributor to disruptions in gastrointestinal integrity during exercise.(33) Indeed, reductions in portal vein blood flow of up to 80% have been reported during 1 h of high intensity (70% $\dot{V}O_{2max}$) exercise, and appear to be further exacerbated by the additional thermoregulatory strain imposed by heat exposure.(1, 30, 34) Exercise duration may contribute to disruptions in gastrointestinal integrity due to the prolonged reduction in splanchnic hypoperfusion.(2) In addition, the mode of exercise, for example running, may aggravate perturbations to gastrointestinal integrity via mechanical impact and associated increases in abdominal vibrations.(2, 27)

Intestinal fatty-acid binding protein (I-FABP) is a small cytosolic protein that is specifically present in mature enterocytes of the small and large intestine, and is rapidly released into systemic circulation

upon damage to the enterocyte.(35) I-FABP has been shown to correlate well with splanchnic perfusion, intestinal ischemia and histological status of the epithelium and has thus, been proposed as a useful marker for assessing injury to the intestinal epithelium.(36, 37) Further, elevated I-FABP levels post-exercise have been associated with impaired nutrient absorption which may have direct implications for exogenous fuel supply, contribute to the development of gastrointestinal symptoms due to nutrient malabsorption, and adversely affect post-exercise recovery processes.(38)

Pre- to post-exercise increases of 88 pg/mL to >800 pg/mL I-FABP have been reported during laboratory-based exercise protocols, providing evidence that exercise consisting of 30 min of resistance training, high intensity interval running and cycling and running at least 3 h can cause injury to the intestinal epithelium.(25, 38-45) Indeed, the highest post-exercise increases in I-FABP (806-995 pg/mL) have been reported after prolonged exercise (1.5 to 3 h), suggesting that exercise duration may be a major contributing factor to intestinal epithelial injury.(25, 43) It has been suggested that heat exposure and higher fitness levels may be contributing factors, due to increased thermoregulatory strain and greater workloads being performed, respectively. For example, a higher pre- to post-exercise increase in I-FABP (806 pg/mL) was observed in more highly trained (≥ 6 sessions per week) recreational athletes after 1.5 h of alternating running and cycling exercise in 30°C T_{amb} , compared with less-trained (≤ 3 sessions per week) recreational athletes (283 pg/mL increase in I-FABP).(43) However, the inclusion of a time-trial in this study resulted in much higher workloads being completed by the more highly trained athletes, thereby making it difficult to differentiate the effect of fitness level from workload on intestinal epithelial injury. Moreover, despite observations of elevated I-FABP during exercise protocols in the heat, no study to date has determined the effects of heat exposure and/or exercise-induced increases in core body temperature on intestinal epithelial injury. Considering splanchnic hypoperfusion is exacerbated during exercise in the heat, it is plausible that prolonged exercise with heat exposure may cause significant damage to the intestinal epithelium.

A breakdown of the tight junction complexes, which adhere the intestinal cells to one another, results in an increased intestinal permeability and loss of intestinal barrier integrity.(46) This increase in permeability is believed to allow the translocation of bacteria and pathogenic substances from the intestinal lumen to the systemic circulation resulting in a systemic inflammatory response.(47) Indeed, elevated intestinal permeability has been observed in a number of chronic inflammatory conditions, including inflammatory bowel disease, coeliac disease, metabolic syndrome and inflammatory joint diseases.(48-51) The dual sugars test is the most direct and commonly used measurement of small intestine permeability in response to exercise stress, and is commonly used in the clinical setting.(2, 52) The administration of this test involves the ingestion of small (e.g. L-rhamnose or mannitol) and large (e.g. lactulose) sugar probe molecules with measurement of their excretion in urine over a 5 to 6 h period.(35) If intestinal barrier and tight junction complexes have been compromised, the large sugar probe molecule will be able to cross between enterocytes via the paracellular pathway, whereas the small molecule is proposed to primarily be absorbed via the transcellular pathway.(51) The combined use of small and large molecules acts to control for factors that may influence absorption and excretion of the sugar probes, such as gastric emptying, intestinal motility and transit and renal function.(35) The ratio of urinary excretion of the large to small molecule is then calculated to provide a measure of small intestine permeability. Similarly, gastric permeability and permeability of the large intestine can be measured using sucrose and sucralose and erythritol, respectively.(40)

Despite the common use of the dual sugars test in measuring gastrointestinal permeability in the clinical and exercise setting, there are several acknowledged limitations to this method that include 1) the need to collect resting permeability data on a separate day to the experimental intervention in order to determine pre- to post-exercise changes; 2) the time burden (i.e., 5-6 h for gastric and small intestine and 24 h for large intestine permeability) associated with urine collection; 3) the sensitivity for detecting small changes in gastrointestinal permeability; 4) variations in dosage and timing of

sugar probe ingestion and length of urine collection; and 5) pre-exercise control of dietary sources of sugar probes (e.g. sucrose).(52)

The current literature suggests that type of activity is important, with running exercise and higher exercise intensities appearing to increase gastric and small intestine permeability, whereas cycling exercise appears to have minimal effect on small and large intestine permeability.(40, 41, 53-56) Specifically, increases in small intestine permeability have only been observed during 60 min running at $\geq 70\% \dot{V}O_{2max}$ in temperate (22-24°C) and hot (30°C) ambient conditions.(54, 55, 57, 58) It appears that factors related to higher exercise intensities, such as dehydration and/or higher core body temperatures may also play a role in elevating small intestine permeability during exercise.(54, 55) A clear example of this is shown in the landmark study by Pals et al.(54), where body mass loss, final rectal temperature and small intestine permeability all increased in proportion to exercise intensity. In addition to the confounding effects of exercise intensity, the influence of exercise duration has not yet been established, with the majority of gastrointestinal permeability research using exercise protocols of 1 h duration. Elevations in small intestine permeability have been observed in soldiers during a period of military combat training compared to a period of rest.(12) Findings from this study highlight the effects of a period of chronic physical and/or psychological stress on disruptions to the gastrointestinal barrier, and the potential translational aspects of this thesis to occupational workers who undertake periods of prolonged physical stress in the presence of heat.

To date, only one study has investigated the effects of heat exposure during exercise on gastrointestinal permeability. Yeh et al.(58) observed increases in plasma claudin-3, a tight junction stabilising protein, after 1 h running in 22°C T_{amb} and 33°C T_{amb} . However, heat exposure had no additional effect on plasma claudin-3, despite observing a significantly higher peak core body temperature in 33°C T_{amb} vs. 22°C T_{amb} (39.1°C vs. 38.4°C, respectively).(58) The use of plasma claudin-3 as a marker of gastrointestinal permeability requires validation, particularly since claudin-

3 is one of several intestinal claudins that interact to maintain tight junction stability and is present in other areas of the body.(59) The effects of heat exposure during exercise on gastrointestinal permeability therefore, warrants further investigation.

Elevated intestinal permeability and/or disruptions to the function of the gastrointestinal barrier can lead to localised inflammation, which may consequently lead to further perturbations to intestinal barrier function.(35) Calprotectin is a protein that plays a regulatory role in inflammatory processes, making faecal calprotectin a useful marker of intestinal inflammation.(35) Indeed, faecal calprotectin is commonly used in the clinical setting to monitor disease activity in inflammatory bowel disease, but has scarcely been used in exercise research. Faecal calprotectin was reported to increase from a median value of 1.07 to 1.48 $\mu\text{g/g}$ following 1 h cycling exercise at 70% $\dot{V}\text{O}_{2\text{max}}$.(40) However, a cut-off value of $>100 \mu\text{g/g}$ is used to detect inflammation in inflammatory bowel disease, suggesting that such minor increases in faecal calprotectin after 1 h cycling exercise are unlikely to be of clinical significance.(60) Considering running appears to create greater gastrointestinal disturbances than cycling, it is possible that faecal calprotectin may be useful in determining associated acute intestinal inflammation.(2) Therefore, further research is required to elucidate the use of faecal calprotectin as a measure of intestinal inflammation in response to running and more prolonged exercise stress.

In summary, I-FABP is a sensitive marker of intestinal epithelial injury, dual sugar tests (i.e., lactulose and L-rhamnose) are the most direct and commonly used measures of gastrointestinal permeability, and faecal calprotectin is commonly used to assess intestinal inflammation in the clinical setting but has not been well-studied in response to exercise stress. Very few studies to date have used a combination of these markers to comprehensively explore the effects of exercise and/or exertional-heat stress on the gastrointestinal system.(40) Considering each marker measures a different distinct perturbation of exercise-induced gastrointestinal syndrome, future studies should aim to use multiple markers in conjunction with assessment of gastrointestinal symptoms.(2, 37) The current literature

suggests moderate to high intensity ($\geq 60\% \dot{V}O_{2\max}$), long duration (≥ 1.5 h) exercise and running mode appear to create the greatest perturbations to gastrointestinal integrity.(2) Additionally, exercise-related factors such as dehydration, elevated core body temperature and/or heat exposure during exercise may further compromise the integrity of the gastrointestinal tract.(29, 43, 44, 54, 55) Despite animal and cell-culture models suggesting hyperthermia perturbs the integrity of the gastrointestinal tract(61-64), only one study has previously investigated the effects of heat exposure in humans during 1 h running exercise on gastrointestinal permeability using a marker (plasma claudin-3) that requires further validation.(58) Considering the health implications associated with the breakdown of the gastrointestinal barrier (2, 50), further research is warranted to determine the effects of heat exposure and associated elevations in core body temperature during physical exertion on the integrity of the gastrointestinal tract.

2.1.2 Gastrointestinal symptoms in response to exercise and heat stress

Gastrointestinal symptoms such as nausea, vomiting, abdominal pain and diarrhoea are amongst the most commonly reported medical complaints during endurance exercise and competition.(24) Observational field studies have consistently reported that 60-96% of endurance athletes experience gastrointestinal symptoms during competition, highlighting that the majority of these athletes are affected by symptoms during exercise.(15, 16, 22, 23) While the severity of these symptoms may vary among individuals, the presence of gastrointestinal symptoms during competition has been shown to adversely affect nutrient intake during and after exercise, impair exercise performance and in more severe cases, cause withdrawal from competition.(23, 24)

Despite such a high prevalence and the reported deleterious consequences of gastrointestinal symptoms on exercise performance, very few studies have investigated the pathophysiology of these symptoms and subsequent prevention and management strategies. The large reduction in splanchnic blood flow arising from the increased sympathetic nervous system activity and/or hormonal and neural changes in gastrointestinal motility during exercise are believed to be major contributors to the development of these symptoms.(33) Further, postural and mechanical factors associated with running exercise may account for the higher occurrence of gastrointestinal symptoms observed during running compared to other endurance-based sports (e.g. cycling and swimming).(27, 53) Recent research suggests that psychological factors such as stress and anxiety may also contribute to the development and/or reporting of running-related gastrointestinal symptoms.(65) These findings are not surprising given that depression and/or anxiety are common in patients with irritable bowel syndrome (IBS) and have been reported to exacerbate symptoms in IBS patients.(66, 67) Moreover, nutrition-related factors can contribute to development of gastrointestinal symptoms, which will be reviewed in **section 2.2.2** of this chapter.

Laboratory-based investigations of symptomatic athletes have shown that gastrointestinal ischemia appears in 50% of symptomatic athletes during submaximal exercise, suggesting an exacerbated reduction in splanchnic blood flow in these athletes.(26) Symptomatic athletes, compared to asymptomatic athletes, have also been shown to have a longer oro-caecal transit time and higher intestinal permeability, suggesting symptomatic athletes may have altered gastrointestinal motility and susceptibility to perturbations in gastrointestinal integrity.(53) Moreover, this research elucidated that disturbances to oro-caecal transit time and intestinal permeability were greater in running than cycling exercise in symptomatic athletes, supporting previous observations of greater gastrointestinal disturbances during running exercise.(53) The complex multifactorial aetiology of exercise-associated gastrointestinal symptoms may also include factors such as exercise intensity, duration, previous history of symptoms, female sex and/or heat exposure.(2) Indeed, the highest reported prevalence of gastrointestinal symptoms have been consistently reported during prolonged running and triathlon (i.e., which includes running) events in the heat.(15, 22) High intensity (i.e., $\geq 70\%$ $\dot{V}O_{2max}$ or equivalent) laboratory-based exercise protocols of ≤ 1 h have reported minimal gastrointestinal symptoms in healthy asymptomatic athletes.(45, 54, 68, 69) Despite observations of a higher incidence of gastrointestinal symptoms occurring during prolonged running in the heat(2), no research to date has been conducted to determine if heat exposure exacerbates exercise-associated gastrointestinal symptoms.

In summary, exercise-induced gastrointestinal symptoms are a very common and often debilitating medical complaint that appear to occur most commonly during prolonged (i.e., ≥ 2 h) submaximal running exercise. The complex multifactorial aetiology of these symptoms is poorly understood, however, splanchnic hypoperfusion, altered gastrointestinal motility and disturbances to gastrointestinal integrity appear to be key contributing factors. These contributing factors are likely exacerbated by the increased thermoregulatory strain imposed by heat exposure, but this requires further investigation.

2.1.3 Systemic circulatory endotoxin and cytokine profile in response to exercise and heat stress

Damage and/or impairment in the function of the gastrointestinal barrier can allow intestinal gram-negative bacteria, which include pathogenic lipopolysaccharides, to translocate from the intestinal lumen into the systemic circulation driving a pro-inflammatory response.(47) This elevation in systemic endotoxins (e.g. lipopolysaccharides) and inflammation has been implicated in the aetiology of heat stroke and septic shock, and linked to other inflammatory health conditions.(14, 47) In addition, some studies have observed a relationship between circulatory endotoxin concentration or cytokinaemia with gastrointestinal symptoms (17, 22) however, these findings have not been consistently reported and require further investigation.(70, 71) Indeed, endotoxaemia, defined as a ≥ 5 pg/mL increase in circulatory endotoxin concentration, has been reported during 60 min running at 70% $\dot{V}O_{2max}$ in the heat (33°C), with increases of up to 122 pg/mL reported after a 24 h ultramarathon competition.(58, 71) Findings from previous studies suggest the extent of exercise-associated endotoxaemia appears to be related to the duration of exercise and is exacerbated by heat exposure or high intensity exercise protocols to exhaustion.(2)

Whilst previous studies have consistently shown increased circulatory endotoxin in response to prolonged exercise in the presence and absence of heat exposure, there are several limitations associated with the use of circulatory endotoxin that should be acknowledged. Firstly, plasma endotoxin may enter the bloodstream via the lymphatic and small intestine lymphatic system.(72, 73) Therefore, the presence of circulatory endotoxin post-exercise may not be an accurate measure of intestinal permeability. Secondly, intestinal-derived circulatory endotoxins must pass through the liver, which plays a significant role in the clearance of endotoxin, prior to reaching systemic circulation.(72) This suggests that post-exercise circulatory endotoxin concentration may only represent a state of flux, rather than indicate the status of the gastrointestinal barrier. Thirdly, the lipopolysaccharide component of gram-negative bacterial endotoxin may bind to and transfer rapidly

between bacterial membranes and lipoproteins.(72) Therefore, the endotoxin neutralizing capacity may vary between individuals due to differences in lipoprotein profiles.(74) Further, the sequestering and neutralization of endotoxin in blood may limit the ability to detect endotoxin using the Limulus amoebocyte lysate (LAL) assay.(72) Fourthly, the LAL assay is highly sensitive to contamination and method of sample analysis (i.e., manufacturer, pre-treatment etc.), thereby limiting the ability to directly compare results between studies.(72, 75) Despite these limitations, the LAL assay is the most commonly used method for determining pre- to post-exercise increases in circulatory endotoxin concentration, which if consistently elevated may lead to a variety of health conditions.(14, 72) Considering the aforementioned limitations and assuming all samples are collected and analysed using the same methods, the pre- to post-exercise increase in circulatory endotoxin concentration may be a useful indicator of ‘excess’ circulatory endotoxin, but is unlikely to accurately reflect gastrointestinal permeability.(72)

The measurement of pre- and post-exercise anti-endotoxin antibody (e.g. IgG and IgM) concentration is another commonly used method of detecting circulatory endotoxin.(35, 72) A pre- to post-exercise reduction in anti-endotoxin antibody concentration signifies the presence and/or clearance of endotoxin from circulation. Indeed, reductions in anti-endotoxin antibodies have been reported after a long-distance triathlon, ultramarathon competition and running at 70% $\dot{V}O_{2max}$ in 35°C T_{amb} until a core temperature of 39.5°C was achieved.(22, 76, 77) In these studies, concurrent increases in circulatory endotoxin concentration were observed in conjunction with a post-exercise reduction in anti-endotoxin antibodies, with one study finding a negative correlation between circulatory endotoxin and anti-endotoxin antibody concentrations.(76) These findings suggest that measurement of anti-endotoxin antibodies in conjunction with circulatory endotoxin, may provide a more accurate indication of endotoxin status and clearance capacity following a bout of exercise than measurement of circulatory endotoxin in isolation.

A rise in circulating endotoxin concentration during exercise has been shown to drive a cytokine-mediated systemic inflammatory response, with significantly elevated interleukin (IL)-1 β , tumour necrosis factor (TNF)- α , IL-8 and IL-6 observed in conjunction with endotoxaemia during endurance events.(22, 70, 71, 78) For example, IL-6, IL-1 β and TNF- α increased by 238%, 64% and 101%, respectively, in conjunction with a 40 pg/mL increase in circulatory endotoxin concentration following stage 1 (37 km) of a multi-stage ultramarathon competition in the heat (30°C to 32°C T_{amb}).⁽⁷⁰⁾ Subsequently, a 1100% and 207% increase in the compensatory anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist (ra), respectively, was observed and remained elevated throughout the multi-stage competition to balance systemic inflammation.⁽⁷⁰⁾ The thermoregulatory strain and elevated stress hormone response (i.e. cortisol and catecholamines) during exertional-heat stress appear to be key factors in the development of cytokinaemia.^(74, 79-81) Indeed, clamping core temperature to a 0.4°C increase during 40 min exercise has been shown to attenuate the rise in circulating stress hormones and systemic cytokines, compared to equivalent exercise in warm conditions resulting in a core temperature increase of 1.9°C.⁽⁸⁰⁾ Further, in this study positive associations were found between exercise-induced changes in core temperature with stress hormones and cytokines, suggesting increases in core temperature are an important mediator of exercise-induced cytokinaemia.⁽⁸⁰⁾

In summary, prolonged exercise, particularly in the presence of heat, appears to increase circulatory endotoxin concentration and reduce anti-endotoxin antibody concentration.^(22, 77) The exercise-associated rise in circulatory endotoxin concentration has been shown to elicit a systemic inflammatory response that is similar to sepsis, and has been implicated in the development of heat stroke and septic shock.⁽⁴⁷⁾ An appropriate compensatory anti-inflammatory cytokine and immune response in healthy well-trained athletes appears to counteract the effects of endotoxaemia and balance systemic inflammatory responses.⁽⁷⁰⁾ While current evidence suggests that heat exposure during exertion increases circulatory endotoxin and systemic inflammatory cytokine responses^{(70,}

74, 82) very few studies have explored these responses in conjunction with markers of gastrointestinal integrity and/or gastrointestinal symptoms. This absence of comprehensive research makes it difficult to establish the effects of exertional-heat stress on gastrointestinal perturbations or to explore appropriate prevention and management strategies. Moreover, the majority of laboratory-controlled research has been of relatively short exercise duration (≤ 1 h) and high intensity ($\geq 70\%$ $\dot{V}O_{2\max}$), whereas the greatest gastrointestinal perturbations have been previously reported during longer duration (≥ 2 h) exercise of moderate intensity ($\geq 60\%$ $\dot{V}O_{2\max}$).⁽²⁾

2.2 The effects of pre- and during exercise nutrition intake on gastrointestinal perturbations

2.2.1 The effects of pre- and during exercise nutrition intake on gastrointestinal integrity

The ingestion of fluid and carbohydrates is common practice during endurance (≥ 1 h duration) exercise and has consistently been shown to improve exercise performance.(83-85) Carbohydrate ingestion provides a rapid exogenous supply of carbohydrates for oxidation, thereby preventing the occurrence of fatigue arising due to limited muscle and liver glycogen stores during prolonged (≥ 2 h) exercise.(83) Fluid intake during prolonged exercise aims to prevent excessive dehydration and minimise electrolyte disturbances, which can adversely affect cardiovascular and thermoregulatory responses, and impair exercise performance.(84, 86) The requirement for both carbohydrate and fluids are increased during prolonged exertional-heat stress due to increased sweat rates, body water losses and endogenous carbohydrate oxidation rates.(84, 87) However, gastric emptying and exogenous carbohydrate oxidation rates may be reduced during exercise in the heat which creates a substantial nutritional challenge.(88, 89) Nevertheless, the increased nutrient requirements and direct interaction with the gastrointestinal tract makes nutrition intake a plausible area for investigating prevention and management strategies for exertional-heat stress induced gastrointestinal perturbations.

Nutrient and fluid intake during prolonged exercise may play a role in reducing the extent and/or exacerbation of splanchnic hypoperfusion(90, 91), which is believed to be a primary cause of disruption to gastrointestinal integrity. For example, fluid ingestion during exercise acts to minimise body water losses and avoid exacerbation of splanchnic hypoperfusion due to systemic hypovolemia.(37) Indeed, the restriction of fluid intake during a 1 h run at 70% $\dot{V}O_{2max}$ resulting in a 1.5% loss of body mass showed significantly increased gastroduodenal and small intestine permeability compared to rest.(55) In the same study gastrointestinal permeability was not increased

from rest when water or carbohydrates were provided.(55) Interestingly, the authors suggested that the absorption of fluid and/or carbohydrates may play a protective role in maintaining intestinal integrity.(55) In contrast, pre-exercise dehydration (3% loss of body mass) induced by sauna exposure before 1.5 h cycling at 70% maximal workload (W_{max}) did not increase intestinal permeability or rectal temperature compared to commencing exercise in a euhydrated condition.(92) Discrepancies in these findings may be due to several methodological factors including 1) higher intestinal permeability observed during running compared to cycling exercise(93), 2) the ingestion of a liquid meal and carbohydrate solution during the cycling study may have mitigated the effects of pre-exercise dehydration on gastrointestinal permeability(92), and 3) the limited sensitivity of urinary measures of intestinal permeability to detect subtle differences between conditions, rather than from rest.(52) Regardless of these conflicting results, it is likely that consuming fluids to minimise loss of body mass will be beneficial in supporting the maintenance of gastrointestinal integrity. For these reasons, future research investigating gastrointestinal responses to exercise should aim to provide and/or control fluid intake where possible.

The ingestion of macronutrients creates a phenomenon known as postprandial hyperaemia, whereby the presence of hydrolytic products of food digestion in the gastrointestinal tract and/or absorption of nutrients increases localised microvilli blood flow.(94) Such a phenomenon may play a role in reducing gastrointestinal damage associated with exercise-induced splanchnic hypoperfusion, however, this requires further research. Interestingly, glucose has been reported as the most potent stimulator of postprandial hyperaemia followed by long-chain fatty acids and protein, whereas water or a saline solution does not stimulate an increase in intestinal blood flow.(94) Indeed, a glucose-based carbohydrate solution ingested every 20 min during 1 h cycling at 70% $\dot{V}O_{2max}$ maintained portal vein blood flow to a greater extent than water, suggesting carbohydrates may be beneficial in ameliorating exercise-induced splanchnic hypoperfusion.(91) However, to date studies investigating gastrointestinal outcomes in response to carbohydrate consumption during exercise have shown

mixed results.(39, 69, 95) For example, ingestion of a carbohydrate solution consisting of sucrose and glucose before and every 10 min during 1 h running at 70% $\dot{V}O_{2max}$ ameliorated gastroduodenal permeability induced by pre-exercise aspirin ingestion.(69) Small intestine permeability was not reduced by carbohydrate consumption compared to water placebo during this study, or in another study that included consumption of a carbohydrates during the first 40 min of a 1.5 h cycling bout.(69, 95) Moreover, ingestion of a commercial energy gel containing maltodextrin and fructose consumed at 20 min during a 1 h run at 70% $\dot{V}O_{2max}$ in the heat (30°C T_{amb}) reportedly increased intestinal epithelial injury (I-FABP increase of 263 pg/mL) compared to a water-based control.(39) Findings from this study, however, should be interpreted with caution due to the very small reported increases in I-FABP (~88 pg/mL) on the water-based control(39), which are well below previously reported I-FABP increases of 179 pg/mL to 306 pg/mL following 1 h exercise.(40-42) While findings from these previous studies have reported variable gastrointestinal outcomes(39, 69, 95), it is possible that the beneficial effects of carbohydrate ingestion may have been masked by the infrequent administration, type of carbohydrate used and/or limited exercise duration. For example, mechanistic research suggests that frequent (e.g. \leq every 20 min) ingestion of a glucose-based carbohydrate solution may be more appropriate than infrequent ingestion of mixed and/or fructose-containing carbohydrates in maintaining splanchnic perfusion during exercise.(91, 94) The translation of this mechanistic research, however, has not yet been studied in relation to the maintenance of gastrointestinal integrity during prolonged exercise or exertional-heat stress.

In addition to carbohydrates, there has been recent interest in the role of amino acids and/or amino acid precursors in preventing exercise and exertional-heat stress induced perturbations to gastrointestinal integrity. The amino acids glutamine, aspartate and glycine have been shown to induce vasodilation of intestinal segments, whereas arginine and L-citrulline, an arginine pre-cursor, have been shown to increase localised nitric oxide production and enhance intestinal microcirculation.(90, 94) A recent study by van Wijck et al.(90) demonstrated prevention of

splanchnic hypoperfusion and attenuation of intestinal epithelial injury during 1 h cycling at 70% $\dot{V}O_{2\max}$ after ingestion of 10g of L-citrulline. However, such benefits did not appear to continue throughout the post-exercise recovery period, and no differences in small intestine permeability were observed between L-citrulline and the placebo.(90)

Promising results in the maintenance of gastrointestinal integrity have also been observed with acute and chronic ingestion of glutamine prior to 1 h running at 70% $\dot{V}O_{2\max}$ in 30°C.(57, 96) For example, ingestion of glutamine 2 h before exercise attenuated the increase in small intestine permeability from resting conditions compared to a non-caloric placebo.(96) The authors of this study suggested that glutamine may upregulate heat shock proteins, stabilise intestinal tight junctions and reduce local and systemic inflammation.(96) Indeed, it is highly plausible that these factors may explain the observed benefits of glutamine ingestion, however, it is also possible that glutamine may have exerted beneficial effects through vasodilation of the intestine which would not have occurred with consumption of a non-caloric placebo.(94) Future studies should therefore aim to match the energy content of a placebo and intervention arm. Moreover, the use of a complete protein or hydrolysed whey protein containing a variety of amino acids has the potential to exert beneficial effects via multiple pathways (i.e., splanchnic perfusion, stabilisation of tight junction protein complexes and reduction in inflammatory pathways) and warrants further investigation.

2.2.2 The effects of pre- and during exercise nutrition intake on gastrointestinal symptoms

Considering the large alterations in gastrointestinal function and integrity during exercise (previously reviewed in **section 2.1**), it is not surprising that food and fluids consumed before or during exercise can contribute to the development of gastrointestinal symptoms. Current sports nutrition guidelines for avoiding the occurrence of gastrointestinal symptoms have primarily been based on observational studies of athletes during competition. Early research by Rehrer et al.(97) found that during a long-distance triathlon; 1) eating within 30 min prior to the start (i.e., swimming) was related to vomiting during the swim; 2) pre-exercise fat and protein intakes were greater in those who vomited or had the urge to vomit throughout the race; 3) hypertonic beverages were associated with vomiting and severity of symptoms; and 4) fibre rich foods were linked to intestinal cramps. Since these early observations, high protein intakes during experimental studies have been shown to contribute to the occurrence of gastrointestinal symptoms, along with high intakes of carbohydrate (90 g/h) and highly concentrated ($\geq 10\%$ concentration) carbohydrates.(25, 85, 98, 99) Indeed, carbohydrate intake at the currently recommended rate of 90 g/h during 3 h running has been shown to cause carbohydrate malabsorption, which was positively correlated with gastrointestinal symptoms.(25) However, more moderate intake rates of 35-45 g/h appear to be more commonly consumed and well tolerated by most athletes during endurance running events such as marathon and ultra-marathon running.(16, 85) Moreover, the form of ingested carbohydrate during exercise may contribute to the development of gastrointestinal symptoms, with solid food (i.e., bar) showing increased occurrence of gastrointestinal symptoms compared to semi-solids (i.e., gels) and liquids (i.e., sports drink).(100)

Recent research has also been conducted on the role of poorly absorbed short-chain carbohydrates, collectively known as FODMAPs (fermentable Oligo-, Di-, Mono-saccharides And Polyols) consumed habitually or prior to exercise.(101, 102) A low FODMAP diet can improve symptoms in patients with irritable bowel syndrome.(103) It has therefore been hypothesised that avoidance of

FODMAPs may reduce gastrointestinal symptoms associated with exercise, particularly in athletes with an undiagnosed FODMAP issue or in those prone to lower-gastrointestinal symptoms.(101) Indeed, a recent study by Lis et al.(101) observed a significant reduction in lower-gastrointestinal symptoms in athletes following a low FODMAP diet for 6 days. It is likely that improvements in lower-gastrointestinal symptoms may occur after only 24 h on a low FODMAP diet, which is commonly used prior to FODMAP breath testing and may reduce the burden of pre-exercise dietary restriction.(104) In addition to specific nutrients, fluid intake and hydration status have also been implicated as contributing factors.(105) Pre-exercise dehydration, exercise-induced dehydration and/or fluid intake above individual tolerance levels appear to disturb gastric emptying and motility, thereby contributing to the development of nausea and upper-gastrointestinal symptoms such as bloating, urge to regurgitate and vomiting.(55, 92, 105, 106) Indeed, the delay in gastric emptying associated with pre-exercise dehydration has previously been positively correlated with the symptom of nausea.(92)

While individual tolerance to food and fluid intakes may vary, an athlete's experience with consuming nutrition products during exercise is also a factor that may contribute to the occurrence of gastrointestinal symptoms. For example, an observational study has reported an association between a higher prevalence of symptoms in those unaccustomed to consuming nutrition products during exercise.(107) Due to the adaptability of the gastrointestinal tract and intestinal nutrient transporters, a 7-14 day period of "gut training" with high carbohydrate and/or fluid intakes may be a suitable prevention strategy in reducing some occurrences of gastrointestinal symptoms.(25, 108) Indeed, a reduction in carbohydrate malabsorption and gastrointestinal symptoms, including improved gut comfort have been reported in response to gut training with carbohydrates and fluid intakes above individual tolerance levels.(25, 106) However, it is currently unknown if gut training can reduce the additional nutrient requirement burden (i.e., increased carbohydrate oxidation, sweat rate and fluid loss) and gastrointestinal symptoms associated with prolonged exercise in the heat.

In summary, it appears that dehydration, highly concentrated carbohydrates (e.g. >10% w/v), high intakes of fat, protein, fibre and potentially FODMAPs prior to exercise may contribute to the development of gastrointestinal symptoms. During exercise, gastrointestinal symptoms may be exacerbated by dehydration, excessive carbohydrate, protein and fluid intakes, and highly concentrated carbohydrates. Although, it is likely that other less commonly consumed nutrients may also contribute to the development of gastrointestinal symptoms during prolonged or strenuous exercise due to the challenges imposed on the gastrointestinal system.

2.2.3 The effects of pre- and during exercise nutrition intake on systemic circulatory endotoxin and cytokine profile

Currently the effects of any nutritional intake before or during exercise on systemic circulatory endotoxin profile appear to be controversial. For example, dehydration has been linked to increased systemic endotoxin in one study(82), whereas another study observed no relationship between circulatory endotoxin and hydration status.(109) Higher pre- to post-exercise increases in circulatory endotoxin concentration have been observed after 1 h running at 70% $\dot{V}O_{2max}$ in the heat with a carbohydrate gel ingested during running compared to a water placebo, however findings were not statistically significant.(39) Additionally, a reduction in plasma endotoxin at 4 h post-exercise with ingestion of glutamine pre-exercise compared to a water-based placebo has been observed.(96) However, no differences in endotoxin profile were observed immediately post- or 2 h post-exercise.(96) Moreover, observational field studies have not observed a relationship between race diet and endotoxin profile.(17, 109) Together, these findings suggest the influence of nutrient intake on systemic endotoxin profile is currently debatable.

It is possible that the effects of nutrient intake before or during exercise on systemic endotoxin profile may be confounded by ingested sources of endotoxin, which may reach systemic circulation via small intestine lymphatics.(72, 73) Indeed, consumption of a high fat meal at rest has been consistently shown to increase systemic endotoxin concentration, with the endotoxin concentration of ingested foods varying greatly.(110, 111) These findings highlight the need for caution in interpreting small increases in circulatory endotoxin during exercise when food or fluid sources containing endotoxins are ingested.

While findings for nutrient intake on endotoxin status appear to be controversial, there is good evidence that ingestion of carbohydrates during exercise can mediate the production and release of

systemic pro- and anti-inflammatory cytokines including IL-6, IL-8, IL-1ra and IL-10.(112-115) Such responses have been proposed to arise due to increased blood glucose levels which provide an exogenous fuel source and may also attenuate the stress hormone response (e.g. catecholamines and cortisol).(115, 116) However, a consistent reduction in systemic cytokines has not been observed with carbohydrate consumption during exercise in the heat.(39, 115) It has been hypothesised that the increased endogenous carbohydrate oxidation with the concurrent decrease in exogenous carbohydrate oxidation during exercise in the heat, may attenuate the beneficial effects of carbohydrate ingestion on systemic cytokine responses.(115, 117)

The effects of protein and/or amino acid intake before or during exercise on systemic cytokine responses has not been studied to the same extent as carbohydrate intake.(114) Increases in IL-6 have been observed with consumption of glutamine and a glutamine-rich protein during 2 h cycling exercise compared to a maltodextrin-based placebo.(118) Ingestion of glutamine prior to running in the heat has also shown reductions in TNF- α at the 4 h post-exercise time point.(96) However, current research suggests that protein and amino acids supplementation appears to have minimal effects on systemic cytokine profile compared to carbohydrate ingestion before or during exercise.(114, 119)

In summary, the effects of nutrition intake before or during exercise on systemic endotoxin profile is controversial and requires further research. Carbohydrate ingestion during exercise appears to attenuate pro- and anti-inflammatory cytokine responses, whereas there appears to be limited effects of protein intake on systemic cytokine profile. A reduction in exogenous carbohydrate oxidation and/or elevated stress hormone responses may however, limit the effects of carbohydrate ingestion during exercise in the heat on systemic cytokine responses.

2.3 The effects of water temperature consumed before and during exercise on gastrointestinal integrity, symptoms and systemic cytokine profile

It has recently been proposed that exercise-induced elevations in core body temperature account for 63% of the variance in intestinal permeability in response to a range of different exercise intensities and ambient conditions.(29) These findings, in conjunction with animal and cell-culture studies suggest that hyperthermia may play a key role in perturbations to gastrointestinal integrity in response to exercise stress.(61-64) Indeed, delayed gastric emptying has been strongly associated with thermoregulatory strain during exercise in the heat, suggesting elevated core body temperature may alter gastrointestinal motility.(88) It is therefore plausible that strategies aimed at attenuating the thermoregulatory strain during exertional-heat stress may be effective at reducing gastrointestinal damage and symptoms.

While it has been shown that fluid intake is important in preventing gastrointestinal perturbations during prolonged exercise(55, 105, 120) the temperature of ingested fluids on gastrointestinal outcomes has not yet been explored and may be a practical strategy which can assist hydration, thermoregulation and possibly gastrointestinal outcomes. Ingestion of ice (-1°C) and cold water (~4°C) prior to exercise (pre-cooling), or during exercise (per-cooling), has been shown to lower core body temperature by up to 0.5°C (i.e., thermoregulatory strain), heart rate (i.e., cardiovascular strain), sweat rates, perceived exertion, thermal sensation and improve exercise performance or time to exhaustion in the heat.(121-125) It has been hypothesised that ingestion of ice or cold fluids acts as a heat sink, providing an extended heat storage capacity.(126) However, the benefits of pre-cooling on core body temperature appears to be limited to a period of ≤ 30 min.(121) Therefore, a per-cooling regime may be required to sustain reductions in core body temperature during prolonged (≥ 2 h) exercise in the heat.

The development and implementation of a practical per-cooling strategy may however, be more difficult than pre-cooling due to the logistical aspects associated with provision of ice and cold fluids in the field setting and consumption of fluids during physical exertion, rather than at rest. The temperature of fluids therefore, has the potential to influence both practical and logistical aspects which are important for the translational outcomes of cooling strategies. Fluids in the range of 0-22°C have been reported to be well tolerated and improve fluid consumption and hydration status during exercise.(127) Whereas, warmer fluids or ice may be more difficult to tolerate during exercise/physical exertion thereby adversely affecting overall fluid intake and hydration status.(127) Indeed, occupational workers have had difficulty consuming and tolerating large volumes of ice resulting in lower overall fluid intake and no additional reductions in core body temperature.(128, 129) Moreover, the logistics of providing and maintaining ice in the field setting may limit the use of ice as a cooling strategy, particularly during prolonged endurance-based events. Considering these important translational aspects, repeated ingestion of cold fluids within the tolerance range of 0-22°C may be a more practical strategy for reducing thermoregulatory strain and attenuating associated gastrointestinal perturbations during prolonged exertional-heat stress.

While the impact of cooling on perturbations to gastrointestinal integrity and symptoms has not yet been explored, several studies have investigated the effects of pre-cooling strategies on systemic cytokine profile and have shown mixed results. Reductions in the absolute change in IL-6 and IL-10 have been observed with an external pre-cooling strategy (1 h cold water immersion protocol) prior to a 1.5 h run at 65% $\dot{V}O_{2max}$ in 32°C T_{amb} compared to no cooling.(130) However, no differences in TNF- α or IL-1ra were observed in this study.(130) In contrast, pre-cooling through application of ice vests, cold towels and ice-packs prior to 30 min intermittent sprint exercise in the heat showed no effect on IL-6 compared to no cooling.(131) Greater distances (i.e., workload) were covered during the cooling arm of this study(131), which may have masked any beneficial effects of cooling on systemic cytokine responses. Similar findings have been observed for stress hormones in response to

sprint-based studies, whereby greater distances covered with cooling interventions may have masked any beneficial effect on stress hormones.(122, 131, 132)

In summary, the effect of pre-cooling and per-cooling strategies on prevention of gastrointestinal injury and symptoms during exertional-heat stress is currently unknown and warrants investigation. The impact of pre-cooling methods on systemic cytokine responses is limited and has shown mixed results however, the effect of per-cooling methods on systemic cytokine responses has not yet been explored. A per-cooling strategy consisting of frequent ingestion of cold water is a practical strategy that may provide benefits to thermoregulation, hydration status, exercise performance and possibly gastrointestinal health.

2.4 Thesis aims

The literature to date suggests heat exposure and the associated thermoregulatory strain during physical exertion may exacerbate elements of exercise-induced gastrointestinal syndrome.(2) However, no previous studies have comprehensively explored the effects of exertional heat-stress on multiple aspects of exercise-induced gastrointestinal syndrome such as intestinal epithelial injury, gastrointestinal permeability, intestinal inflammation, gastrointestinal symptoms and the associated systemic endotoxin and cytokine profiles. Further, the majority of exertional-heat stress studies have been conducted in relatively short duration (≤ 1 h) high intensity ($\geq 70\% \dot{V}O_{2\max}$) exercise(44, 58, 77), whereas the greatest gastrointestinal perturbations have most commonly been reported in response to prolonged (≥ 2 h) submaximal exercise.(2) Moreover, there is a clear lack of effective prevention and management strategies that are designed to address the primary underlying causes of exertional-heat stress induced gastrointestinal perturbations, which likely include splanchnic hypoperfusion, hyperthermia and increased stress hormone responses.

Considering current gaps in the research, the broad aims of this thesis are firstly, to determine the impact of varying degrees of prolonged exertional-heat stress on gastrointestinal perturbations as the primary outcome and associated systemic responses as a secondary outcome. Secondly, this thesis aims to explore the feasibility of novel nutrition-related prevention and management strategies for exertional-heat stress induced gastrointestinal perturbations. Specifically, the aims of each experimental study contained within this thesis were to investigate: 1) The effects of exertional-heat stress ($35^{\circ}\text{C } T_{\text{amb}}$) on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profile, and explore relationships between exercise-induced increases in core body temperature and gastrointestinal perturbations; 2) the effects of mild exertional-heat stress ($30^{\circ}\text{C } T_{\text{amb}}$) on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profile; 3) the effects of carbohydrate and protein intake during exertional-heat stress on

gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profile; and 4) the effects of the temperature of ingested water during exertional-heat stress on gastrointestinal integrity, gastrointestinal symptoms and systemic cytokine profile.

It was hypothesised that: 1) Exertional-heat stress would result in greater perturbations to gastrointestinal integrity, increased gastrointestinal symptoms and systemic responses compared to exertion in temperate conditions, and that the observed gastrointestinal perturbations would be positively correlated with exercise-induced increases in rectal temperature. 2) Mild exertional-heat stress would result in greater perturbations to gastrointestinal integrity, increased gastrointestinal symptoms and systemic responses compared to exertion in temperate conditions, but such perturbations would be lower than those observed in study 1. 3) Carbohydrate (i.e., glucose) and protein (i.e., hydrolysed whey) intake before and during exertional-heat stress would ameliorate perturbations to gastrointestinal integrity and systemic responses compared to water, but that protein intake would increase the incidence and severity of gastrointestinal symptoms compared to those associated with carbohydrate and water ingestion. 4) Cold (0°C) and cool (7°C) water ingestion before and during exertional-heat stress would reduce intestinal epithelial injury, gastrointestinal symptoms and systemic cytokine responses compared to temperate (22°C) water consumption, with cold water showing greater reductions than cool water.

CHAPTER THREE

General Methods

Additional details of the general methods of all experimental studies that are not contained within **Chapters 4-7** of this thesis are included within this chapter. The general methods applied to the design of each study have been based on previous literature in the fields of exercise physiology, clinical gastroenterology and exercise immunology, whilst also considering the validity and any limitations associated with the method.

3.1 Ethical approval

Approval was obtained from the Monash University Human Research Ethics Committee prior to the commencement of each experimental study (**appendix A**). The purpose and nature of each study was fully explained to participants, in written form through the participant information sheet (**appendix B**) and verbally. Participants were made aware that they were free to withdraw from any study at any time. All participants provided written informed consent and completed a medical questionnaire prior to the commencement of each study (**appendix C and D**). Healthy participants of both sexes aged 18-45 years were deemed eligible to initiate and complete the experimental procedures if they were 1) free from gastrointestinal infections, diseases and (or) disorders; 2) had not consumed potential modifiers of gastrointestinal integrity (such as prebiotics, probiotics, and/or antibiotics) or were not adhering to gastrointestinal-focused dietary regimes (such as fibre-modified or gluten-free diets) within the three months prior to or during the study; and 3) had not consumed non-steroidal anti-inflammatory medications and/or stool altering medications (e.g., laxatives and anti-diarrhoea) within one month before or during the study.⁽²⁵⁾ A mixed-sex cohort was included in each study to represent the population group participating in endurance running events. Indeed, participation in endurance

running events from the female sex has risen in recent years and may sometimes exceed that of male competitors.(133)

3.2 Preliminary measures

Participants' anthropometry, body composition and $\dot{V}O_{2\max}$ were measured in temperate ambient conditions (range 21-23°C) at least one week prior to the first experimental trial as described in **Chapters 4-7**.

3.3 Experimental studies diet

During each study, participants were provided with a low FODMAP diet for the 24 h period prior to each experimental trial. The low FODMAP diet (in-house generated diet containing <5g/day of FODMAPs for Chapters 4-6, and BASE Essentials[®] FODMAP modified PowerPackets[®] and ExtremeMeals[®] FODMAP content <2g/meal, Monash University, Notting Hill, Victoria, Australia for Chapter 7) was provided to reduce the risk of gastrointestinal symptoms arising from pre-exercise nutrition intake and was individually tailored to provide a total of 6 g/kg body mass carbohydrates.(101, 104)

3.4 Sample collection and analysis

Sample collection was conducted in the same order to maintain consistency in procedures across experimental trials. Prior to all experimental trials, a faecal sample was collected first, followed by baseline gastrointestinal symptoms during a 5 min period of seated rest before a venous blood sample was collected. This was followed by nude body mass measurement, thermocouple insertion and fitting of a heart rate monitor prior to exercise commencement. Upon cessation of exercise a venous blood

sample was collected, followed by removal of the thermocouple, towel drying and measurement of nude body mass. All water and fluids provided to participants were weighed on digital scales sensitive to 0.1 g (APTK461, AMPUT, Guangdong, China). Any remaining water at the cessation of exercise was weighed and subtracted from the provided water to determine total fluid intake during exercise for each experimental trial. Exercise-induced body mass loss was determined by calculating the difference between pre-exercise and post-exercise nude body mass, which was then divided by pre-exercise nude body mass and multiplied by 100 to obtain a percentage value.

To determine small intestine permeability, participants consumed a dual-sugars solution containing 5 g lactulose (Duphalac, Abbott Biologicals, Olst, Netherlands) and 1 g L-rhamnose (MP Biomedicals, LLC, Solon, USA) in 100 ml water, 90 min into exercise as described in **Chapters 4-7**. The 1 g L-rhamnose and 5g of lactulose were weighed on digital scales sensitive to 0.1 g (APTK461, AMPUT, Guangdong, China) and added to a small plastic cup, where 100 ml of weighed water was added before stirring the solution to dissolve the L-rhamnose and lactulose. The dual-sugars drink mixture was made within the 5 min period prior to participant consumption. A 5 h urine collection period commenced post-ingestion, where the final volume was weighed, and 30 ml aliquoted and stored frozen at -20°C until analysis.

As described in **Chapters 4-7**, whole blood samples were used to determine haematocrit in triplicate, and haemoglobin and blood glucose in duplicate with a 1.4%, 4.3% and 2.2% coefficient of variation (CV), respectively for all experimental chapters. Haemoglobin was determined by aspirating approximately 500µg of lithium heparin blood into a HemoCue201 microcuvette (Hemocue, Ängelholm, Sweden) and read using a HemoCue HB 201⁺ haemoglobin analyser (Hemocue, Ängelholm, Sweden). Haematocrit was determined by aspirating approximately 500µg of lithium heparin blood into three capillary tubes which were then sealed with a capillary tube sealant at one end. Capillary tubes were centrifuged for 2 minutes at 10,000 rpm before reading haematocrit values

by placing capillary tubes on a micro-haematocrit reader. Plasma was used to determine plasma osmolality, concentrations of cortisol, I-FABP, gram-negative bacterial endotoxin, endotoxin core antibody and cytokine profile (IL-6, IL-1 β , TNF- α , IL-8, IL-10, and IL-1ra) in duplicate with a CV of 2.9%, 3.2%, 4.8%, 3.2%, 4.6%, 3.2%, 3.6%, 4.0%, 3.2%, 1.6% and 3.0% for all experimental chapters. Urine samples were collected as described in **Chapters 4-6** to determine small intestine permeability with a CV of 13.8% for all experimental chapters. Faecal samples were collected by study participants using a faecal sample collection container (Sarstedt, Nümbrecht, Germany) within the 12 h period prior to exercise (stored at 4°C if sample was collected \geq 1 h prior to exercise) and from the first stool \geq 4 h post-exercise. Faecal samples were frozen at -80°C until analysis as described in **Chapter 4**. Faecal samples were thawed at room temperature before transferring faecal samples to an incubation solution using the Euorspital device for stool collection as per manufacturer's instructions (Eurospital S.p.a., Via Flavia, Trieste, Italy). Faecal samples were incubated at 4°C for 12 h prior to conducting analysis of faecal calprotectin concentration by enzyme linked immunosorbent assay (ELISA) as per manufacturer's instructions (DE849, Demeditec, Kiel, Germany) with a CV of 9% for all experimental chapters.

3.5 Gastrointestinal symptoms

A 10-point Likert-type rating scale (**appendix E**) was used to quantify self-perceived gastrointestinal symptoms adapted from a 10 cm visual analogue scale(134, 135), with 0 indicative of no symptoms to 10 indicative of extreme symptoms warranting cessation of exercise (5 indicative of severe symptoms) as described further in **Chapters 4-7**.(25) The individual participant cumulative score for gastrointestinal symptoms were determined by calculating the total severity score for each gastrointestinal symptom during exercise. Ratings of appetite and thirst were also collected in conjunction with gastrointestinal symptoms.

3.6 Statistical analysis

A power calculation was also conducted for I-FABP based on a standard deviation of 118 pg/mL(40) and using a standard alpha (0.05) and beta value (0.8), a sample size of n= 6 was estimated to have adequate statistical precision to detect a >100% increase in I-FABP values post-exercise (www.dssresearch.com/toolkit/sscalc). Additionally, based on the typical standard deviation of 0.7 EU/mL for circulatory endotoxin responses to exertional-stress in the field(71), and using standard alpha (0.05) and beta values (0.8), a sample size of n= 8 was calculated to have adequate statistical precision to detect a >10% difference in circulatory endotoxin concentration in response to exertional-heat stress in the target population. Such increases in circulatory endotoxin concentration have consistently been associated with systemic inflammatory and compensatory anti-inflammatory responses.(70, 71)

In each experimental study, participants completed all trials. However, some blood and faecal samples at certain time-points were missed due to difficulty obtaining a sample. Therefore, only participants with full data sets within each specific variable were used in the data analysis. All data were checked for normal distribution by calculating skewness and kurtosis coefficients. Where data violated the assumption of normality (positive skewness and kurtosis), data were log-transformed, and the transformed data were used for the analysis. The data were examined using two-way repeated-measures ANOVA for multiple time points or paired sample t-test for single time points, except for gastrointestinal symptoms which were examined using non-parametric equivalents (e.g. Friedman's test or Wilcoxon signed rank test). Assumptions of homogeneity and sphericity were checked, and when appropriate adjustments to the degrees of freedom were made using the Greenhouse-Geisser correction method. Significant main effects were analysed using a post hoc Tukey's HSD test or Wilcoxon signed rank test for gastrointestinal symptoms. Statistics were analysed using SPSS statistical software (V.23.0, Chicago, Illinois, USA) with significance accepted at $p \leq 0.05$. Data in

the text and tables are presented as mean and 95% confidence interval (CI) or \pm standard deviation (SD), and accumulative score and individual participant range for gastrointestinal symptoms. For clarity, data in figures are presented as mean \pm standard error of the mean (SEM).

In summary, healthy athletes were screened, and written consent was obtained, prior to anthropometry measures and a $\dot{V}O_{2\max}$ test, which was conducted at least one week prior to the first experimental trial. Primary outcome measures of intestinal epithelial injury, small intestine permeability, intestinal inflammation and gastrointestinal symptoms were measured by plasma I-FABP, urinary lactulose to L-rhamnose ratio, faecal calprotectin and a 10-point Likert-type rating scale, respectively. Systemic endotoxin (including endotoxin and anti-endotoxin antibodies) and cytokine profile (IL-6, IL-1 β , TNF- α , IL-8, IL-10, and IL-1ra) were measured from blood plasma as secondary outcome measures.

CHAPTER FOUR

The impact of exertional-heat stress on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profile.

4.1 Background

Gastrointestinal issues are so common in endurance running that Bill Rodgers, a former Olympic marathoner and world record holder once proclaimed, “More marathons are won or lost in the porta-toilets than at the dinner table”.(136) Indeed, gastrointestinal symptoms are so common that they affect up to six out of every ten endurance runners during competition and up to nine out of ten athletes when endurance events are conducted in the heat.(15, 22) Exposure to hot ambient conditions during prolonged physical exertion has also been shown to increase systemic endotoxaemia and cytokinaemia.(58, 70, 74, 82) However, the effects of heat exposure during physical exertion on gastrointestinal integrity, including intestinal epithelial injury, intestinal permeability and inflammation, which are key components of exercise-induced gastrointestinal syndrome are currently unknown. Moreover, the role of heat exposure and the associated thermoregulatory strain as a contributor to the development of exercise-induced gastrointestinal symptoms has not yet been investigated.

The first experimental study within this thesis, therefore, aims to address these research gaps by investigating the effect of exposure to hot ($35^{\circ}\text{C T}_{\text{amb}}$) and temperate ($22^{\circ}\text{C T}_{\text{amb}}$) ambient conditions during prolonged running on several markers of gastrointestinal integrity, in conjunction with gastrointestinal symptoms and systemic endotoxin and cytokine profiles. The comprehensive findings from this foundation study will build on the existing exertional-heat stress literature and provide a

deeper understanding on the effects of heat exposure on the gastrointestinal system during prolonged running. Such findings are important, not only for enhancing knowledge on the effects of exertional-heat stress on gastrointestinal health, but also in devising appropriate prevention and management strategies which target multiple aspects of exercise-induced gastrointestinal syndrome.

4.2 Abstract

Purpose: The study aimed to determine the effects of exertional-heat stress on gastrointestinal integrity, symptoms, systemic endotoxin and inflammatory responses; and assess the relationship between changes in body temperature and gastrointestinal perturbations.

Methods: Ten endurance runners completed 2 h running at 60% $\dot{V}O_{2max}$ in hot (HOT: 35°C) and temperate (TEMP: 22°C) ambient conditions. Rectal temperature (T_{re}) and gastrointestinal symptoms were recorded every 10 min during exercise. Blood samples were collected pre- and post-exercise, and during recovery to determine plasma intestinal fatty acid binding protein (I-FABP), cortisol, bacterial endotoxin and cytokine profile. Calprotectin was determined from pre- and post-exercise faecal samples. Urinary lactulose:L-rhamnose ratio was used to measure intestinal permeability.

Results: HOT significantly increased T_{re} compared to TEMP (mean \pm SD; $2.4 \pm 0.8^\circ\text{C}$ vs. $1.4 \pm 0.5^\circ\text{C}$, $p < 0.001$), cortisol (82% vs. 26%, $p < 0.001$), I-FABP (432% vs. 127%, $p < 0.001$), incidence (90% vs. 70%) and severity (720 counts vs. 58 counts, $p = 0.008$) of total gastrointestinal symptoms. Faecal calprotectin and circulating endotoxin increased post-exercise in both trials (mean increase $1.5 \pm 2.5 \mu\text{g/g}$, $p = 0.032$, and $6.9 \pm 10.3 \text{ pg/mL}$, $p = 0.047$, respectively), while anti-endotoxin antibodies increased 28% post-exercise with TEMP and decreased 21% with HOT ($p = 0.027$). However, intestinal permeability did not differ between trials ($p = 0.185$). Inflammatory cytokines were greater with HOT compared to TEMP ($p < 0.05$). Increases in T_{re} were positively associated with I-FABP, IL-10, cortisol, nausea and urge to regurgitate ($p < 0.05$).

Conclusions: Exertional-heat stress induces a thermoregulatory strain that subsequently injures the intestinal epithelium, reduces endotoxin clearance capacity, promotes greater cytokinaemia, and development of gastrointestinal symptoms.

4.3 Introduction

Physical exertion in hot ambient conditions is a common feature of many recreational and professional activities (e.g., occupational, adventure, and expedition) and sports (e.g., team and endurance). Recently, there have been concerns raised over the substantial incidence of gastrointestinal symptoms, and subsequent medical support, observed during exertional-heat stress; although such reports have primarily been limited to endurance and ultra-endurance events.(15, 22, 23) Indeed, prolonged exercise reduces splanchnic blood flow, leading to ischaemia and disruption of the gastrointestinal barrier, potentially allowing for the translocation of pathogenic endotoxins across the epithelium, promoting local and systemic inflammatory responses.(34, 40, 70, 71, 76, 137) Such perturbations have been linked to the onset of acute health complications (e.g., exertional heat stroke and fatal septic shock, ischaemic colitis, gastritis and paralytic ileus) and may have the potential to exacerbate chronic (e.g., inflammatory bowel disease and chronic fatigue syndrome) health complications in those with a predisposition to and/or current chronic gastrointestinal condition.(6, 8, 11, 14, 32, 47, 138, 139)

Exercise duration, intensity, and running mode appear to be key exacerbating factors, with a threshold of ≥ 2 h running at 60% maximal oxygen uptake ($\dot{V}O_{2\max}$), or equivalent, causing substantial gastrointestinal disturbance.(2) For example, repetitive high intensity exercise (i.e., 18 x 400 m running bouts at 120% $\dot{V}O_{2\max}$) results in minimal disturbances to gastrointestinal integrity(45), in comparison with endurance and ultra-endurance exercise.(2) Moreover, gastrointestinal symptoms such as nausea, regurgitation, pain, diarrhoea, and (or) faecal blood loss are the end result of such

exercise-associated gastrointestinal perturbations(11), with symptom incidence of $\geq 60\%$ consistently reported during and after endurance events.(15, 16, 22, 23) The incidence and severity of gastrointestinal symptoms can adversely affect performance, nutrition intake during and after exertion, and cause withdrawal from exertional activities.(15, 16, 22-24)

Prolonged physical exertion in hot ($\geq 35^{\circ}\text{C}$) ambient conditions poses additional challenges due to increased thermoregulatory strain, body water losses, and the associated hypovolemia that can further exacerbate splanchnic hypoperfusion.(1, 37, 88, 140) Current evidence indicates that hyperthermia directly contributes to intestinal epithelial injury, disrupts epithelial tight junction proteins and increases intestinal permeability; although the majority of research to date has been in animal and cell-culture studies.(29, 61-64) Additionally, exertional-heat stress studies in humans to date have predominantly focused on endotoxaemia as a primary outcome, and (or) intestinal permeability as a secondary marker, rather than directly and (or) comprehensively measuring intestinal injury, permeability, inflammation, and accompanying gastrointestinal symptoms collectively, which may have broader ranging health implications.(44, 58, 74) Therefore, the full extent of gastrointestinal perturbations and associated health implications arising from exertional-heat stress remains to be elucidated. Furthermore, the majority of exercise-associated gastrointestinal perturbation research has been of relatively short exercise durations (≤ 1 h) at high intensity ($\geq 70\% \dot{V}O_{2\text{max}}$). However, the populations most likely at risk of exertional-heat stress induced gastrointestinal perturbations and potential health implications are endurance athletes and active occupational workers (i.e., mining, military, firefighters, construction, and agricultural workers) who undertake prolonged workloads at moderate intensity in hot ambient conditions.

Considering the lack of research comprehensively investigating gastrointestinal perturbations arising from prolonged submaximal exertional-heat stress, the current study aimed to first determine the effects of prolonged exertional-heat stress on several measures of gastrointestinal integrity, including

intestinal fatty acid binding protein as a marker of intestinal epithelial injury, lactulose to L-rhamnose ratio as a measure of small intestinal permeability, and faecal calprotectin as a measure of intestinal inflammation; in conjunction with perceptive gastrointestinal symptoms, and associated systemic circulatory endotoxin, anti-endotoxin antibodies, and cytokine profile (IL-6, IL-1 β , TNF- α , IL-8, IL-10, and IL-1ra). A secondary aim of this study was to assess the relationship between body temperature increases induced by exercising in temperate and hot ambient conditions and the aforementioned markers of gastrointestinal perturbation. It was hypothesised that running in the heat would result in greater perturbations to gastrointestinal integrity, increased systemic responses, and gastrointestinal symptoms compared to running in temperate conditions. Additionally, greater increases in body temperature would result in greater gastrointestinal perturbations and symptoms.

4.4 Methods

Participants

Ten non-heat acclimatised endurance trained runners [mean \pm SD: (male $n= 6$, female $n= 4$) age 31 ± 6 years, nude body mass 66.3 ± 10.5 kg, height 1.71 ± 0.10 m, % body fat mass $18 \pm 6\%$, $\dot{V}O_{2max}$ 56 ± 8 ml/kg/min] volunteered to participate in the study. All participants gave written informed consent, which received approval from the local ethics committee and conformed to the 2008 Helsinki Declaration for Human Research Ethics. The standardised exclusion criteria were in accordance with Costa et al.(25)

Preliminary measures

One week before the first experimental trial, height and nude body mass (Seca 515 MBCA, Seca Group, Hamburg, Germany) were recorded. $\dot{V}O_{2max}$ (Vmax Encore Metabolic Cart, Carefusion, San Diego, California, US) was estimated by a continuous incremental exercise test to volitional exhaustion on a motorized treadmill (Forma Run 500, Technogym, Seattle, Washington, US). To

determine running speed for the experimental trials, the treadmill speed at approximately 60% $\dot{V}O_{2\max}$ and 1% gradient was extrapolated from the $\dot{V}O_2$ -work rate relationship and then verified (10.4 ± 0.8 km/h).

Experimental procedure

To reduce any potential gastrointestinal symptoms arising from pre-exercise food and fluid intake, participants were provided with a low fermentable oligo-, di-, mono-saccharide, and polyol (FODMAP) diet for the 24 h period before each experimental trial (12.6 ± 1.8 MJ, 461 ± 69 g carbohydrate, 114 ± 10 g protein, 72 ± 13 g fat). Participants refrained from strenuous exercise for 48 h before each experimental trial with compliance determined by a dietary and exercise log. Participants reported to the laboratory at 08:00h after consuming the low FODMAP breakfast (2.5 MJ, 125 g carbohydrate, 14 g protein, and 4 g fat) with 400 ml of water (consumed at 07:00h). A pre-exercise faecal sample was collected and stored frozen at -80°C until analysis. Participants were asked to void before nude body mass measurements and completion of a self-reported gastrointestinal symptom assessment tool. A 10-point Likert-type rating scale was used to quantify self-perceived gastrointestinal symptoms (adapted from a 10 cm visual analogue scale (134, 135)), with 0 indicative of no symptoms to 10 indicative of extreme symptoms. Blood was then collected by venepuncture from an antecubital vein into a vacutainer (6 ml, 1.5 IU/ml heparin). To monitor rectal temperature (T_{re}) during running, participants inserted a thermocouple 12 cm beyond the external anal sphincter (Grant REC soft insertion probe thermocouple; Grant 2010 Squirrel data logger, Shepreth, UK).

In a randomised order with a one-week washout, participants completed 2 h (initiated at 09:00h) running exercise on a motorised treadmill at the previously determined speed within an environmental chamber in hot (HOT: $35.4 \pm 1.8^\circ\text{C}$ and $26 \pm 4\%$ relative humidity) and temperate (TEMP: $22.2 \pm 1.0^\circ\text{C}$ and $44 \pm 6\%$ relative humidity) ambient conditions. In the absence of repeated gut-training with a set fluid volume, an *ad libitum* water intake regime (HOT: 1.6 ± 0.4 l and TEMP: 0.8 ± 0.1 l).

was employed to provide autonomy over drinking patterns to minimise occurrence of gastrointestinal symptoms.(25, 106) Heart rate, rating of perceived exertion (RPE; 20-point Likert-type perceived exertion rating scale, with 7 indicative of very very light and 19 indicative of very very hard)(141), thermal comfort rating (13-point Likert-type thermal rating, with 7 indicative of comfortable, 10 indicative of hot, and 13 indicative of unbearably hot) (adapted from (142)), T_{re} , and gastrointestinal symptoms were measured and recorded every 10 min during running. To determine small intestinal permeability, participants consumed a dual-sugars solution containing 5 g lactulose (Duphalac, Abbott, Botany, Australia) and 1 g L-rhamnose (MP Biomedicals, LLC, Solon, USA) in 100 ml water, 90 min into exercise. A 5 h urine collection commenced post-ingestion, where the final volume was weighed, and 30 ml aliquoted and stored frozen at -20°C until analysis. Immediately after exercise, a blood sample was collected and nude body mass was recorded. Participants remained seated during the recovery period and consumed water *ad libitum*. Blood was also collected 1 h, 2 h, and 4 h post-exercise, with a faecal sample collected from the first stool ≥ 4 h post-exercise. To reduce any seasonal heat acclimatisation, the experimental procedures were conducted over the cooler seasonal periods (temperatures consistently $\leq 20^{\circ}\text{C}$).

Sample analysis

Whole blood haemoglobin and haematocrit values were used to estimate changes in plasma volume, and used to correct plasma variables. Blood glucose concentration was determined pre- and post-exercise using a handheld monitor (Accu-Chek Proforma, Roche Diagnostics). The remaining blood samples were centrifuged at 4000 rpm for 10 min within 15 min of sample collection. Plasma was aliquoted and frozen at -80°C until analysis, except for 50 μl that were used to determine plasma osmolality (P_{Osmol}), in duplicate (coefficient of variation (CV): 2.7%), by freeze-point osmometry (Osmomat 030, Gonotec, Berlin, Germany). Recovery of lactulose and L-rhamnose in urine were determined by ultra-performance liquid chromatography in duplicate (CV: 13.8%). Plasma concentrations of IL-6, IL-1 β , TNF- α , IL-8, IL-10, and IL-1ra were determined by multiplex ELISA

(HCYTOMAG-60K, EMD Millipore, Darmstadt, Germany). Circulating gram-negative bacterial endotoxin concentration was determined by LAL chromogenic endpoint assay (HIT302, Hycult Biotech, Uden, Netherlands). Plasma concentrations of I-FABP (HK406, Hycult Biotech, Uden, Netherlands), endotoxin core antibody (HK504, Hycult Biotech, Uden, Netherlands), cortisol (RE52061, IBL International, Hamburg, Germany), and faecal concentrations of calprotectin (DE849, Demeditec, Kiel, Germany) (CV:9.0%) were determined by ELISA. All variables were analysed in duplicate as per manufacturer's instructions on the same day, with standards and controls on each plate, and each participant assayed on the same plate. The CVs for endotoxin, I-FABP, endotoxin core antibody, cortisol, IL-10, IL-1ra, IL-1 β , IL-6, IL-8 and TNF- α were 3.2%, 5.4%, 6.0%, 3.1%, 2.0%, 2.3%, 1.4%, 1.7%, 0.6% and 1.1%, respectively.

Statistical analysis

Data in the text are presented as mean and 95% confidence interval (CI), and accumulative score and individual participant range for gastrointestinal symptoms, otherwise specified. For clarity, data in figures are presented as mean \pm standard error of the mean (SEM). All data were checked for normal distribution by calculating skewness and kurtosis coefficients. Where data violated the assumption of normality (positive skewness and kurtosis), data were log-transformed prior to analysis. Variables with singular data points were examined using paired sample t-tests (or non-parametric equivalents); while variables with multiple data points were examined using a two-way repeated-measures ANOVA. Assumptions of homogeneity and sphericity were checked, and when appropriate adjustments to the degrees of freedom were made using the Greenhouse-Geisser correction method. Significant main effects were analysed using a post hoc Tukey's HSD test. Pearson product-moment correlation was used to assess associations between variables, except for gastrointestinal symptoms which were analysed using Spearman's rank correlation. Statistics were analysed using SPSS statistical software (V.23.0, Chicago, Illinois, USA) with significance accepted at $p \leq 0.05$.

4.5 Results

Hydration status, cardiovascular and thermoregulatory strain

Exercise-induced body mass loss (HOT: 1.6 (1.0-2.1)% and TEMP: 1.6 (1.0-2.1)%) did not differ between trials ($p=0.968$). Plasma osmolality did not differ pre- to post-exercise with HOT (293 (287-299) mOsmol/kg and 295 (289-301) mOsmol/kg, respectively) and TEMP (292 (287-299) mOsmol/kg and 295 (289-301) mOsmol/kg, respectively) or between trials (trial x time, $p=0.639$). A main effect of time was observed for change in plasma volume ($p<0.001$), with the greatest reduction seen immediately after exercise (TEMP: -7.7% and HOT: -10.0%), and returning to similar baseline level 1 h and 2 h into recovery with TEMP and HOT, respectively. A higher peak T_{re} (39.6 (39.1-40.0) $^{\circ}$ C vs. 38.5 (38.2-38.7) $^{\circ}$ C, $p<0.001$) and exercise-induced increase in T_{re} (2.4 (1.8-2.9) $^{\circ}$ C vs. 1.4 (1.1-1.8) $^{\circ}$ C, $p<0.001$) was observed with HOT compared to TEMP, respectively. T_{re} (**Figure. 4.1a**), heart rate (**Figure. 4.1b**), and RPE (**Figure. 4.1c**) increased during exercise and were higher with HOT compared to TEMP (trial x time, $p<0.001$). Thermal comfort rating increased during exercise (time effect, $p<0.001$), with a greater thermal rating observed with HOT compared to TEMP (trial effect, $p=0.001$) (**Figure. 4.1d**).

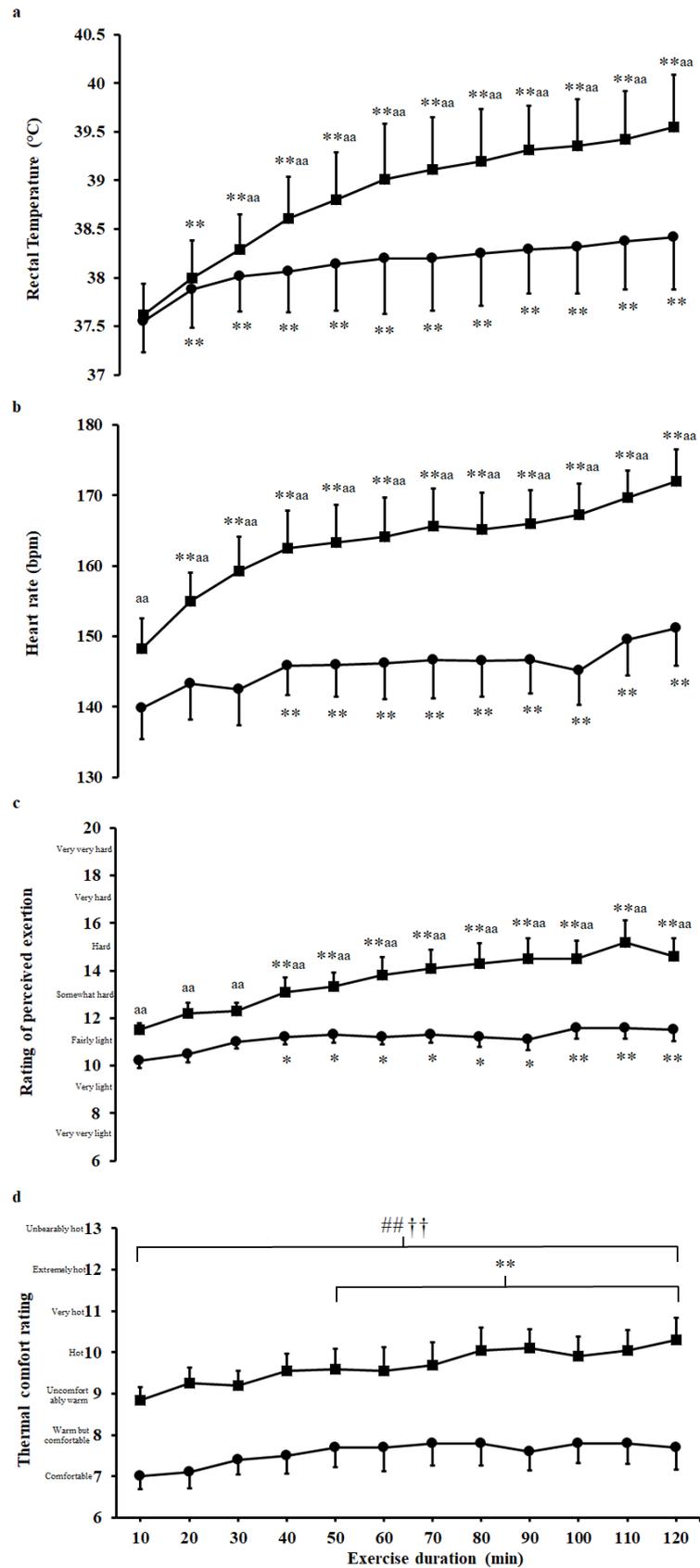


Figure 4.1 Rectal temperature (a), heart rate (b), rating of perceived exertion (c), and thermal comfort rating (d) responses during 2 h running at 60% $\dot{V}O_{2max}$ in hot (HOT: 35°C ■) and temperate (TEMP: 22°C ●) ambient conditions. Mean \pm SEM (n= 10): †† main effect of time p< 0.001 vs. pre-exercise, ## main effect of trial p= 0.001, * p< 0.01 and * p< 0.05 vs. pre-exercise, ^{aa} p< 0.01 vs. TEMP.

Plasma cortisol and blood glucose concentration

Plasma cortisol concentration increased pre- to post-exercise with HOT (363 (251-475) nmol/ml vs 660 (444-876) nmol/ml, $p < 0.01$) and TEMP (362 (276-447) nmol/ml vs. 456 (355-556) nmol/ml, $p < 0.01$), with HOT showing significantly higher concentrations post-exercise than TEMP (trial x time, $p < 0.001$). Blood glucose concentration increased (time effect, $p = 0.004$) pre- to post-exercise with HOT (5.1 (4.3-5.8) mmol/l vs. 6.2 (5.8-6.5) mmol/l) and TEMP (4.8 (4.0-5.6) mmol/l vs. 5.7 (5.2-6.1) mmol/l), with no difference between trials (trial x time, $p = 0.757$).

Gastrointestinal integrity and symptoms

I-FABP significantly increased pre- to post-exercise with HOT (285 (137-432) pg/ml vs. 1515 (965-2065) pg/ml, $p < 0.01$), and was greater with HOT than TEMP (Δ pre- to post-exercise: 432% (1230 (710-1750) pg/ml) vs. 127% (274 (127-422) pg/ml); trial x time, $p < 0.001$) (**Figure 4.2**). Faecal calprotectin increased pre- to post-exercise (time effect, $p = 0.032$) with HOT (2.3 (1.4-3.2) $\mu\text{g/g}$ and 3.3 (1.4-5.2) $\mu\text{g/g}$, respectively) and TEMP (1.9 (1.3-2.6) $\mu\text{g/g}$ and 3.8 (1.3-6.3) $\mu\text{g/g}$, respectively), with no difference between trials (trial x time, $p = 0.518$). Small intestinal permeability (i.e., lactulose:L-rhamnose ratio) was not significantly different between HOT (0.032 (0.022-0.042)) and TEMP (0.025 (0.014-0.035), $p = 0.185$). Gut discomfort, total, upper-, and lower-gastrointestinal symptoms were significantly higher with HOT compared to TEMP (all $p < 0.05$), and greater during the 2nd h of exercise compared to the 1st h with HOT only (**Table 4.1**).

Table 4.1 Incidence and severity of gut discomfort, total, upper-, and lower-gastrointestinal symptoms in response to 2 h running at 60% $\dot{V}O_{2max}$ in hot (HOT: 35°C) and temperate (TEMP: 22°C) ambient conditions.

	TEMP				HOT			
	Incidence	1 st h (range)	2 nd h (range)	Total (range)	Incidence	1 st h (range)	2 nd h (range)	Total (range)
Gut discomfort	NA	33 (0-13)	23 (0-11)	56 (0-24)	NA	81 (0-15)	172 (0-52) [‡]	253 (0-67) ^{aa}
Total gastrointestinal symptoms	70%	35 (0-13)	23 (0-11)	58 (0-24)	90%	185 (0-54)	535 (0-200) [‡]	720 (0-254) ^{aa}
Upper-gastrointestinal symptoms	40%	14 (0-8)	9 (0-6)	23 (0-10)	90%	92 (0-30)	248 (0-98) [‡]	340 (0-128) ^{aa}
Belching	40%	7 (0-2)	5 (0-6)	12 (0-6)	80%	23 (0-6)	62 (0-27) ^c	85 (0-33) ^a
Heartburn	0%	0 (0-0)	0 (0-0)	0 (0-0)	10%	5 (0-5)	12 (0-12)	17 (0-17)
Bloating	20%	9 (0-6)	2 (0-2)	11 (0-8)	70%	33 (0-13)	70 (0-27) [‡]	103 (0-40) ^a
Upper abdominal pain	0%	0 (0-0)	0 (0-0)	0 (0-0)	20%	15 (0-10)	33 (0-18)	48 (0-28)
Urge to regurgitate	0%	0 (0-0)	0 (0-0)	0 (0-0)	30%	6 (0-3)	71 (0-45)	77 (0-48)
Regurgitation	0%	0 (0-0)	0 (0-0)	0 (0-0)	10%	10 (0-10)	0 (0-0)	10 (0-10)
Lower-gastrointestinal symptoms	30%	21 (0-21)	11 (0-11)	32 (0-24)	70%	31 (0-15)	60 (0-22) [‡]	91 (0-37) ^a
Flatulence	0%	0 (0-0)	0 (0-0)	0 (0-0)	30%	0 (0-0)	6 (0-2)	6 (0-2)
Urge to defecate	30%	21 (0-13)	11 (0-11)	32 (0-24)	40%	26 (0-15)	44 (0-22) ^c	70 (0-37)
Abdominal pain/s	0%	0 (0-0)	0 (0-0)	0 (0-0)	20%	5 (0-5)	10 (0-9)	15 (0-14)
Abnormal defecation ¹	0%	0 (0-0)	0 (0-0)	0 (0-0)	0%	0 (0-0)	0 (0-0)	0 (0-0)
Others								
Nausea	10%	0 (0-0)	1 (0-1)	1 (0-1)	40%	23 (0-10)	102 (0-52) ^c	125 (0-62) ^b
Dizziness	0%	0 (0-0)	0 (0-0)	0 (0-0)	60%	26 (0-12)	103 (0-32) [‡]	129 (0-43) ^a
Abdominal stitch	10%	0 (0-0)	2 (0-2)	2 (0-2)	30%	13 (0-5)	22 (0-12)	35 (0-17) ^a
Appetite	NA	62 (0-17)	132 (0-32) [‡]	194 (0-49)	NA	47 (0-13)	60 (0-23)	107 (0-36) ^a
Thirst	NA	112 (0-21)	110 (0-21)	222 (0-39)	NA	168 (9-26)	185 (9-27)	353 (18-53) ^a

Overall participant summative accumulation of rating scale point score of measured time periods and individual participant range (n=10). ^{‡‡} p< 0.01 and [‡] p< 0.05 vs. 1st h, ^{aa} p< 0.01 and ^a p< 0.05 vs. TEMP, ^b p= 0.068 vs. TEMP, ^c p< 0.07 vs. 1st h. ¹ abnormal defecation including loose watery stools, diarrhoea and blood in stools. NA: not applicable.

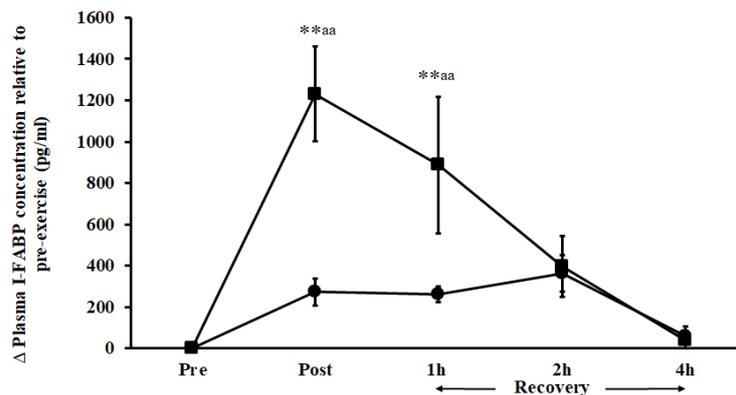


Figure. 4.2 Change in plasma intestinal fatty acid binding protein (I-FABP) in response to 2 h running at 60% $\dot{V}O_{2max}$ in hot (HOT: 35°C ■) and temperate (TEMP: 22°C ●) ambient conditions. Mean \pm SEM ($n = 10$): ** $p < 0.01$ vs. pre-exercise, ^{aa} $p < 0.01$ vs. TEMP.

Systemic endotoxin and cytokine profile

A rise in circulating endotoxin concentration was observed post-exercise with TEMP (5% (4.1 (-2.5-10.7) pg/ml)) and HOT (11% (9.6 (0.7-18.6) pg/ml); time effect, $p = 0.047$); however, no interaction effect was observed (trial \times time, $p = 0.142$) (**Figure. 4.3a**). Plasma anti-endotoxin antibody concentration increased pre- to post-exercise with TEMP (28% (34 (14-55) pg/ml), however decreased with HOT (21% (-16 (-36-4) pg/ml); trial \times time, $p = 0.027$) (**Figure. 4.3b**). A trial \times time interaction was observed for plasma concentrations of IL-6 ($p = 0.034$), IL-1 β ($p = 0.033$), IL-10 ($p = 0.003$), and IL-1ra ($p = 0.030$); whereas a main effect of time was observed for plasma TNF- α ($p = 0.011$) and IL-8 ($p = 0.008$) concentrations (**Figure. 4.4**). Running in 35°C resulted in a greater compensatory anti-inflammatory cytokine response, as indicated by significantly lower IL-1 β :IL-10 and TNF- α :IL-10 ratio with HOT compared to TEMP (trial \times time, $p = 0.002$ and $p < 0.001$, respectively) (**Figure. 4.5**). Peak post-exercise values for plasma endotoxin, anti-endotoxin antibody, IL-6, IL-1 β , TNF- α and IL-8 occurred immediately post-exercise, with IL-10 and IL-1ra peaking at 1 h post-exercise.

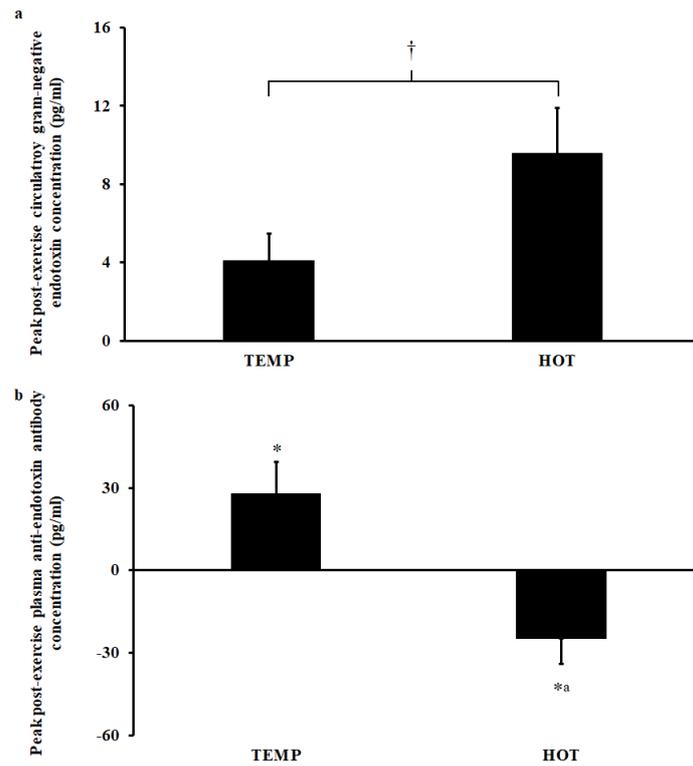


Figure. 4.3 Peak post-exercise circulating gram-negative bacterial endotoxin (a) and pre- to post-exercise change in plasma anti-endotoxin antibody (b) concentration in response to 2 h running at 60% $\dot{V}O_{2max}$ in hot (HOT: 35°C) and temperate (TEMP: 22°C) ambient conditions. Mean \pm SEM ($n=10$): † main effect of time $p < 0.05$ vs. pre-exercise, * $p < 0.05$ vs. pre-exercise, ^a $p < 0.05$ vs. TEMP.

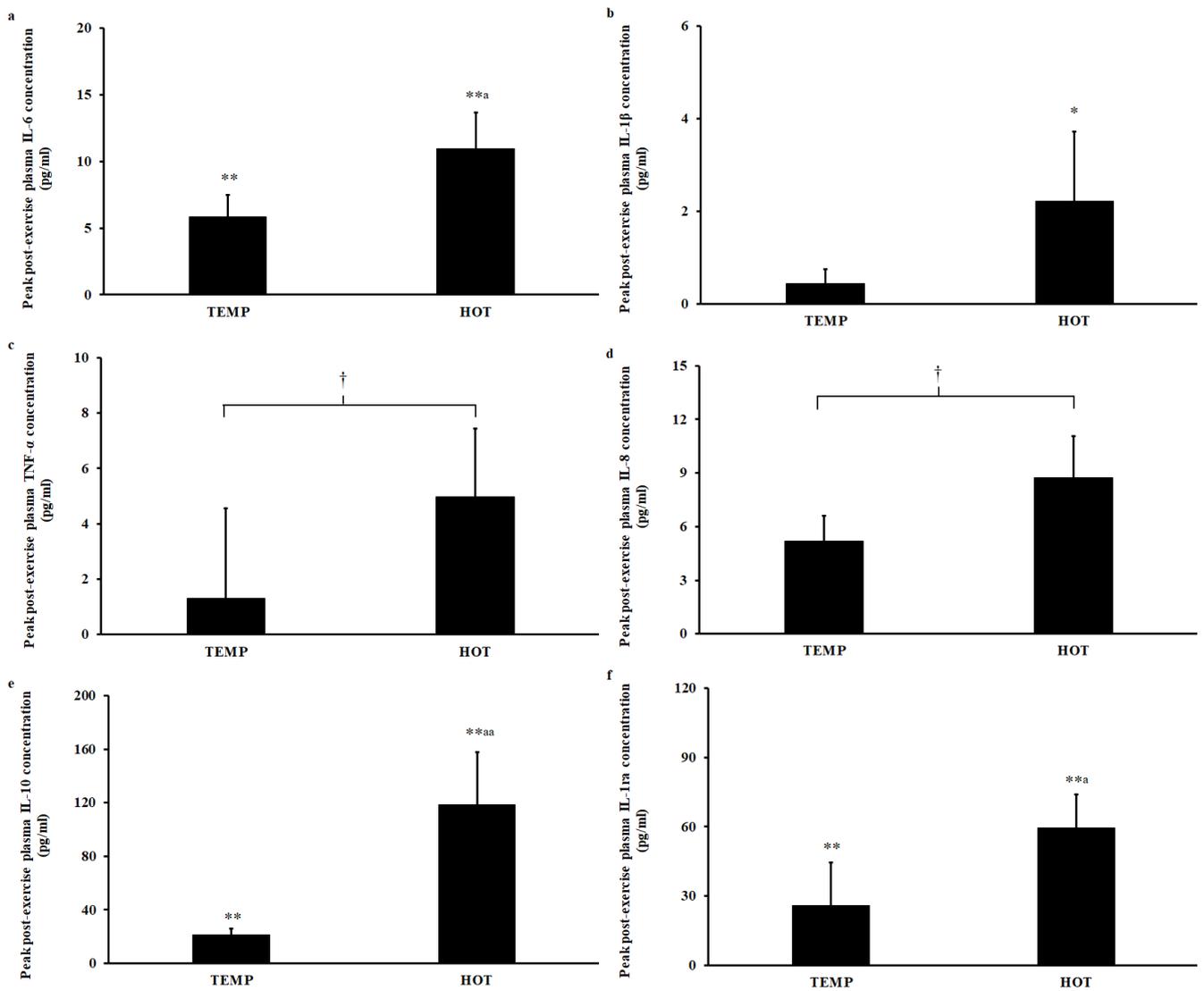


Figure. 4.4 Peak post-exercise plasma IL-6 (a), IL-1 β (b), TNF- α (c), IL-8 (d), IL-10 (e), and IL-1ra (f) concentrations in response to 2 h running at 60% $\dot{V}O_{2max}$ in hot (HOT: 35°C) and temperate (TEMP: 22°C) ambient conditions. Mean \pm SEM ($n=9$): † main effect of time $p < 0.05$ vs. pre-exercise, ** $p < 0.01$ and * $p < 0.05$ vs. pre-exercise, ^{aa} $p < 0.01$ and ^a $p < 0.05$ vs. TEMP.

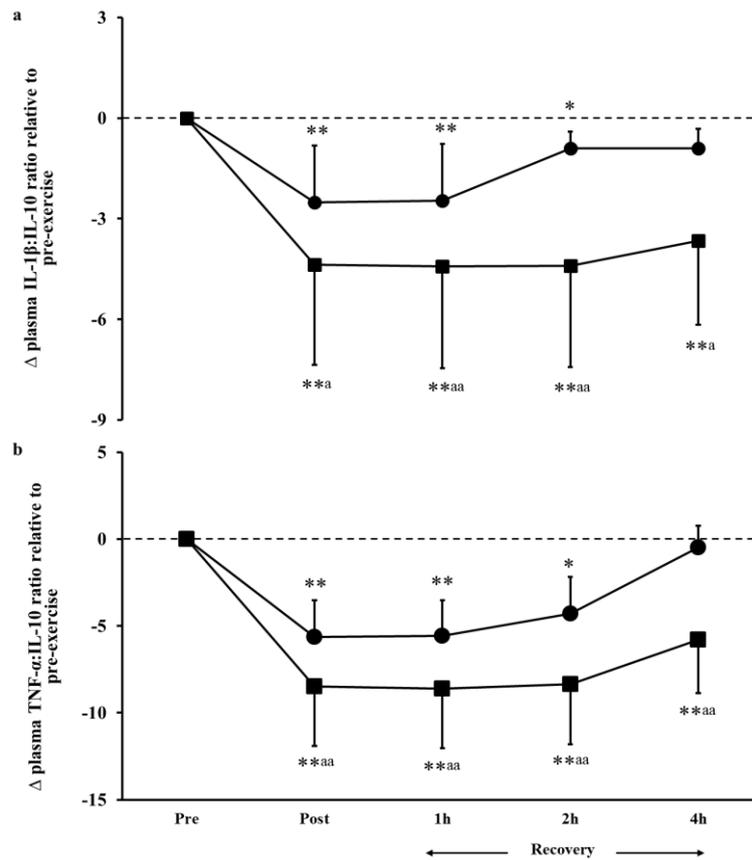


Figure. 4.5 Plasma IL-1β:IL-10 (a) and TNF-α:IL-10 (b) ratio in response to 2 h running at 60% $\dot{V}O_{2max}$ in hot (HOT: 35°C ■) and temperate (TEMP: 22°C ●) ambient conditions. Mean \pm SEM ($n=9$): ** $p < 0.01$ and * $p < 0.05$ vs. pre-exercise, ^{aa} $p < 0.01$ and ^a $p < 0.05$ vs. TEMP.

Correlation analysis

Significant correlations were observed between the exercise-induced increase in T_{re} (ΔT_{re} from 0-120 min exercise) with plasma I-FABP concentration ($r = 0.625$, $n = 20$, $p = 0.003$), plasma IL-10 concentration ($r = 0.643$, $n = 18$, $p = 0.002$), plasma cortisol concentration ($r = 0.786$, $n = 20$, $p < 0.001$), nausea ($r = 0.465$, $n = 20$, $p = 0.039$) and urge to regurgitate ($r = 0.482$, $n = 20$, $p = 0.032$).

4.6 Discussion

The current study aimed to comprehensively determine the effects of prolonged exertional-heat stress on gastrointestinal integrity, symptoms, and systemic responses; and to assess the relationship between body temperature increases induced by exercising in temperate and hot ambient conditions and the aforementioned markers of gastrointestinal perturbation. In accordance with our hypothesis, exertional-heat stress exhibited greater intestinal epithelial injury, gastrointestinal symptoms, cytokinaemia, and reduced anti-endotoxin antibodies than exercising in temperate conditions. However, there were no differences in intestinal inflammation and small intestinal permeability. Additionally, intestinal epithelial injury, stress hormone and anti-inflammatory cytokine responses, and gastrointestinal symptoms (i.e., nausea and urge to regurgitate) appear to correlate with increases in body temperature. The findings suggest that prolonged submaximal physical exertion in the heat creates a greater gastrointestinal (i.e., intestinal epithelial injury and symptoms) and systemic burden than exertion in thermoneutral conditions, which potentially has health and performance implications.(6, 8, 11, 14, 32, 47, 138)

The intestinal epithelial injury observed after running in 35°C compared to 22°C ambient conditions is a novel finding, whereby the magnitude of injury with HOT (mean increase I-FABP: 1230 pg/ml) exceeds that of previous research reporting values ranging from 88 pg/ml to 806 pg/ml.(39-45) It is likely that the additional thermoregulatory strain induced by heat exposure may have exacerbated splanchnic hypoperfusion, intestinal ischaemia, and hyperthermic injury to the intestinal epithelium.(1, 61, 96) This is supported by previous research showing I-FABP levels correlating with splanchnic hypoperfusion, intestinal ischaemia, and histological damage.(36, 40) Moreover, I-FABP levels were positively associated with rectal temperature, and considering the highest I-FABP levels previously reported were found after prolonged exercise (90 min) in the heat (30°C)(43), these observations support a link between thermoregulatory strain and intestinal injury. From a practical

perspective, it has previously been shown that intestinal epithelial damage may promote a transient impairment in nutrient absorption.(36, 38, 143) Such damage has the potential to dampen the provisions of recovery nutrition, which may have greater implications for optimised recovery after exertional-heat stress.

The combination of hyperthermia and prolonged intestinal ischaemia disrupts the intestinal epithelial and immunological barrier(46, 61), resulting in the release of local inflammatory cytokines and the perpetuation of local intestinal inflammation.(36, 144) We observed evidence of intestinal epithelial damage in the heat, but only modest increases in faecal calprotectin (1.0 µg/g), which occurred under both conditions, although were under the clinical reference range (>100 µg/g).(60) van Wijck et al.(40) also found evidence of intestinal epithelial injury with modest increases in intestinal inflammation (faecal calprotectin: 0.41 µg/g) after 1 h cycling at 70% maximum workload (W_{max}). Such a limited rise, especially in comparison to clinical populations that present chronic elevations in faecal calprotectin of clinical relevance (i.e., inflammatory diseases of the gastrointestinal tract)(145), raise questions over the sensitivity of faecal calprotectin as a marker of transient exercise-induced intestinal inflammation. It is possible that alterations in gastrointestinal transit during exercise and timing of the post-exercise faecal sample may influence the ability to appropriately capture the stool corresponding to such transient inflammation.(146, 147)

A strength of the current study was the determination of small intestine permeability in response to heat stress, and not merely determining the impact of the exercise *per se* (Δ pre- to post-exercise) on permeability, which has been frequently reported.(2) Despite a higher small intestine permeability observed with HOT, no significant difference between trials was detected. Such findings may possibly be due to methodological issues associated with dual-sugars permeability tests that make it difficult to detect subtle differences.(52) Or, despite the clear evidence of intestinal epithelial injury, the exertional heat stress and increased core temperature, may have been insufficient to reach

threshold levels that would elicit damage and/or perturbations to intestinal epithelial tight-junction stability and/or regulatory proteins (i.e., claudin and occludin), subsequently increasing small intestine permeability.(2, 29, 46, 52) Other studies using dual-sugars have predominantly detected post-exercise increases in small intestine permeability compared with resting levels, rather than post-exercise permeability between trial conditions.(40, 54, 55, 58, 93, 148) A limitation of the dual-sugar method is the inability to measure pre-exercise small intestine permeability which may vary between trials and could have subsequently affected the results of this study. The outcomes of the current study are in accordance with van Wijck et al.(90), who observed increases in splanchnic hypoperfusion (i.e., gastric air tonometry) and intestinal injury (i.e., I-FABP) in response to 60 min cycling at 70% W_{max} , while there was no change in small intestine permeability (i.e., lactulose to L-rhamnose urinary excretion ratio). These findings, and those of the current study, suggest dual-sugar permeability tests may not provide a clear overview of disturbances to gut integrity, since they attempt to measure stability of tight-junction barrier and its regulation, which is a mechanistic factor of gastrointestinal integrity, and not a factor indicative of potential health implications (e.g., splanchnic ischaemia injury, repetitive epithelial cell damage, acute and (or) chronic systemic responses).(2, 33, 36) Moreover, a correlation between core temperature and small intestinal permeability has previously been reported, which was not observed in the current study.(29, 54) Discrepancies may be due to the inclusion of resting values within data interpretation.(29) For example, findings from a recent review suggest that over one-third of subjects in the TEMP trial had increased intestinal permeability from pre-exercise rest with peak rectal temperatures of 38.5°C.(29) Therefore, exercise-induced increases in intestinal permeability may have occurred with TEMP and HOT, suggesting additional heat exposure with HOT may have had a negligible effect on small intestine permeability.

The increase in circulating endotoxin with HOT (9.6 pg/ml) in the current study is similar to previous exertional-heat stress studies, which include 1 to 2 h running at 60-70% $\dot{V}O_{2max}$, and elicit similar peak body temperatures of $\geq 39.0^{\circ}C$.(58, 77, 82) However, the endotoxaemia was lower than reported

after ultra-endurance events and exertional-heat stress protocols to exhaustion.(2) This highlights that endotoxin responses are likely related to the magnitude of exercise stress, with heat exposure acting as an exacerbating factor. However, differences in the assay kits, analysis methods and general health of the study participants should be considered when comparing and interpreting endotoxin results.(72, 149) The full extent of endotoxaemia may be difficult to capture, since reported values represent only a small proportion of endotoxins that may be bound to various lipoproteins, soluble/membrane CD14 or exist as outer member vesicles.(74, 150, 151) Despite these limitations, our findings show a significant reduction in anti-endotoxin antibodies (e.g., IgM) with HOT and an increase with TEMP, demonstrating a reduced endotoxin clearance capacity after heat exposure. Indeed, similar endotoxin profiles (i.e., elevated endotoxin and reduced anti-endotoxin antibodies) have been observed after endurance events.(22, 76, 77) Increased fitness status may protect against exercise-associated endotoxaemia by exhibiting lower endotoxin, higher anti-endotoxin antibody levels, and similar favourable cytokine responses to those observed in the current study (i.e., enhanced compensatory anti-inflammatory responses).(74, 77, 81, 152) Moreover, intestinal epithelial cells may play a role in the regulation and adaptation of mucosal immune responses and cytokine signalling.(153) Therefore, the greater compensatory anti-inflammatory response observed after exertional-heat stress in healthy endurance-trained athletes may be important in preventing the onset of gastrointestinal originated immune disturbance with potential disease pathophysiology in extreme, although rare, cases (i.e., acute septic shock, inflammatory gastrointestinal diseases, chronic fatigue, and autoimmune conditions)(14, 70, 71, 138, 139, 153), while the safety of exertional-heat stress in those with a predisposition to or existing condition (e.g., inflammatory gastrointestinal diseases) is questionable and requires further research.(2)

Gastrointestinal symptoms are a common feature of prolonged physical exertion, especially running exercise.(15, 22, 23) In the current study, a low FODMAP pre-exercise diet was provided to reduce the risk of gastrointestinal symptoms associated with pre-exercise food intake.(97, 102) This

controlled diet strengthens the findings however, the incidence and severity of gastrointestinal symptoms in this study may be lower than what may have been observed if the study participants consumed their regular diet.(102, 154) A novel finding in the current study was confirmation that running in 35°C with *ad libitum* water provisions, increased the incidence and severity of gastrointestinal symptoms compared with running in 22°C. In addition, symptom incidence and severity was greater in the 2nd h of exercise compared to the 1st h with HOT only, indicating exercise duration may be a contributing factor. These outcomes are consistent with observational field research that reports gastrointestinal symptoms are experienced by the majority of participants during endurance events that present heat exposure.(15, 16, 22-24, 70) Despite an *ad libitum* drinking regime, it is possible that the increased thirst and fluid intake observed with HOT may have partly contributed to the increased gastrointestinal symptoms. Repeated consumption of large fluid volumes or consistently promoting intra-gastric pressure during running has been shown to improve stomach comfort and may therefore be a useful strategy for reducing the risk of gastrointestinal symptoms associated with large fluid requirements during exertional-heat stress.(25, 106) The rise in rectal temperature in the current study was associated with nausea and urge to regurgitate, suggesting that increases in body temperature are likely to have a substantial contribution to the development of these symptoms. However, other potential factors associated with rising body temperature (e.g., disruptions to acid-base balance, membrane permeability, increased reactive oxygen species, and (or) hypocapnia) may have also contributed to the development of such symptoms. Since upper-gastrointestinal symptoms were the most common, it is plausible that dysfunction to enteric control of gastrointestinal motility (e.g., gastric emptying and intestinal transit) may have also contributed to symptom incidence and severity in response to *ad libitum* water intake.(146, 147) This is concerning for individuals undertaking prolonged (≥ 2 h) physical exertion whereby food and fluid consumption during a period of compromised gastrointestinal integrity and function may further exacerbate gastrointestinal symptoms.(25)

In conclusion, exposure to hot ambient temperatures during running at 60% $\dot{V}O_{2max}$, induces a thermoregulatory strain that subsequently injures the intestinal epithelium, contributes to the development of gastrointestinal symptoms, reduces endotoxin clearance capacity and promotes cytokinaemia. Considering the associated acute and chronic adverse health implications, future studies should aim to investigate thermoregulatory strain and (or) splanchnic hypoperfusion prevention and management strategies (e.g., heat acclimation, internal/external cooling strategies, fluid and nutrient intake).

4.7 Conclusion

Findings from this experimental study show that heat exposure exacerbates features of exercise-induced gastrointestinal syndrome, including intestinal injury, gastrointestinal symptoms and systemic responses. These novel findings and a focus on gastrointestinal outcomes, rather than systemic responses, provide new insight into the role of heat exposure during physical exertion on the gastrointestinal system. The comprehensive nature of this study and inclusion of systemic responses, which have been linked to acute and chronic health implications, also complements existing exertional-heat stress literature by providing a greater range of responses across different exercise modes, intensities and duration. While the findings from this study are important in moving forward to identify prevention and management strategies; the threshold ambient temperature (and /or core body temperature) at which clinically relevant exertional-heat stress induced gastrointestinal perturbations occur is currently unknown and warrants further investigation.

CHAPTER FIVE

The impact of mild heat stress during prolonged running on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profiles.

5.1 Background

The highest levels of thermoregulatory strain (i.e., core body temperatures $\geq 39.5^{\circ}\text{C}$) in Australian occupational workers has recently been reported to occur in warm to temperate ($\leq 30^{\circ}\text{C}$), rather than hot ($\geq 35^{\circ}\text{C}$) ambient conditions, coinciding with higher observations of exertional-heat stroke.(155) While these findings appear paradoxical, it is likely that self-regulation of workload, cooling strategies and/or modified work to rest ratios during exposure to hot ambient conditions may reduce the risk of adverse health outcomes.(155) Less precaution may be observed during occupational exertion and/or competitive athletic events held in warm ambient conditions. Considering exposure to hot ambient conditions during prolonged submaximal running exacerbates gastrointestinal damage and symptoms the second experimental study within this thesis aimed to investigate if exposure to warm (30°C) ambient conditions results in similar gastrointestinal perturbations.

5.2 Abstract

The study aimed to determine the effects of mild exertional-heat stress on intestinal injury, permeability, gastrointestinal symptoms, systemic endotoxin and cytokine responses. Ten endurance runners completed 2 h running at 60% $\dot{V}\text{O}_{2\text{max}}$ in warm (WARM: 30°C) and temperate (TEMP: 22°C) ambient conditions. Rectal temperature (T_{re}) and gastrointestinal symptoms were recorded every 10 min during exercise. Blood samples were collected pre- and post-exercise, and during recovery to determine plasma intestinal fatty-acid binding protein (I-FABP) and cortisol concentrations, and

systemic endotoxin and inflammatory cytokine profiles. Urinary lactulose:L-rhamnose ratio (L/R) was used to measure small intestine permeability. WARM significantly increased T_{re} compared to TEMP from 50 min onwards ($38.4 \pm 0.5^{\circ}\text{C}$ vs. $38.1 \pm 0.3^{\circ}\text{C}$, respectively; $p < 0.01$), gastrointestinal symptoms ($p = 0.017$), post-exercise plasma cortisol (59% vs. 26%, respectively; $p < 0.001$) and I-FABP (184% vs. 127%, respectively; $p < 0.001$) concentrations. Circulatory anti-endotoxin antibodies increased post-exercise ($p < 0.001$) with WARM (20%) and TEMP (28%). No differences between WARM and TEMP were observed for plasma endotoxin concentration (6% vs. 5% increase, respectively) or small intestine permeability (L/R 0.026 ± 0.010 and 0.025 ± 0.015 , respectively). Both pro- and anti-inflammatory cytokines increased post-exercise, with inflammatory response cytokines TNF- α ($p = 0.015$) and IL-8 ($p = 0.044$), and compensatory anti-inflammatory cytokines IL-10 ($p = 0.065$), and IL-1ra higher with WARM than TEMP. These findings suggest that exposure to warm ambient conditions during prolonged submaximal running induces transient intestinal epithelial injury, increases gastrointestinal symptoms, and promotes greater perturbations to the systemic cytokine profile compared to running in temperate conditions.

5.3 Introduction

Cooling behaviours such as consumption of cold fluids, application of ice packs, fanning, water spraying, and (or) reduced workload are common practices during physical exertion in hot ambient conditions ($\geq 35^{\circ}\text{C}$ ambient temperature (T_{amb})), with the intention to mitigate increases in core body temperature and reduce the risk of heat-related illness.(123, 140, 156, 157) Such cooling behaviours may not be of prime concern during physical exertion in milder ambient conditions ($\sim 30^{\circ}\text{C}$ T_{amb}), resulting in similar increases in core body temperature (i.e., $\geq 39^{\circ}\text{C}$) and cases of exertional-heat illness to hotter ambient conditions.(158, 159) In association with ambient temperature, high relative humidity (i.e., $\geq 70\%$) can impair heat dissipation thereby increasing thermal stress and contributing to high core body temperatures during low to moderate intensity exercise.(160) We have recently

observed that exposure to dry ($26 \pm 4\%$ relative humidity) hot ambient conditions during prolonged submaximal (i.e., 2 h at 60% maximal oxygen uptake ($\dot{V}O_{2max}$)) running creates a greater thermoregulatory strain and subsequent gastrointestinal and systemic cytokine burden compared to running in temperate conditions of low humidity ($\leq 50\%$ relative humidity) (**Chapter 4**). It is therefore plausible that similar exercise-associated gastrointestinal disturbance may occur with exposure to milder conditions (i.e., 30°C T_{amb} , relative humidity $\leq 50\%$)(58), with potential, although uncommon, health implications including fatal septic shock, gastritis and reversible ischaemic colitis or ischaemic bowel disease.(2, 6, 7, 10, 11, 32, 161)

Recently, concerns have been raised over the large incidence of gastrointestinal symptoms (i.e., nausea, regurgitation, abdominal pain, diarrhoea, etc.), reportedly affecting 85-96% of athletes during endurance and ultra-endurance running events with some degree of mild heat-stress.(2) The occurrence of gastrointestinal symptoms has been found to adversely affect exercise performance, nutrient intake during and after exercise, and is a primary reason for withdrawal from ultra-endurance running events.(22-24) Although gastrointestinal symptoms have a multifactorial aetiology, exercise-associated splanchnic hypoperfusion and increased sympathetic drive have been implicated as primary contributors.(2) Considering heat exposure generates a thermoregulatory strain that exacerbates these primary mechanisms(1, 2), it is plausible that exertional-heat stress may directly and (or) indirectly contribute to the development of gastrointestinal symptoms. Indeed, we observed a greater incidence and severity of gastrointestinal symptoms during 2 h running at 60% $\dot{V}O_{2max}$ in 35°C T_{amb} , compared to running in temperate conditions (**Chapter 4**). However, it is currently unknown if gastrointestinal symptoms are exacerbated at a lower degree of heat exposure (i.e., 30°C T_{amb}), which is more commonly experienced by athletes during training and competition.

Despite such high reports of gastrointestinal symptoms occurring during prolonged running in the heat, very few studies have investigated these in conjunction with gastrointestinal integrity and

associated systemic endotoxaemia and cytokinaemia in response to exertional-heat stress. Furthermore, the majority of research to date has focused on exposure to hot, rather than warm, ambient conditions and high intensity short duration exercise. For example, running for ≤ 1 h at $\geq 70\%$ $\dot{V}O_{2\max}$ in 33°C to 40°C T_{amb} has been shown to injure the intestinal epithelium (i.e., induce increases in intestinal-fatty acid binding protein (I-FABP)), and increase systemic endotoxin and (or) inflammatory cytokines.(44, 58) However, the populations (i.e., endurance athletes and active occupational workers) most likely at risk of potential health implications undertake prolonged workloads at moderate intensity, where exposure to warm ambient conditions (i.e., mild exertional-heat stress) is more common than hot ambient conditions. It is, therefore, possible that exertion during milder heat exposure ($\sim 30^{\circ}\text{C}$ T_{amb}) may pose a similar or potentially greater gastrointestinal burden and health risk. The threshold T_{amb} at which clinically significant gastrointestinal perturbations (i.e., requiring medical attention) occur is currently unknown, and warrants investigation. With this in mind, the current study aimed to determine the effects of prolonged mild exertional-heat stress on gastrointestinal integrity (i.e., intestinal epithelial injury and small intestine permeability), systemic responses (i.e., endotoxaemia and cytokinaemia), and gastrointestinal symptoms. It was hypothesised that running in mild heat-stress would result in greater perturbations to gastrointestinal integrity, increased systemic responses, and gastrointestinal symptoms, compared to running in temperate conditions.

5.4 Methods

Participants

Ten (male $n=6$, female $n=4$) non-heat acclimatised endurance trained runners [mean \pm SD: age 31 ± 6 years, nude body mass 66.3 ± 10.5 kg, height 1.71 ± 0.10 m, % body fat mass $18 \pm 6\%$, $\dot{V}O_{2\max}$ 56 ± 8 ml·kg⁻¹·min⁻¹] volunteered to participate in the study. All participants gave written informed consent, which received approval from the local ethics committee, and conformed to the 2008 Helsinki Declaration for Human Research Ethics and meet the ethical standards of the International Journal of Sports Medicine.(162) The standardised exclusion criteria were in accordance with Costa et al.(25)

Preliminary measures

One week before the first experimental trial, height, nude body mass, and body fat mass (Seca 515 MBCA, Seca Group, Hamburg, Germany) were recorded. $\dot{V}O_{2\max}$ (Vmax Encore Metabolic Cart, Carefusion, San Diego, California, US) was estimated by a continuous incremental exercise test to volitional exhaustion on a motorized treadmill (Forma Run 500, Technogym, Seattle, Washington, US). The incremental exercise test commenced with a treadmill speed of 6 km·h⁻¹ and 1% inclination. Speed was increased by 2 km·h⁻¹ every 3 min until a speed of 16 km·h⁻¹, upon which inclination was increased by 2.5% every 3 min until participants reached volitional exhaustion. Criteria for attaining $\dot{V}O_{2\max}$ included participants reaching volitional exhaustion (rating of perceived exertion (RPE) at 20)(141), a heart rate within 10 beats per min of age predicted maximum heart rate, and respiratory exchange ratio ≥ 1.15 .(163) To determine running speed for the experimental trials, the treadmill speed at 60% $\dot{V}O_{2\max}$ and 1% gradient was extrapolated from the $\dot{V}O_2$ -work rate relationship and then verified (10.4 ± 0.8 km·h⁻¹). A treadmill gradient of 1% was used to match the energy-cost of outdoor running.(164) All participants reported previous experience with treadmill running.

Experimental procedure

To reduce any potential gastrointestinal symptoms arising from pre-exercise food and fluid intake, participants were provided with a low fermentable oligo-, di-, mono-saccharide, and polyol (FODMAP) diet for the 24 h period before each experimental trial (12.6 ± 1.8 MJ, 461 ± 69 g carbohydrate, 114 ± 10 g protein, 72 ± 13 g fat). Participants also refrained from strenuous exercise for 48 h before each experimental trial. Compliance was determined by a dietary and exercise log. Participants reported to the laboratory at 08:00h after consuming the low FODMAP breakfast (2.5 MJ, 125 g carbohydrate, 14 g protein, and 4 g fat) with 400 mL of water (consumed at 07:00h). Participants were asked to void before nude body mass measurements and completion of a self-reported gastrointestinal symptom assessment tool.⁽²⁵⁾ A 10-point Likert-type rating scale was used to quantify self-perceived gastrointestinal symptoms (adapted from a 10 cm visual analogue scale, with 0 indicative of no symptoms to 10 indicative of extreme symptoms), as previously reported.^(23, 25, 165) Blood was then collected by venepuncture from an antecubital vein into a vacutainer (6 mL, $1.5 \text{ IU} \cdot \text{mL}^{-1}$ heparin). To monitor rectal temperature (T_{re}) during running, participants inserted a thermocouple 12 cm beyond the external anal sphincter (Grant REC soft insertion probe thermocouple; Grant 2010 Squirrel data logger, Shepreth, UK).

In a randomised order with a one-week washout, participants completed 2 h (initiated at 09:00h) running exercise on a motorised treadmill at the previously determined speed within an environmental chamber in warm (WARM: $30.2 \pm 0.4^\circ\text{C}$ and $35 \pm 6\%$ relative humidity) and temperate (TEMP: $22.2 \pm 1.0^\circ\text{C}$ and $44 \pm 6\%$ relative humidity) ambient conditions. Water was consumed *ad libitum* (1.3 ± 0.3 L and 0.8 ± 0.1 L, respectively). Heart rate, RPE, thermal comfort rating⁽¹⁴²⁾, T_{re} , and gastrointestinal symptoms were measured and recorded every 10 min during running. To determine small intestine permeability, participants consumed a dual-sugars solution containing 5 g lactulose (Duphalac, Abbott Biologicals, Olst, Netherlands) and 1 g L-rhamnose (MP Biomedicals, LLC, Solon, USA) in 100 mL water, 90 min into exercise. A 5 h urine collection commenced post-

ingestion, where the final volume was weighed, and 30 mL were aliquoted and stored frozen at -20°C until analysis. Immediately after exercise, a blood sample was collected and nude body mass was recorded. Participants remained seated during the recovery period and consumed water *ad libitum*. Blood was also collected 1 h, 2 h, and 4 h post-exercise. To reduce any seasonal heat acclimatisation, the experimental procedures were conducted over the cooler seasonal periods (temperatures consistently $\leq 20^{\circ}\text{C}$). Participants were unaccustomed to running in warm environments, and were not blinded to the broad aims of the study due to the comprehensive gastrointestinal-focused exclusion criteria, dietary control, frequent gastrointestinal symptom assessment, and exercise protocol in two differing ambient conditions.

Sample analysis

Whole blood haemoglobin and haematocrit values were used to estimate changes in plasma volume, and used to correct plasma variables.⁽¹⁶⁶⁾ Blood glucose concentration was determined pre- and post-exercise using a handheld monitor (Accu-Chek Proforma, Roche Diagnostics, Basel, Switzerland). The remaining blood samples were centrifuged at 4000 rpm for 10 min within 15 min of sample collection. Plasma was aliquoted and frozen at -80°C until analysis, except for 50 μL that were used to determine plasma osmolality in duplicate (coefficient of variation (CV): 2.7%), by freeze-point osmometry (Osmomat 3000, Gonotec, Berlin, Germany). Recovery of lactulose and L-rhamnose in urine were determined by ultra-performance liquid chromatography (CV: 13.8%).⁽¹⁶⁷⁾ Plasma concentrations of interleukin (IL)-6, IL-1 β , tumor necrosis factor (TNF)- α , IL-8, IL-10, and IL-1 receptor antagonist (ra) were determined by multiplex enzyme linked immunosorbent assay (ELISA: HCYTOMAG-60K, EMD Millipore, Darmstadt, Germany). Circulating gram-negative bacterial endotoxin concentration was determined by Limulus amoebocyte lysate (LAL) chromogenic endpoint assay (HIT302, Hycult Biotech, Uden, Netherlands). Plasma concentrations of I-FABP (HK406, Hycult Biotech, Uden, Netherlands), endotoxin core antibody (HK504, Hycult Biotech, Uden, Netherlands) and cortisol (RE52061, IBL International, Hamburg, Germany) were determined

by ELISA. All variables were analysed in duplicate as per manufacturer's instructions on the same day, with standards and controls on each plate, and each participant assayed on the same plate. The CVs for endotoxin, I-FABP, endotoxin core antibody, cortisol, IL-10, IL-1ra, IL-1 β , IL-6, IL-8 and TNF- α were 3.2%, 5.4%, 6.0%, 3.1%, 2.0%, 2.3%, 1.4%, 1.7%, 0.6% and 1.1%, respectively.

Statistical analysis

Based on a standard deviation of 118 pg·mL⁻¹ for post-exercise I-FABP(40) and using a standard alpha (0.05) and beta value (0.8), a sample size of n= 6 was calculated (www.dssresearch.com/toolkit/sscalc) to have adequate statistical precision to detect a >100% increase in I-FABP post-exercise. Such increases in I-FABP after 1 h exercise have been correlated with the magnitude of splanchnic hypoperfusion and increases in intestinal permeability.(40) Current participant numbers are also in accordance with sufficient statistical precision to detect significant changes in pre- to post-exercise plasma endotoxin and cytokine concentrations(82), and gastrointestinal symptoms.(25, 165) Data in the text and tables are presented as mean and 95% confidence interval (CI), and cumulative score and individual participant range for gastrointestinal symptoms. For clarity, data in figures are presented as mean \pm standard error of the mean (SEM). Standardised data management, pre-analysis diagnostic tests, and statistical analysis was performed as previously reported.(25, 71, 168) In short, a two-way repeated measures ANOVA with post hoc Tukey's HSD test was used for multiple data points, t-tests for singular data points, with non-parametric equivalents for gastrointestinal symptom analysis. Sex sub-group analysis revealed no differences in primary and secondary variable between sexes, therefore combined data was used for statistical analysis. Data were analysed using SPSS statistical software (V.23.0, Chicago, Illinois, USA) with significance accepted at $p \leq 0.05$.

5.5 Results

Hydration status, cardiovascular and thermoregulatory strain

Exercise-induced body mass loss (WARM: 1.6 (1.3-2.0)% and TEMP: 1.6 (1.0-2.1)%) did not differ between trials. Plasma osmolality did not differ pre- to post-exercise with WARM (292 (287-297) mOsmol·kg⁻¹ and 294 (290-297) mOsmol·kg⁻¹, respectively) and TEMP (292 (287-299) mOsmol·kg⁻¹ and 295 (289-301) mOsmol·kg⁻¹, respectively) or between trials. A trial x time interaction ($p < 0.001$) was observed for T_{re} (**Figure 5.1a**), which increased during both trials and was higher with WARM compared to TEMP from 50 min exercise onwards. Main effects of time and trial were observed for heart rate ($p < 0.001$ and $p = 0.006$, respectively; **Figure 5.1b**) and thermal comfort rating ($p < 0.001$ and $p = 0.004$, respectively; **Figure 5.1c**). Heart rate and thermal comfort rating were significantly elevated from 30 min and 40 min exercise, respectively, compared to 10 min, and higher throughout WARM compared to TEMP. RPE (**Figure 5.1d**) increased during exercise and was higher with WARM compared to TEMP (trial x time, $p = 0.032$).

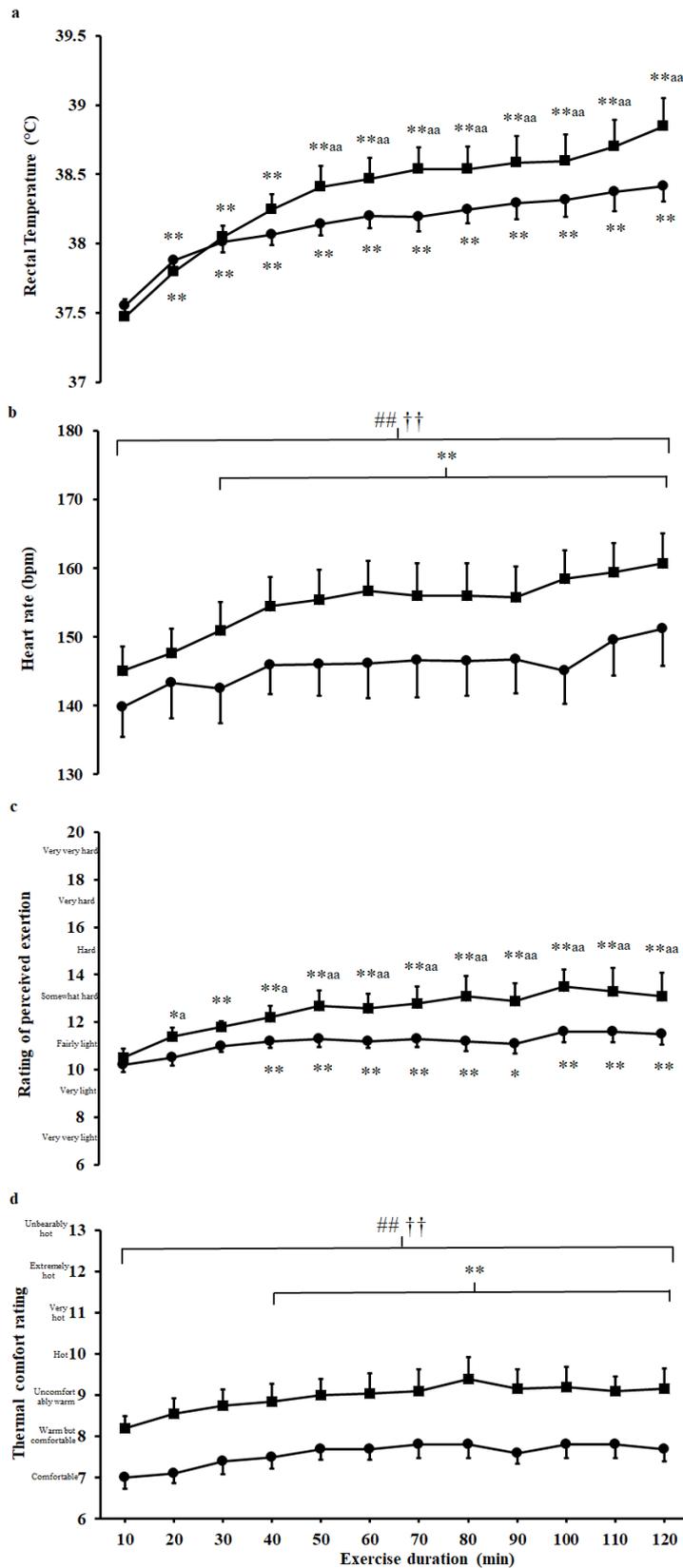


Figure 5.1. Rectal temperature (a), heart rate (b), rating of perceived exertion (c), and thermal comfort rating (d) responses during 2 h running at 60% $\dot{V}O_{2max}$ in warm (WARM: 30°C ■) and temperate (TEMP: 22°C ●) ambient conditions. Mean \pm SEM (n= 10): †† main effect of time p< 0.01 vs. 10 min, ### main effect of trial p< 0.01, ** p< 0.01 and * p< 0.05 vs. 10 min, ^{aa} p< 0.01 and ^a p< 0.05 vs. TEMP.

Plasma cortisol and blood glucose concentration

A trial x time interaction ($p = 0.049$) was observed for plasma cortisol concentration which significantly increased pre- to post-exercise with WARM (322 (242 - 402) $\text{nmol}\cdot\text{mL}^{-1}$ vs. 513 (327 - 699) $\text{nmol}\cdot\text{mL}^{-1}$, $p < 0.01$) but not TEMP (362 (276 - 447) $\text{nmol}\cdot\text{mL}^{-1}$ vs. 456 (355 - 556) $\text{nmol}\cdot\text{mL}^{-1}$, $p > 0.05$) and was significantly higher at 1 h post-exercise with WARM (439 (312 - 566) $\text{nmol}\cdot\text{mL}^{-1}$) compared to TEMP (354 (254 - 455) $\text{nmol}\cdot\text{mL}^{-1}$). Blood glucose concentration increased pre- to post-exercise (time effect, $p = 0.019$) with WARM (5.0 (4.5 - 5.4) $\text{mmol}\cdot\text{L}^{-1}$ vs. 6.0 (5.7 - 6.4) $\text{mmol}\cdot\text{L}^{-1}$) and TEMP (4.8 (4.0 - 5.6) $\text{mmol}\cdot\text{L}^{-1}$ vs. 5.7 (5.2 - 6.1) $\text{mmol}\cdot\text{L}^{-1}$), with no difference between trials.

Gastrointestinal integrity and symptoms

I-FABP significantly increased pre- to post-exercise in both trials, and was greater with WARM than TEMP (Δ pre- to post-exercise: 184% vs. 127% , respectively; trial x time, $p = 0.02$) (**Figure 5.2**). Small intestine permeability (i.e., lactulose:L-rhamnose ratio) was not significantly different between WARM (0.026 (0.018 - 0.033)) and TEMP (0.025 (0.014 - 0.035)). Total and upper-gastrointestinal symptoms were significantly higher ($p < 0.05$) and appetite lower ($p < 0.05$) with WARM compared to TEMP (**Table 5.1**). Gut discomfort and total gastrointestinal symptoms were greater during the 2nd h of exercise compared to the 1st h with WARM only (**Table 5.1**).

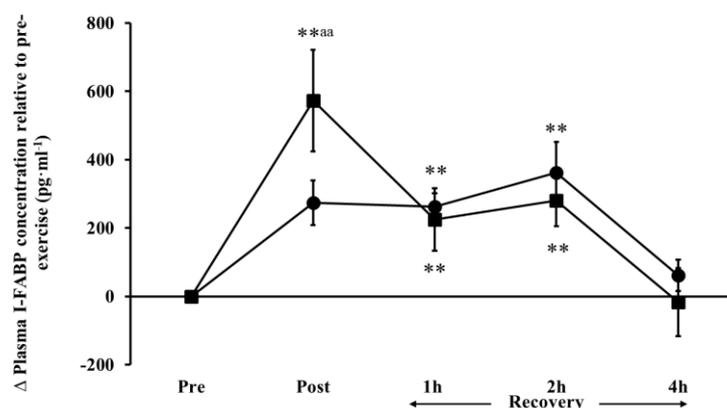


Figure 5.2. Change in plasma intestinal fatty acid binding protein (I-FABP) in response to 2 h running at $60\% \dot{V}O_{2\max}$ in warm (WARM: 30°C ■) and temperate (TEMP: 22°C ●) ambient conditions. Mean \pm SEM ($n = 10$): ** $p < 0.01$ vs. pre-exercise, ^{aa} $p < 0.01$ vs. TEMP.

Table 5.1. Incidence and severity of gut discomfort, total, upper-, and lower-gastrointestinal symptoms in response to 2 h running at 60% $\dot{V}O_{2\max}$ in warm (WARM: 30°C) and temperate (TEMP: 22°C) ambient conditions.

	WARM				TEMP			
	Incidence	1 st h (range)	2 nd h (range)	Total (range)	Incidence	1 st h (range)	2 nd h (range)	Total (range)
Gut discomfort	NA	52 (0-13)	125 (0-13) [‡]	177 (0-30) ^b	NA	33 (0-13)	23 (0-11)	56 (0-24)
Total gastrointestinal symptoms¹	80%	81 (0-24)	294 (0-93) [‡]	375 (0-104) ^a	70%	35 (0-13)	23 (0-11)	58 (0-24)
Upper-gastrointestinal symptoms²	70%	50 (0-14)	122 (0-54)	172 (0-58) ^a	40%	14 (0-8)	9 (0-6)	23 (0-10)
Belching	60%	17 (0-13)	46 (0-13) [#]	63 (0-26) ^a	40%	7 (0-2)	5 (0-6)	12 (0-6)
Heartburn	20%	6 (0-6)	4 (0-2)	10 (0-8)	0%	0 (0-0)	0 (0-0)	0 (0-0)
Bloating	50%	23 (0-13)	52 (0-23) [#]	75 (0-33)	20%	9 (0-6)	2 (0-2)	11 (0-8)
Upper abdominal pain	30%	4 (0-3)	9 (0-5)	13 (0-5)	0%	0 (0-0)	0 (0-0)	0 (0-0)
Urge to regurgitate	10%	0 (0-0)	11 (0-11)	11 (0-11)	0%	0 (0-0)	0 (0-0)	0 (0-0)
Regurgitation	0%	0 (0-0)	0 (0-0)	0 (0-0)	0%	0 (0-0)	0 (0-0)	0 (0-0)
Lower-gastrointestinal symptoms²	70%	10 (0-7)	45 (0-13)	55 (0-13)	30%	21 (0-21)	11 (0-11)	32 (0-24)
Flatulence	10%	0 (0-0)	4 (0-4)	4 (0-4)	0%	0 (0-0)	0 (0-0)	0 (0-0)
Urge to defecate	30%	7 (0-7)	25 (0-11)	32 (0-12)	30%	21 (0-13)	11 (0-11)	32 (0-24)
Abdominal pain/s	40%	3 (0-3)	16 (0-8)	19 (0-8) ^b	0%	0 (0-0)	0 (0-0)	0 (0-0)
Abnormal defecation ³	0%	0 (0-0)	0 (0-0)	0 (0-0)	0%	0 (0-0)	0 (0-0)	0 (0-0)
Others								
Nausea	40%	5 (0-5)	70 (0-30) [#]	75 (0-35) ^b	10%	0 (0-0)	1 (0-1)	1 (0-1)
Dizziness	40%	9 (0-4)	38 (0-19) [#]	47 (0-23) ^b	0%	0 (0-0)	0 (0-0)	0 (0-0)
Abdominal stitch	40%	7 (0-4)	19 (0-10)	26 (0-14) ^b	10%	0 (0-0)	2 (0-2)	2 (0-2)
Appetite	NA	37 (0-12)	59 (0-21)	96 (0-31) ^a	NA	62 (0-17)	132 (0-32) [‡]	194 (0-49)
Thirst	NA	130 (3-24)	141 (4-28)	271 (7-55)	NA	112 (0-21)	110 (0-21)	222 (0-39)

Overall participant summative accumulation of rating scale point score of measured time periods and individual participant range (n=10). [‡] p< 0.05 vs. 1st h and [#] trend p< 0.07 vs. 1st h, ^a p< 0.05 vs. TEMP and ^b trend p< 0.07 vs. TEMP. ¹ Summative accumulation of upper-, lower- and other gastrointestinal symptoms, ² summative accumulation of upper- or lower- gastrointestinal symptoms, ³ abnormal defecation including loose watery stools, diarrhoea and blood in stools. NA: not applicable.

Systemic endotoxin and cytokine profile

No differences were observed pre- to post-exercise or between trials for peak circulating endotoxin concentration (**Figure 5.3a**). Plasma anti-endotoxin antibody concentration increased post-exercise in WARM (20%) and TEMP (28%, time effect $p < 0.001$) with no difference between trials (**Figure 5.3b**). A trial x time interaction was observed for plasma IL-8 concentration ($p = 0.044$), with a trend for plasma IL-10 concentration ($p = 0.065$) whereby higher pre- to post-exercise peak values were observed with WARM compared to TEMP (**Figure 5.4**). A main effect of trial was observed for plasma TNF- α ($p = 0.015$) and IL-1ra ($p = 0.044$) concentrations; and a main effect of time was observed for plasma IL-6 ($p = 0.009$), TNF- α ($p < 0.001$), IL-10 ($p = 0.004$), and IL-1ra ($p = 0.004$) concentrations (**Figure 5.4**). No main effects were observed for TNF- α :IL-10 and IL-1 β :IL-10 ratios, suggesting WARM and TEMP did not elicit a significant compensatory anti-inflammatory response. Peak post-exercise values for plasma endotoxin, anti-endotoxin antibody, IL-6, IL-1 β , TNF- α and IL-8 occurred immediately post-exercise, with IL-10 and IL-1ra peaking at 1 h post-exercise.

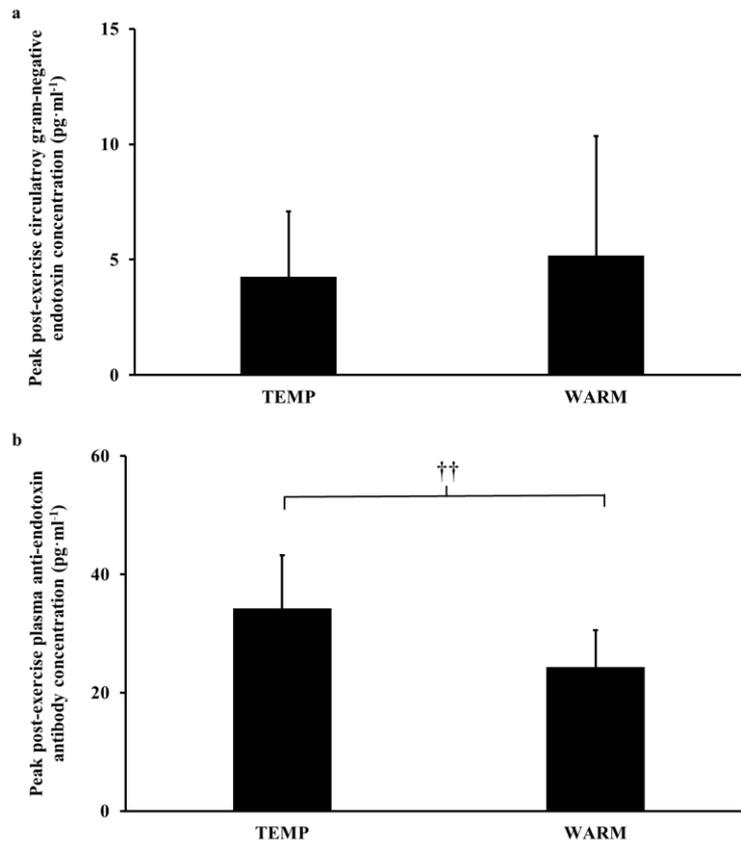


Figure 5.3. Peak post-exercise circulating gram-negative bacterial endotoxin (a) and plasma anti-endotoxin antibody (b) concentration in response to 2 h running at 60% $\dot{V}O_{2max}$ in warm (WARM: 30°C) and temperate (TEMP: 22°C) ambient conditions. Mean \pm SEM (n= 9): †† main effect of time $p < 0.01$ vs. pre-exercise.

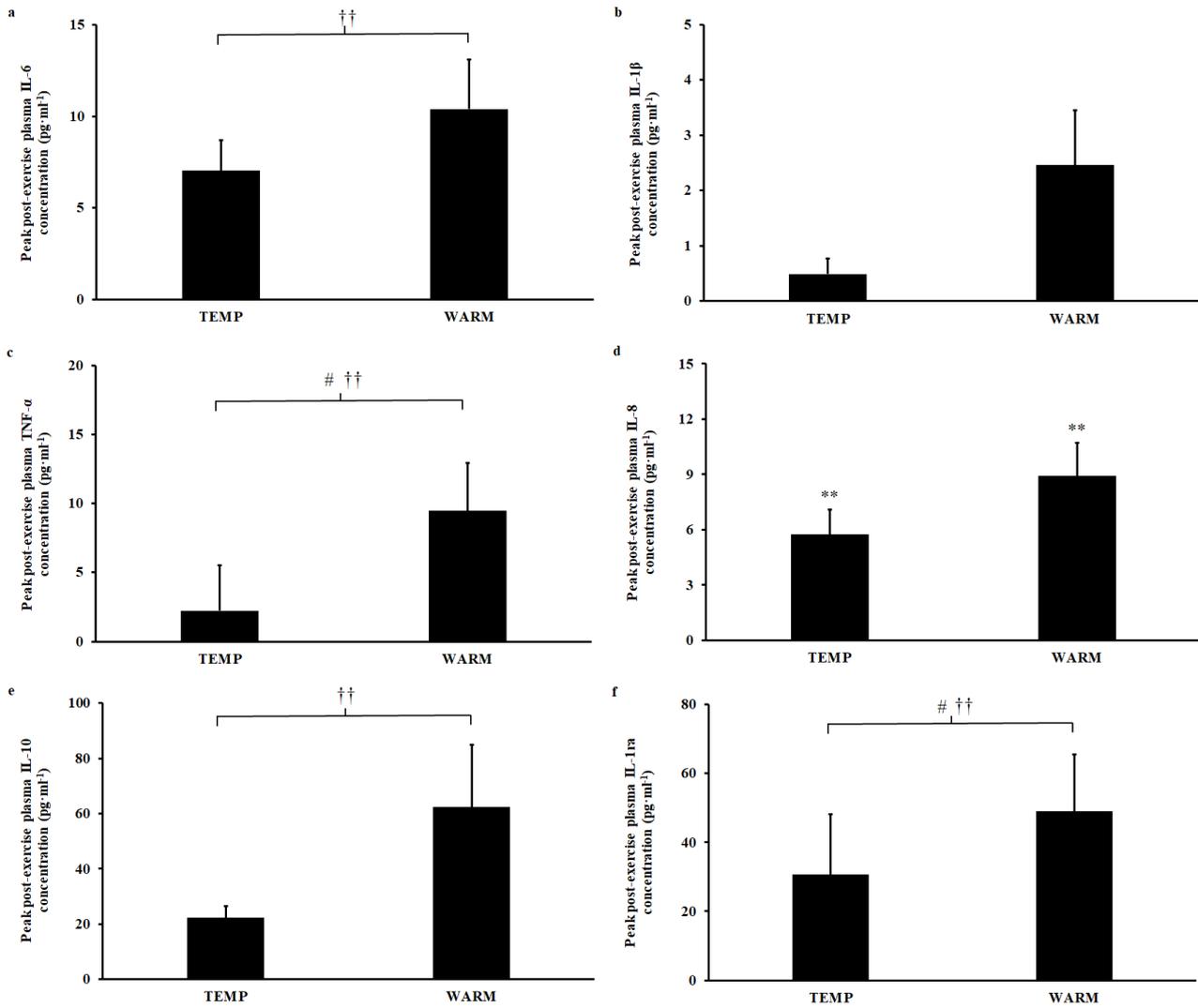


Figure 5.4. Peak post-exercise plasma IL-6 (a), IL-1 β (b), TNF- α (c), IL-8 (d), IL-10 (e), and IL-1ra (f) concentrations in response to 2 h running at 60% $\dot{V}O_{2max}$ in warm (WARM: 30°C) and temperate (TEMP: 22°C) ambient conditions. Mean \pm SEM (n= 9): # main effect of trial p < 0.05, †† main effect of time p < 0.01 and † p < 0.05 vs. pre-exercise, ** p < 0.01 vs. pre-exercise.

5.6 Discussion

The current study aimed to determine the effects of mild exertional-heat stress on gastrointestinal integrity, symptoms and systemic responses. In accordance with our hypothesis, mild exertional-heat stress resulted in greater intestinal epithelial injury and gastrointestinal symptoms, and modest perturbations to systemic cytokine profile, compared with exercising in temperate conditions. Mild exertional-heat stress did not further perturb small intestine permeability or systemic endotoxin profile above responses seen with exercise in temperate conditions. These findings suggest that prolonged submaximal physical exertion in warm conditions has modest effects on gastrointestinal integrity and systemic responses. However, the increased intestinal epithelial injury and gastrointestinal symptoms has the potential to adversely affect nutrient intake and absorption during and after exercise, which may potentially impact exercise performance and recovery processes.(23, 25, 38, 143, 165)

The increased I-FABP observed after running in 30°C T_{amb} (Δ I-FABP 573 pg·mL⁻¹) compared to 22°C T_{amb} (Δ I-FABP 274 pg·mL⁻¹), suggests exposure to warm ambient conditions during running at the same relative speed exacerbates intestinal epithelial injury. Plasma I-FABP with WARM returned to similar concentrations as TEMP by 1 h post-exercise, signifying any additional damage to the intestinal epithelium was transient. We have previously observed (**Chapter 4**) that running for 2 h at 60% $\dot{V}O_{2max}$ in 35°C T_{amb} significantly injures the intestinal epithelium, with I-FABP increases of 1230 pg·mL⁻¹ observed post-exercise, that remained elevated beyond 2 h post-exercise. The current study therefore, highlights that exposure to warm ambient temperatures and (or) the associated thermoregulatory strain can injure the intestinal epithelium, but not to the extent of exercising in hot ambient conditions ($\geq 35^{\circ}\text{C } T_{amb}$). It is hypothesised that exposure to high relative humidity ($\geq 70\%$) during mild exertional-heat stress may contribute to greater intestinal injury due to higher core body temperatures, however this requires further investigation.(160) Our findings are supported by other

studies showing exertional-heat stress increases splanchnic hypoperfusion and injury to the intestinal epithelium.(1, 43, 44) Such damage has been shown to temporarily impair nutrient absorption.(36, 38, 143) Therefore, the increased intestinal epithelial injury induced by exertional-heat stress may impair uptake of nutrition during exercise and recovery, which has the potential to impact performance and delay post-exercise recovery processes.(23, 38) Moreover, the associated malabsorption has the potential to exacerbate gastrointestinal symptoms prompted by reductions in gastrointestinal function (e.g., gastric emptying and oro-caecal transit) in response to the ‘ileal brake’.(25, 165, 169)

It has been suggested that a loss of gastrointestinal barrier integrity allows the translocation of bacterial endotoxins from the intestinal lumen in to the systemic circulation, promoting an inflammatory response, which has been linked to the development of several acute and chronic health conditions.(2) In the current study, we observed no differences in small intestine permeability or circulating endotoxin concentrations pre- to post-exercise or between trials; and minimal increases in inflammatory cytokines. These findings are consistent with other studies that have observed no difference in small intestine permeability after running for 1 to 2 h in 33°C T_{amb} and 35°C T_{amb} compared to running in temperate conditions, despite significant elevations in core temperature (**Chapter 4**). (58) Increases in core body temperature have been proposed to account for 63% of the variance in intestinal permeability; however, a variety of exercise intensities and ambient conditions were included, and comparisons were made using both pre- and post-exercise values.(29) These factors, therefore, make it difficult to separate the effects of exercise stress and T_{amb} on subsequent core body temperature and associations with intestinal permeability.(29, 54)

The systemic endotoxin and cytokine findings from this study contrast with other studies conducted in hotter conditions of 33°C to 40°C T_{amb} , that have demonstrated significant increases in circulating endotoxin in conjunction with an elevated inflammatory (i.e., IL-6, IL-1 β , TNF- α and IL-8) and

compensatory anti-inflammatory cytokine profile (i.e., IL-10, and IL-1ra) in response to exertional-heat stress.(44, 58, 74, 82) Furthermore, the increase in anti-endotoxin antibodies observed post-exercise with WARM and TEMP in this study contrast with findings from our previous study (**Chapter 4**) where a concurrent reduction in anti-endotoxin antibodies was observed with increased circulatory endotoxin (mean increase $9.6 \text{ pg}\cdot\text{mL}^{-1}$) after 2 h running at 60% $\dot{V}O_{2\text{max}}$ in $35^{\circ}\text{C } T_{\text{amb}}$. Together these findings suggest that mild heat exposure during exertional activities appears to have minimal impact on small intestine permeability, circulatory endotoxin concentration, and systemic inflammatory responses despite elevated core body temperature. Exertion in slightly higher temperatures (i.e., 33°C to $35^{\circ}\text{C } T_{\text{amb}}$) appears to increase circulatory endotoxin concentrations, possibly via a reduction in immune and (or) hepatic clearance of circulatory endotoxin and increased inflammatory responses, with potential for associated acute and chronic health implications.(2, 58, 82) Despite the current participant cohort numbers being similar to previous exertional-heat stress studies(44, 74, 82), it cannot be discounted that the number of participants used in the current study, may not have provided sufficient statistical power to detect any subtle significances in systemic responses.

Gastrointestinal symptoms are common during endurance running and have been implicated as a primary performance-limiting factor during competitive events.(22-24) In the current study, WARM significantly increased total- and upper-gastrointestinal symptoms, but not lower-gastrointestinal symptoms, compared to TEMP, suggesting mild heat stress exacerbates the development of gastrointestinal symptoms. This is possibly through increased sympathetic drive and stress hormone responses(2, 146, 147), as evidenced by higher cortisol responses with WARM compared to TEMP. Furthermore, gut discomfort and gastrointestinal symptoms were higher during the 2nd h of exercise, compared to the 1st h with WARM only, indicating the duration of exercise in mild heat stress may be a contributing factor. Similar outcomes were observed during equivalent running in $35^{\circ}\text{C } T_{\text{amb}}$ (**Chapter 4**). This suggests the threshold for the development of gastrointestinal symptoms may occur

at a lower degree of exertional-heat stress than perturbations to gastrointestinal integrity and systemic responses. Indeed, 85-96% of athletes have previously reported gastrointestinal symptoms during endurance running whilst exposed to heat at some point during the event, compared with ~60% during events conducted in temperate conditions.(15, 22-24) Despite such high prevalence rates, the underlying and precise cause/s of these symptoms remain elusive, possibly due to their multifactorial aetiology (i.e., exercise-associated gastrointestinal syndrome).(2) Given that upper-gastrointestinal symptoms have been most commonly reported during running in the heat, it is plausible that dysfunction to hormonal and (or) neural control of gastrointestinal motility may arise from the increased thermoregulatory strain (i.e., increased rectal temperature and heart rate), however this requires further investigation.(88, 146, 147) A predisposition to gastrointestinal symptoms arising from mild heat exposure during exercise is concerning for endurance athletes due to higher fluid and nutrient requirements during training and (or) competition, compared with cooler ambient conditions. The presence of gastrointestinal symptoms has been shown to compromise nutrient intake, adversely affect performance and may directly or indirectly result in withdrawal from competition due to symptom severity or an inability to sustain fluid and nutrient requirements.(23-25)

In conclusion, prolonged submaximal running in 30°C T_{amb} increases intestinal epithelial injury, gastrointestinal symptoms, and promotes modest perturbations to the systemic inflammatory profile, compared to running in temperate conditions (i.e., 22°C T_{amb}). Considering that small intestine permeability and circulatory endotoxin profile were not affected by mild exertional-heat stress, such transient gastrointestinal perturbations are unlikely to be of clinical significance. However, the increased gastrointestinal symptoms and reduction in appetite during running in the heat may have implications for nutrient intake during and after exercise, and subsequently exercise performance implications.

5.7 Conclusion

In summary, the findings from this experimental study show that perturbations to gastrointestinal integrity, symptoms and systemic cytokine responses are exacerbated with exposure to warm ambient conditions during prolonged submaximal running. However, such modest and transient damage to the intestinal epithelium and mild increases in upper-gastrointestinal symptoms and cytokinaemia are unlikely to be of any clinical significance. Exercise at a higher intensity in warm conditions may elicit a greater thermoregulatory strain (i.e., core body temperature), likely resulting in similar gastrointestinal perturbations as was observed in study one of this thesis (**Chapter 4**). Indeed a comparison between the findings from study one (**Chapter 4**) and two (**Chapter 5**) of this thesis highlight that exposure to hotter ambient conditions (i.e., a 5°C increase in T_{amb}) at the same relative exercise intensity and duration markedly increased core body temperature, resulting in greater and more prolonged injury to the intestinal epithelium, higher occurrence of gastrointestinal symptoms, perturbations to endotoxin clearance capacity and an exaggerated compensatory anti-inflammatory response. Therefore, to ascertain the effectiveness of a prevention and/or management strategy, future studies should be conducted in hot ambient conditions that elicit a significant thermoregulatory strain (i.e., core body temperature $\geq 39.0^{\circ}\text{C}$). The underlying basis of these studies should aim to address conceivable primary mechanisms or exacerbating factors of exercise-induced gastrointestinal syndrome, such as splanchnic hypoperfusion, thermoregulatory strain and/or stress hormone responses.

CHAPTER SIX

Carbohydrate and protein intake during exertional-heat stress ameliorates intestinal epithelial injury and small intestine permeability.

6.1 Background

Glucose is the primary fuel source for working muscles during moderate to high intensity exercise.(170) It is therefore, not surprising that consumption of carbohydrates during prolonged exercise, where endogenous carbohydrate stores are limited, improves performance.(83) The intestinal absorption of carbohydrates, particularly in the form of glucose, may also exert beneficial effects on the gastrointestinal system by attenuating splanchnic hypoperfusion, a primary contributor in exercise-induced gastrointestinal syndrome.(91) Despite carbohydrates being commonly consumed during exercise, no studies to date have primarily explored the effects of frequent carbohydrate consumption aimed at improving splanchnic perfusion during exercise on gastrointestinal outcomes.

In contrast to carbohydrates, the use of amino acids and amino acid precursors have been explored in relation to the maintenance of intestinal integrity and splanchnic perfusion during exercise and exertional-heat stress, with the proposed benefits occurring via multiple pathways (i.e., stabilisation of tight junction proteins, reduction in localised inflammation and/or maintaining splanchnic perfusion).(90, 96) However, the use of a whole protein which is more commonly found in foods and/or a hydrolysed protein containing a variety of amino acids and peptides, which may improve digestion and absorption has not been explored.

The third experimental study within this thesis therefore, aimed to identify if frequent carbohydrate ingestion, in the form of glucose, during exertional-heat stress could attenuate gastrointestinal perturbations that are primarily induced by exacerbated splanchnic hypoperfusion. Additionally, this study aimed to identify if the energy content or specific nutrient-related factors were important in mediating perturbations to gastrointestinal integrity. This was achieved by comparing frequent glucose ingestion against an energy-matched hydrolysed whey protein.

6.2 Abstract

Background: Exertional-heat stress (EHS) disturbs the integrity of the gastrointestinal tract leading to endotoxaemia and cytokinaemia, which have symptomatic and health implications. This study aimed to determine the effects of carbohydrate and protein intake during EHS on gastrointestinal integrity, symptoms and systemic responses. **Methods:** Eleven (male $n=6$, female $n=5$) endurance runners completed 2 h running at 60% $\dot{V}O_{2max}$ in 35°C ambient temperature on three occasions in randomised order, consuming water (WATER) or 15 g glucose (GLUC) or energy-matched whey protein hydrolysate (WPH) before and every 20 min during EHS. Rectal temperature and gastrointestinal symptoms were recorded every 10 min during EHS. Blood was collected pre- and post-EHS, and during recovery to determine plasma concentrations of intestinal fatty-acid binding protein (I-FABP) as a marker of intestinal epithelial injury, cortisol, endotoxin, and inflammatory cytokines. Urinary lactulose:L-rhamnose was used to measure small intestine permeability. **Results:** GLUC and WPH ameliorated EHS-associated intestinal epithelial injury compared to WATER (I-FABP: $123 \pm 197 \text{ pg}\cdot\text{mL}^{-1}$ and $82 \pm 156 \text{ pg}\cdot\text{mL}^{-1}$ vs $897 \pm 478 \text{ pg}\cdot\text{mL}^{-1}$, respectively, $p < 0.001$) and small intestine permeability (lactulose:L-rhamnose ratio: 0.017 ± 0.005 and 0.008 ± 0.002 vs 0.034 ± 0.014 , respectively, $p = 0.001$). A main effect of time was observed for endotoxin concentration (mean increase $10.2 \pm 12.8 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.001$). Post-EHS anti-endotoxin antibodies were higher ($p < 0.01$) and cortisol and IL-6 lower ($p < 0.05$) with GLUC than WATER only. Total and upper-

gastrointestinal symptoms were greater with WPH, compared to GLUC and WATER ($p < 0.05$), in response to EHS. **Conclusion:** Carbohydrate and protein intake during EHS ameliorates intestinal injury and permeability. Carbohydrate also supports endotoxin clearance and reduces stress markers, while protein appears to increase gastrointestinal symptoms therefore, carbohydrate is more suitable to consume during prolonged EHS.

6.3 Introduction

Exposure to hot ambient temperatures is common in many sports and exertional activities. Exertional heat stress (EHS) exacerbates splanchnic hypoperfusion, hyperthermic injury and perturbations of intestinal barrier function that may lead to endotoxaemia and subsequent systemic inflammatory responses (i.e., cytokinaemia), and the development of gastrointestinal symptoms.(2) These gastrointestinal disturbances and systemic responses have been linked to several acute health conditions, including septic shock, ischaemic colitis, gastritis and paralytic ileus, however, reported cases of these conditions are uncommon and appear to be limited to endurance running.(6-11, 32, 161) Indeed, endotoxaemia, cytokinaemia, and gastrointestinal symptoms have been consistently observed during and after endurance events with exposure to hot ambient conditions.(15, 16, 22, 23, 70) Gastrointestinal symptoms during these events consistently affect $\geq 60\%$ of athletes, making them a major medical issue that can compromise exercise performance, nutrient intake during and after exercise, and, in severe cases, cause withdrawal from competition.(2)

Considering postprandial hyperaemia increases splanchnic perfusion and microvascular blood flow in intestinal villi, it is plausible that repeated macronutrient intake during EHS may ameliorate gastrointestinal perturbations by improving splanchnic perfusion.(37, 94, 171) Furthermore, absorption of glucose and certain amino acids and (or) amino acid precursors (i.e., glutamine, aspartate, glycine, L-arginine and L-citrulline) have been shown to produce localised metabolic

vasodilators, principally nitric oxide, which may also improve intestinal microvillous perfusion during exercise.(37, 94) Indeed, a 10 g bolus of L-citrulline, an L-arginine pre-cursor, before 1 h cycling at 70% maximal workload (W_{max}) improved splanchnic perfusion and reduced circulating levels of intestinal fatty-acid binding protein (I-FABP), a sensitive marker of intestinal epithelial injury and damage, during exercise.(36, 90)

In addition to postprandial hyperaemia, it has been proposed that certain amino acids, for example glutamine, may enhance the expression of heat shock proteins which can prevent injury to the intestinal epithelium; and subsequently reduce intestinal permeability, and localised and systemic inflammatory responses.(57, 61) Despite some promising effects on gastrointestinal integrity, the practical utility of amino acid and (or) protein intake during prolonged exercise may be somewhat limited due to reports of gastrointestinal symptoms.(97, 99) However, to date no studies have investigated the effects of hydrolysed whey protein, which contains a variety of amino acids and peptides in a more rapidly digested form (172), on gastrointestinal integrity and symptoms, and systemic profiles in response to EHS.

Carbohydrate is more commonly consumed than protein during endurance exercise and has been shown to improve physical performance.(83) However, recommended intakes of $90 \text{ g}\cdot\text{h}^{-1}$ multiple transportable carbohydrates during prolonged exercise (i.e., $\geq 3 \text{ h}$) has been associated with the development of gastrointestinal symptoms, due to the inability to tolerate such high carbohydrate intakes.(25) Moreover, few studies have investigated the effects of carbohydrate intake before or during exercise on gastrointestinal integrity. Observations by Rehrer et al.(91) reported that consumption of a glucose solution pre-exercise and every 20 min during 60 min cycling at 70% $\dot{V}O_{2max}$ tended to maintain portal vein blood flow to a greater extent than water, highlighting the efficacy of glucose in stimulating postprandial hyperaemia. However, other studies have observed no benefit to gastroduodenal permeability, except when aspirin was ingested pre-exercise(69); and it

appears that small infrequent carbohydrate (i.e., ~10-27g) intakes during 60-90 min steady state exercise has minimal impact on intestinal integrity.(39, 95) Consumption of an energy gel during 60 min running at 70% $\dot{V}O_{2max}$ has been suggested as being harmful to gastrointestinal integrity.(39) While direct comparisons are not possible due to differences in assay kits and methodology between studies, the small transient post-exercise increase in I-FABP (~263 pg·ml⁻¹) and circulatory endotoxin (~5 pg·ml⁻¹)(39) observed with gel consumption are well below the 1230 pg·ml⁻¹ and 10 pg·ml⁻¹ increase that we previously observed after 2 h running at 60% $\dot{V}O_{2max}$ in 35°C T_{amb} with water intake alone.(173) In addition to macronutrient intake, inadequate, excessive, and (or) prescribed fluid intakes above tolerance levels during exercise have been shown to contribute to the development of gastrointestinal symptoms, with restricted fluid intake also increasing small intestine permeability.(25, 55, 106) It is evident that there is currently a clear gap in the research regarding the most appropriate nutrient intake to recommend for maintenance of gastrointestinal integrity and ameliorating the associated systemic responses associated with EHS.

The current study, therefore, aimed to determine the effects of carbohydrate and protein intake before and during EHS on gastrointestinal integrity (i.e., using I-FABP as a marker of intestinal epithelial injury and lactulose:L-rhamnose ratio as a measure of small intestine permeability), systemic responses (i.e., circulatory endotoxin and inflammatory cytokine profiles) and gastrointestinal symptoms compared to *ad libitum* water intake. It was hypothesised that carbohydrate and protein intake would ameliorate perturbations to gastrointestinal integrity and systemic responses compared to water, but based on previous observations, protein intake would increase the incidence and severity of gastrointestinal symptoms compared to those associated with carbohydrate and water ingestion.

6.4 Methods

Participants

Eleven non-heat acclimatised endurance-trained runners [mean \pm SD: (male $n=6$, female $n=5$) age 31 ± 5 years, nude body mass 65.7 ± 12.0 kg, height 1.72 ± 0.09 m, % body fat mass $17 \pm 6\%$, $\dot{V}O_{2\max}$ 54 ± 6 ml·kg·min⁻¹] volunteered to participate in the study. All participants gave written informed consent. The study protocol received approval from the local ethics committee and conformed to the 2008 Helsinki Declaration for Human Research Ethics. Participants were excluded if they confirmed having gastrointestinal infections, diseases and/or disorders, consumed potential modifiers of gastrointestinal integrity (such as prebiotics, probiotics, and/or antibiotics), were adhering to gastrointestinal-focused dietary regimes (such as fibre-modified or gluten-free diets) within the previous three months, or consumed non-steroidal anti-inflammatory medications and/or stool altering medications such as laxatives and anti-diarrhoeal medication within one month before the experimental protocol.

Preliminary measures

One week before the first experimental trial, height and nude body mass (Seca 515 MBCA, Seca Group, Hamburg, Germany) were recorded. Maximal oxygen uptake ($\dot{V}O_{2\max}$; Vmax Encore Metabolic Cart, Carefusion, San Diego, California, US) was estimated by means of a continuous incremental exercise test to volitional exhaustion on a motorized treadmill (Forma Run 500, Technogym, Seattle, Washington, US), as previously reported (163). To determine running speed for the exercise trials, the speed at approximately 60% $\dot{V}O_{2\max}$ and 1% gradient was determined and verified from the $\dot{V}O_2$ -work rate relationship (10.3 ± 0.7 km·h⁻¹).

Experimental procedure

Participants were provided an individualised diet low in fermentable carbohydrates (i.e., FODMAPs) for the 24 h period before each experimental trial (Mean \pm SD: 12.4 \pm 1.8 MJ, 454 \pm 69 g carbohydrate, 113 \pm 10 g protein, 71 \pm 13 g fat) to reduce gastrointestinal symptoms arising from pre-exercise food and fluid intake.(102) Participants were asked to refrain from consuming additional high FODMAP foods, alcohol, and caffeinated beverages during the diet controlled period, and refrain from strenuous exercise during the 48 h period before each experimental trial. Compliance was determined by a dietary and exercise log. Participants reported to the laboratory at 8:00h after consuming the standardised low FODMAP breakfast (2.5 MJ, 125 g carbohydrate, 14 g protein, and 4 g fat) with 400 ml of water (consumed at 07:00h). Participants were asked to void before nude body mass measurements and completion of a self-reported gastrointestinal symptom assessment tool. A 10-point Likert-type rating scale was used to quantify self-perceived gastrointestinal symptoms (adapted from a 10 cm visual analogue scale (134, 135), with 0 indicative of no symptoms to 10 indicative of extreme symptoms, as previously reported (23, 25)). Blood was then collected by venepuncture from an antecubital vein into a vacutainer (6 ml, 1.5 IU·ml⁻¹ heparin). To monitor rectal temperature (T_{re}) during running, participants inserted a thermocouple 12 cm beyond the external anal sphincter (Grant REC soft insertion probe thermocouple; Grant 2010 Squirrel data logger, Shepreth, UK).

In a randomised order, participants completed three experimental trials separated by one-week, consisting of 2 h (initiated at 09:00h) running exercise on a motorised treadmill at the previously determined speed that elicited 60% $\dot{V}O_{2max}$ within an environmental chamber at 35.5 \pm 1.4°C T_{amb} and 27 \pm 5% relative humidity. Participants were provided with an in-house formulated non-commercial energy-matched glucose (GLUC: 255 kJ of which protein 0 g, carbohydrate 15.0 g and fat 0 g, 6.0% w/v; Glucodin, Valeant, Laval, Quebec, Canada) or whey protein hydrolysate (WPH: 255 kJ of which protein 14.8 g, carbohydrate 0.1 g and fat 0.1 g, 6.4% w/v; Tatura HWP406,

Morrinsville, New Zealand) solution immediately pre-exercise and every 20 min during running with additional water *ad libitum*, or water (WATER) *ad libitum* and requested to drink to maintain euhydration. In the absence of repeated gut-training with a set fluid volume, an *ad libitum* water intake regime was employed to provide autonomy over drinking patterns to minimise occurrence of gastrointestinal symptoms.(25, 106) Participants were not advised of the contents of the GLUC and WPH solutions; however, the test solutions were not blinded due to an inability to mask the flavour of the WPH solution. Heart rate, rating of perceived exertion (RPE; 20-point Likert-type perceived exertion rating scale, with 7 indicative of very very light and 19 indicative of very very hard)(141), thermal comfort rating (13-point Likert-type thermal rating, with 7 indicative of comfortable, 10 indicative of hot, and 13 indicative of unbearably hot) (adapted from (142)), T_{re} , and gastrointestinal symptoms were measured and recorded every 10 min during running. To determine small intestine permeability, participants were asked to consume a dual-sugars solution containing 5 g lactulose (Duphalac, Abbott Biologicals, Olst, Netherlands) and 1 g L-rhamnose (MP Biomedicals, LLC, Solon, USA) in 100 ml water, 90 min into exercise. A 5 h urine collection period commenced post-ingestion, where the final volume was weighed, and 30 ml aliquoted and stored frozen at -20°C until analysis. Immediately after EHS, a blood sample was collected and nude body mass was recorded. Participants remained seated during the recovery period and were provided with water *ad libitum*. Blood was also collected 1 h, 2 h, and 4 h post-EHS. To reduce any seasonal heat acclimatisation, the experimental procedures were conducted over the cooler seasonal periods (temperatures were consistently $\leq 20^{\circ}\text{C}$). (158)

Sample analysis

Whole blood haemoglobin and haematocrit values were used to estimate changes in plasma volume relative to baseline, and used to correct plasma variables. Blood glucose concentration was determined pre- and post-EHS using a handheld glucose monitor (Accu-Chek Proforma, Roche Diagnostics, Indianapolis, Indiana, USA). The remaining blood samples were centrifuged at 4000

rpm for 10 min within 15 min of sample collection. Plasma was aliquoted into eppendorfs and frozen at -80°C until analysis, except for 50 µl that were used to determine plasma osmolality (P_{Osmol}), in duplicate (coefficient of variation (CV): 2.7%), by freeze-point osmometry (Osmomat 030, Gonotec, Berlin, Germany). Recovery of lactulose and L-rhamnose in urine was determined by ultra-performance liquid chromatography in duplicate (CV: 13.8%). Plasma concentrations of interleukin (IL)-6, IL-1 β , tumour necrosis factor (TNF)- α , IL-8, IL-10, and IL-1 receptor antagonist (ra) were determined by multiplex enzyme-linked immunosorbent assay (ELISA; HCYTOMAG-60K, EMD Millipore, Darmstadt, Germany). Circulating gram-negative bacterial endotoxin concentration was determined by limulus amoebocyte lysate (LAL) chromogenic endpoint assay (HIT302, Hycult Biotech, Uden, Netherlands). Plasma concentrations of I-FABP (HK406, Hycult Biotech, Uden, Netherlands), endotoxin core antibody (HK504, Hycult Biotech, Uden, Netherlands) and cortisol (RE52061, IBL International, Hamburg, Germany) were determined by ELISA. All variables were analysed in duplicate as per manufacturer's instructions on the same day, with standards and controls on each plate, and each participant assayed on the same plate. The CVs for endotoxin, I-FABP, endotoxin core antibody, cortisol, IL-10, IL-1ra, IL-1 β , IL-6, IL-8 and TNF- α were 3.2%, 5.4%, 6.0%, 3.1%, 2.0%, 2.3%, 1.4%, 1.7%, 0.6% and 1.1%, respectively.

Statistical analysis

Based on the typical standard deviation of 0.7 EU·ml⁻¹ for circulatory endotoxin responses to exertional-stress (70, 71), and using standard alpha (0.05) and beta values (0.8) (www.dssresearch.com), a sample size of n= 8 was estimated to provide adequate statistical precision to detect a >10% difference in circulatory endotoxin concentration in response to EHS in the target population. Such increases in circulatory endotoxin concentration have consistently been associated with systemic inflammatory responses.(70, 71, 82) Data in the text and tables are presented as mean and 95% confidence interval (CI), and cumulative score and individual participant range for gastrointestinal symptoms. For clarity, data in figures are presented as the magnitude of change with

mean \pm standard error of the mean (SEM). Due to commonly reported individual variations in plasma cytokine responses to exercise(70, 71, 82), figures for cytokine profile has been presented as individual responses, with the removal of outliers for clarity. Only raw data and participants with full data sets within each specific variable were used in the data analysis. All data were checked for normal distribution by calculating skewness and kurtosis coefficients. Where data violated the assumption of normality (positive skewness and kurtosis), data were log-transformed prior to analysis. Variables with singular data points were examined using a one-way ANOVA; while variables with multiple data points were examined using a two-way repeated-measures ANOVA, except for gastrointestinal symptoms that were examined using Friedman's test. Assumptions of homogeneity and sphericity were checked, and when appropriate adjustments to the degrees of freedom were made using the Greenhouse-Geisser correction method. Significant main effects were analysed using a post hoc Tukey's HSD test or Wilcoxon signed rank test for gastrointestinal symptoms. Data were also analysed for order effect using a two-way repeated measures ANOVA, with no significant trial order effect observed. Statistics were analysed using SPSS statistical software (V.23.0, Chicago, Illinois, USA) with significance accepted at $p \leq 0.05$.

6.5 Results

Hydration status, cardiovascular and thermoregulatory strain

Water intake was higher ($p= 0.005$) and EHS-induced loss of body mass was lower ($p< 0.001$) with GLUC and WPH compared to WATER (**Table 6.1**). Plasma osmolality did not differ pre- to post-EHS within or between trials ($p= 0.393$), indicating maintenance of euhydration. A main effect of time was observed for T_{re} ($p< 0.001$), which was significantly elevated from 20 min EHS onwards, compared to 10 min ($p< 0.01$; **Figure 6.1A**). No significant difference was observed between trials for peak T_{re} (GLUC: 38.9 (38.5-39.4)°C, WPH: 39.0 (38.5-39.4)°C and WATER: 39.3 (38.8-39.8)°C, $p= 0.103$) and change in T_{re} from pre-EHS (GLUC: 2.0 (1.6-2.4)°C, WPH: 2.1 (1.6-2.5)°C, and WATER: 2.2 (1.7-2.7)°C, $p= 0.175$). A main effect of time was found for heart rate ($p< 0.001$; **Figure 6.1B**), rating of perceived exertion ($p< 0.001$; **Figure 6.1C**), and thermal comfort rating ($p< 0.001$; **Figure 6.1D**), which were significantly elevated in all trials from 40 min EHS onwards, compared to 10 min.

Table 6.1. Water intake, body mass loss and plasma osmolality in response to 2 h running at 60% $\dot{V}O_{2max}$ in 35°C T_{amb} (exertional-heat stress: EHS) with ingestion of glucose (GLUC), whey protein hydrolysate (WPH) and water (WATER).

	GLUC		WPH		WATER		ANOVA
	Pre-EHS	Post-EHS	Pre-EHS	Post-EHS	Pre-EHS	Post-EHS	p-value
Total water intake (L)	NA	1.84 (1.66-2.03) ^{aa}	NA	1.89 (1.64-2.15) ^{aa}	NA	1.59 (1.30-1.88)	0.005
Body mass loss (%)	NA	0.5 (-0.1-1.2) ^{aa}	NA	0.4 (-0.1-1.0) ^{aa}	NA	1.3 (1.0-1.6)	0.000
Plasma Osmolality (mOsmol·kg⁻¹)	285 (277-293)	284 (275-294)	287 (280-295)	290 (281-298)	290 (284-297)	291 (284-298)	0.393

Mean and (95% confidence interval) (n=11). ^{aa}p< 0.01 vs. WATER. NA: not applicable.

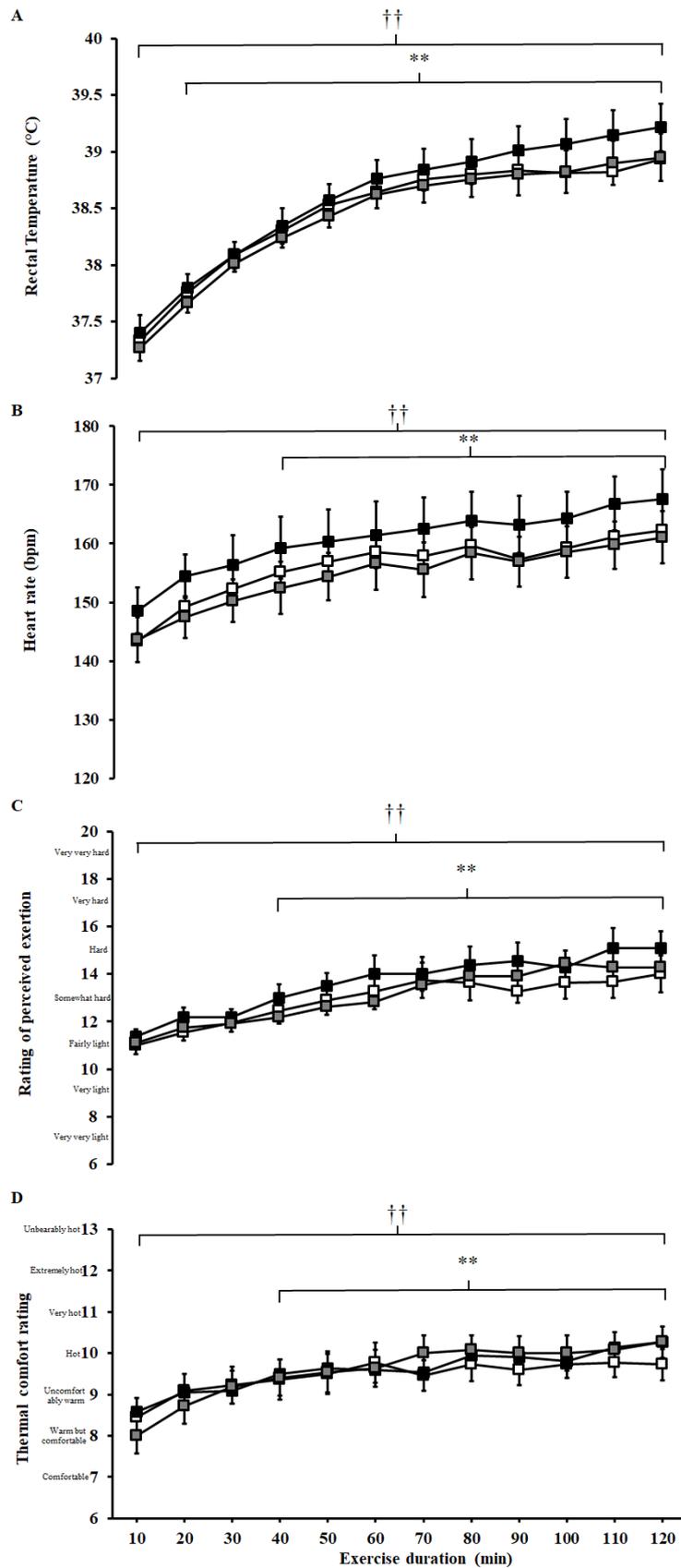


Figure 6.1. Rectal temperature (A), heart rate (B), rating of perceived exertion (C), and thermal comfort rating (D) in response to 2 h running at 60% $\dot{V}O_{2max}$ in 35°C T_{amb} with glucose (GLUC: white squares), hydrolysed whey protein (WPH: grey squares) or water (WATER: black squares). Mean \pm SEM (n= 11): †† main effect of time p< 0.01 vs. 10 min, ** p< 0.01 vs. 10 min.

Plasma cortisol and blood glucose concentration

A trial x time interaction was observed for plasma cortisol ($p= 0.003$) and blood glucose concentrations ($p= 0.014$; **Figure 6.2**). Plasma cortisol concentration increased pre- to post-EHS during all trials and was significantly lower immediately, 1 h and 2 h post-EHS with GLUC ($p< 0.01$), and at 1 h post-EHS with WPH ($p< 0.05$) compared to WATER. Blood glucose concentration increased pre- to post-EHS during all trials and was higher with GLUC compared to WPH and WATER ($p< 0.01$).

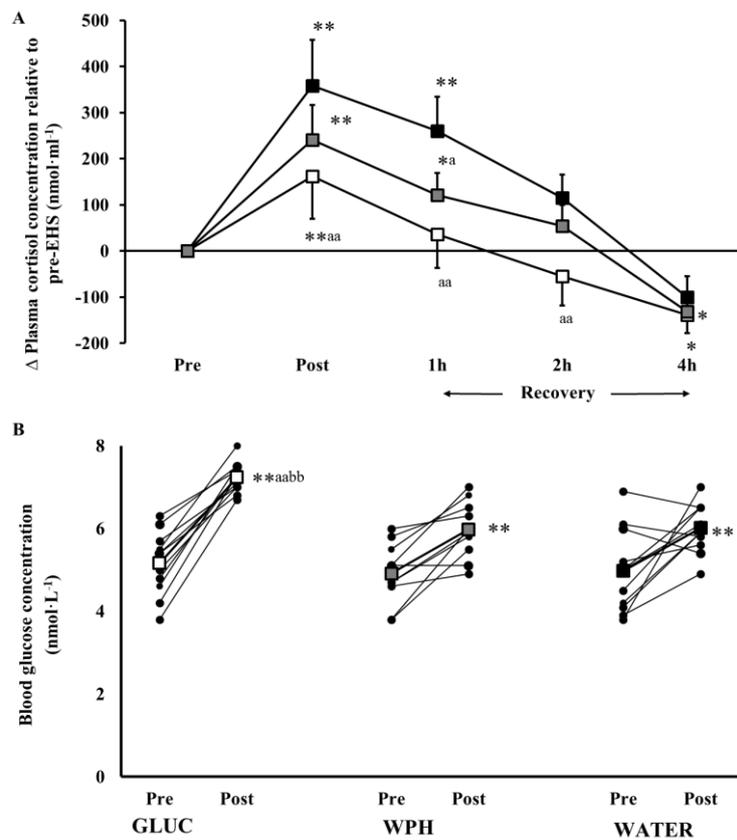


Figure 6.2. Change in plasma cortisol (A) and blood glucose (B) in response to 2 h running at 60% $\dot{V}O_{2max}$ in 35°C T_{amb} with glucose (GLUC: white squares), hydrolysed whey protein (WPH: grey squares) or water (WATER: black squares). Mean \pm SEM (A) and mean and individual responses (B) ($n= 11$): ** $p< 0.01$ and * $p< 0.05$ vs. pre-EHS, ^{aa} $p< 0.01$ and ^a $p< 0.05$ vs. WATER, ^{bb} $p< 0.01$ vs. WPH.

Gastrointestinal integrity and symptoms

A trial x time interaction was found for plasma I-FABP concentrations ($p < 0.001$; **Figure 6.3**), which showed it was significantly elevated immediately, 1 h and 2 h post-EHS, compared to pre-EHS values with WATER (288%, 236% and 97%, respectively, $p < 0.01$). However, they did not change with GLUC and WPH. Compared to WATER, I-FABP levels were significantly lower immediately, 1 h and 2 h post-EHS with GLUC and WPH ($p < 0.01$). A significantly lower lactulose:L-rhamnose ratio was observed with GLUC (0.017 (0.013-0.021)) and WPH (0.008 (0.004-0.011)) compared to WATER (0.034 (0.022-0.046); $p = 0.001$) (**Figure 6.4**). Gut discomfort was significantly higher with WPH compared to WATER ($p = 0.003$), while total and upper-gastrointestinal symptoms were significantly higher ($p < 0.01$) with WPH and appetite were significantly lower ($p < 0.05$) compared to GLUC and WATER (**Table 6.2**).

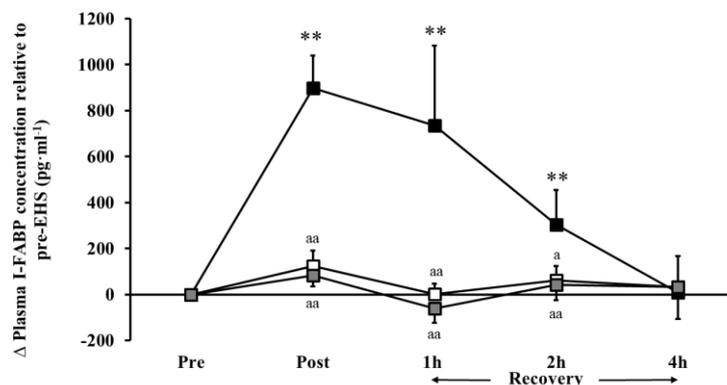


Figure 6.3. Change in plasma intestinal fatty acid binding protein (I-FABP) in response to 2 h running at 60% $\dot{V}O_{2\max}$ in 35°C T_{amb} with glucose (GLUC: white squares), hydrolysed whey protein (WPH: grey squares) or water (WATER: black squares). Mean \pm SEM ($n = 11$): ** $p < 0.01$ vs. pre-EHS, ^{aa} $p < 0.01$ and ^a $p < 0.05$ vs. WATER.

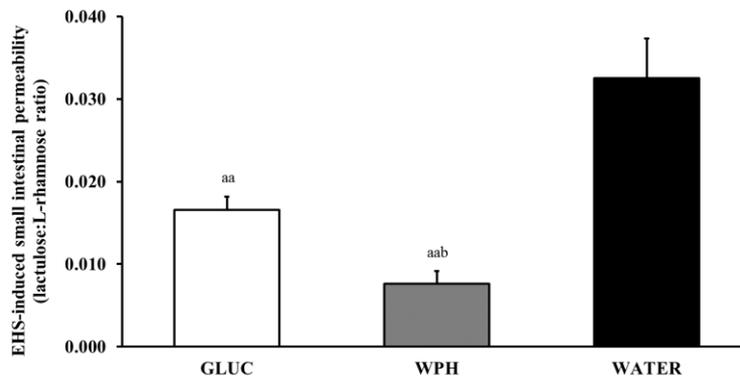


Figure 6.4: EHS-induced small intestine permeability (lactulose:L-rhamnose ratio) in response to 2 h running at 60% $\dot{V}O_{2max}$ in 35°C T_{amb} with glucose (GLUC), hydrolysed whey protein (WPH) or water (WATER). Mean \pm SEM (n= 8): ^{aa} $p < 0.01$ vs. WATER, ^b $p < 0.05$ vs. GLUC.

Table 6.2. Incidence of severe gastrointestinal symptoms and severity of gut discomfort, total, upper-, and lower-gastrointestinal symptoms in response to 2 h running at 60% $\dot{V}O_{2max}$ in 35°C T_{amb} with ingestion of glucose (GLUC), whey protein hydrolysate (WPH) and water (WATER).

	GLUC		WPH		WATER		Friedman
	Incidence ¹	Total (range)	Incidence ¹	Total (range)	Incidence ¹	Total (range)	<i>p</i> -value
Gut discomfort	NA	402 (11-58)	NA	516 (1-88) ^{aa}	NA	286 (0-67)	0.003
Total gastrointestinal symptoms	73%	641 (4-153)	91%	1370 (14-269) ^{aabb}	82%	875 (0-254)	0.003
Upper-gastrointestinal symptoms	45%	362 (3-90)	91%	861 (0-167) ^{aabb}	45%	378 (0-128)	0.002
Belching	27%	149 (0-41)	45%	267 (0-48)	18%	94 (0-33)	0.058
Heartburn	0%	6 (0-4)	9%	18 (0-18)	0%	25 (0-17)	0.607
Bloating	9%	122 (0-29)	64%	311 (0-71) ^{aabb}	18%	106 (0-40)	0.001
Upper abdominal pain	0%	21 (0-7)	9%	93 (0-21)	0%	53 (0-28)	0.066
Urge to regurgitate	9%	64 (0-27)	45%	172 (0-40) ^b	18%	90 (0-48)	0.034
Regurgitation	0%	0 (0-0)	0%	0 (0-0)	9%	10 (0-10)	0.368
Lower-gastrointestinal symptoms	27%	135 (0-45)	45%	201 (0-45)	18%	156 (0-39)	0.338
Flatulence	0%	3 (0-3)	0%	28 (0-25)	0%	6 (0-2)	0.424
Urge to defecate	0%	58 (0-36)	9%	56 (0-36)	0%	83 (0-29)	0.130
Abdominal pain/s	0%	29 (0-9)	18%	72 (0-22)	0%	30 (0-15)	0.417
Abnormal defecation ²	0%	0 (0-0)	0%	0 (0-0)	0%	0 (0-0)	---
Others							
Nausea	9%	82 (0-38)	36%	177 (0-53)	27%	155 (0-43)	0.210
Dizziness	9%	48 (0-30) ^a	18%	72 (0-37) ^a	27%	155 (0-43)	0.003
Abdominal stitch	0%	14 (0-6)	27%	59 (0-22)	0%	37 (0-17)	0.582
Appetite	NA	180 (0-53)	NA	120 (0-41) ^{ab}	NA	198 (0-50)	0.020
Thirst	NA	397 (6-80) ^a	NA	344 (1-66) ^{aa}	NA	520 (20-87)	0.006

Overall participant summative accumulation of rating scale point score of measured time periods and individual participant range (n=11). ^{aa} *p*< 0.01 and ^a *p*< 0.05 vs WATER, ^{bb} *p*< 0.01 and ^b *p*< 0.05 vs GLUC. ¹ Incidence of severe (≥ 5 out of 10) gastrointestinal symptoms and ² abnormal defecation including loose watery stools, diarrhoea and blood in stools. NA: not applicable.

Circulating endotoxin profile

A main effect of time was observed for plasma bacterial endotoxin concentration, which increased by a mean of 10.2 (5.1-15.3) $\text{pg}\cdot\text{ml}^{-1}$ ($p=0.001$), peaking immediately post-EHS (**Figure 6.5A**). A trial \times time interaction was observed for plasma anti-endotoxin antibody concentration ($p=0.012$) which increased post-EHS with GLUC (23%, $p<0.01$) and WPH (9%, $p>0.05$), and decreased with WATER (12%, $p>0.05$) (**Figure 6.5B**). Post-EHS anti-endotoxin antibodies were significantly higher with GLUC compared to WATER only ($p<0.01$).

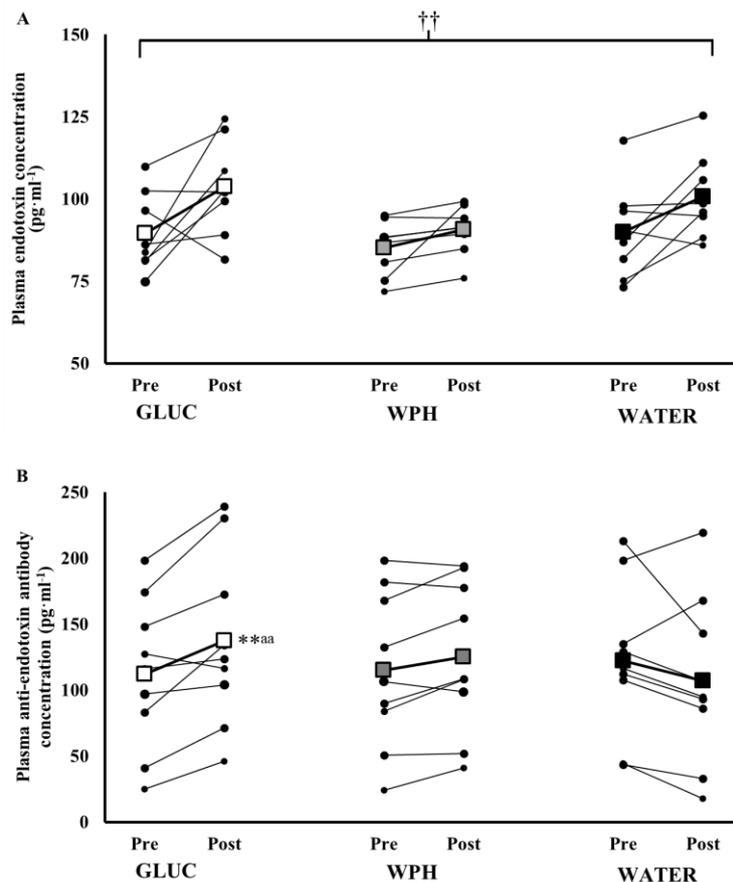


Figure 6.5: Pre- to post-EHS circulatory gram-negative bacterial endotoxin (A) and anti-endotoxin antibody (B) concentration in response to 2 h running exercise at 60% $\dot{V}O_{2\max}$ in 35°C T_{amb} with glucose (GLUC), hydrolysed whey protein (WPH) or water (WATER). Mean and individual responses ($n=9$): †† main effect of time $p<0.01$ vs. pre-EHS, ** $p<0.01$ vs. pre-EHS, ^{aa} $p<0.01$ vs. WATER.

Cytokine profile

A trial x time interaction was observed for plasma IL-6 concentration ($p= 0.048$; **Figure 6.6A**), whereas, a main effect of time was observed for plasma IL-8 ($p= 0.004$), IL-10 ($p= 0.003$) and IL-1ra ($p= 0.002$) concentrations, while no difference was observed for IL-1 β (peaking post-exercise) and TNF- α (peaking post-exercise) concentrations. (**Figure 6.6**). Plasma IL-6 concentration significantly increased pre- to post-EHS with WATER only ($p< 0.01$), and was greater than GLUC, but not WPH. Pre- to post-EHS increases in the plasma concentrations of IL-8 (85%, peaking post-exercise), IL-10 (926%, peaking 1 h post-exercise) and IL-1ra (78%, peaking 2 h post-exercise) were observed ($p< 0.05$), with no difference between trials. A compensatory anti-inflammatory response was observed after EHS with WATER, GLUC, and WPH as evident by a significant reduction in TNF- α :IL-10 ratio (main effect of time; $p< 0.001$). However, this reduction was not significant for IL-1 β :IL-10 ratio.

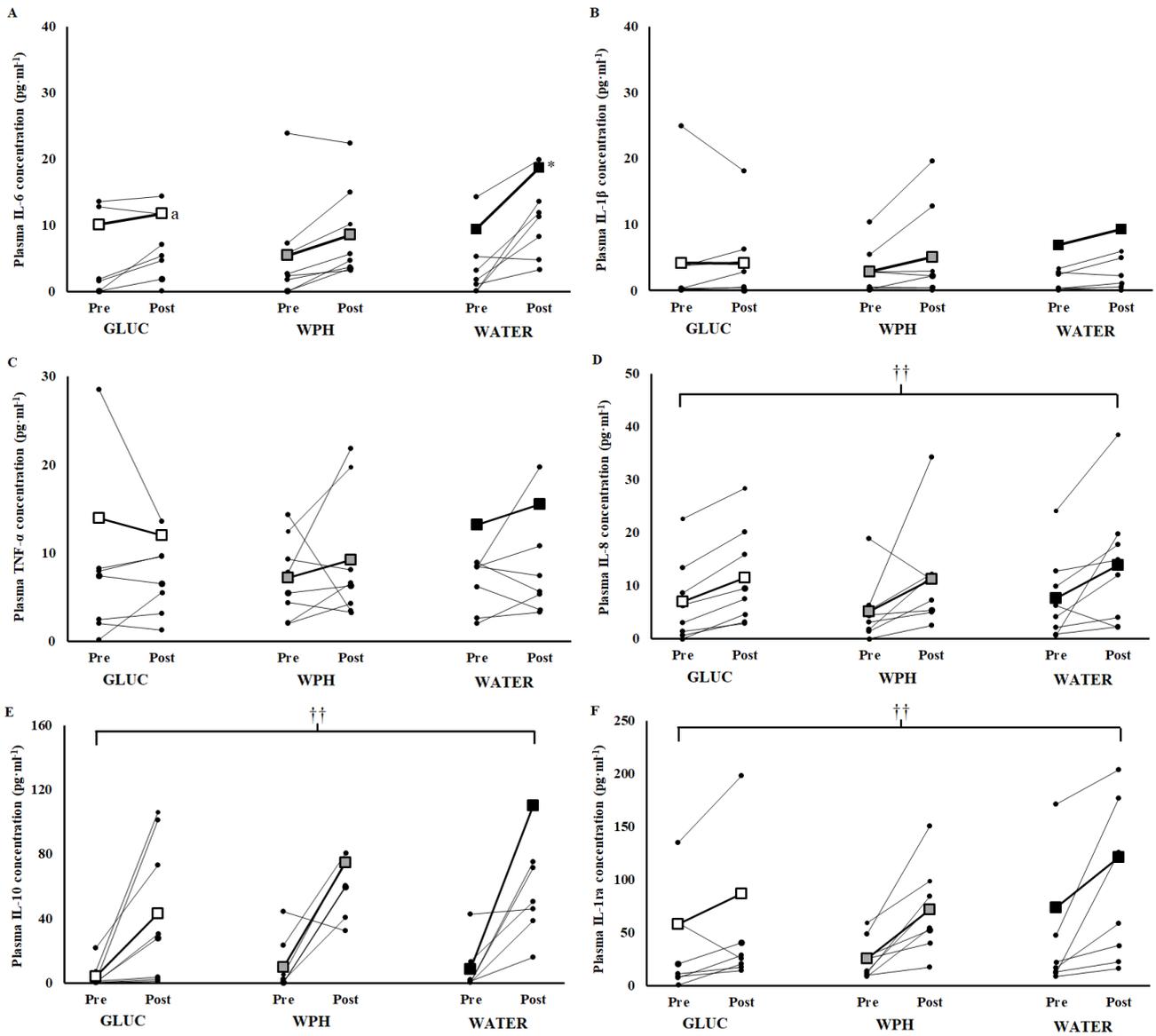


Figure 6.6: Pre- and post-EHS plasma IL-6 (A), IL-1β (B), TNF-α (C), IL-8 (D), IL-10 (E), and IL-1ra (F) concentrations in response to 2 h running exercise at 60% $\dot{V}O_{2max}$ in 35°C T_{amb} with glucose (GLUC), hydrolysed whey protein (WPH) or water (WATER). Individual responses (n= 9): †† main effect of time p < 0.01 vs. pre-EHS, *p < 0.05 vs. pre-EHS, ^a p < 0.05 vs. WATER.

6.6 Discussion

The current study aimed to determine the effects of carbohydrate and protein intake before and during EHS on gastrointestinal integrity, systemic responses, and gastrointestinal symptoms, compared to those with *ad libitum* water intake. The novel findings of the current study are that frequent ingestion of GLUC before and during EHS ameliorates intestinal epithelial injury and small intestine permeability, and enhances anti-endotoxin antibody responses compared to WATER. Further, energy-matched WPH also ameliorates intestinal epithelial injury and small intestine permeability compared to WATER. However, WPH increases the incidence and severity of gastrointestinal symptoms compared to GLUC and WATER. These findings indicate that, while frequent carbohydrate and protein ingestion both support gastrointestinal integrity during EHS, carbohydrate may be a more suitable recommendation for four reasons: 1) it was not associated with the triggering and heightened severity of gastrointestinal symptoms as observed with whey protein hydrolysate intake; 2) it supports endotoxin clearance, and results in reduced cortisol and IL-6 responses; 3) it contributes to blood glucose maintenance; and 4) it provides a rapid exogenous energy source to support skeletal muscle workload, all of which may contribute to improvements in exercise performance.(25, 83)

Intestinal epithelial injury

A novel finding was the substantial reduction in intestinal epithelial injury observed after running in 35°C T_{amb} with GLUC and WPH compared to WATER. Additionally, we observed no significant pre- to post-EHS increase in I-FABP with GLUC and WPH, indicating frequent feeding of macronutrients abolishes exercise-associated intestinal epithelial injury. Considering that 1) repeated ingestion of a glucose solution maintains portal vein blood flow during exercise(91); 2) I-FABP levels correlate well with splanchnic perfusion(40); and 3) no differences in I-FABP were found between

GLUC and WPH, it is likely repeated macronutrient feeding ameliorates intestinal injury by maintaining microvascular hyperaemia in the intestine villi.

Indeed, a reduced splanchnic hypoperfusion during exercise has been observed after ingestion of 10 g of L-citrulline compared with 20 g L-alanine (placebo), providing further evidence on the beneficial effects of macronutrient feeding on intestinal blood flow and subsequent intestinal injury.(90) However, it appears that pre-exercise macronutrient intake and (or) infrequent macronutrient consumption may have limited and (or) short-lived effects on splanchnic perfusion, intestinal ischemia, and subsequent intestinal epithelial injury.(39, 90) Indeed, this is supported by previous research suggesting the cessation of carbohydrate intake during prolonged (≥ 3 h) steady state running may contribute to substantial increases in intestinal injury (i.e., up to $4443 \text{ pg}\cdot\text{ml}^{-1}$ increase in plasma I-FABP concentration).(25) Therefore, from a practical perspective, it may be important to recommend early and frequent macronutrient consumption during prolonged exercise to avoid intestinal epithelial injury and associated gastrointestinal implications, including impaired nutrient absorption and exacerbation of gastrointestinal symptoms.(25, 38, 143) It is also yet to be determined if lower concentrations of macronutrients consumed before and frequently during prolonged strenuous exercise results in similar outcomes (e.g., 5 g or $10 \text{ g}\cdot 20 \text{ min}^{-1}$).

Small intestine permeability

The reduction in small intestine permeability observed with GLUC and WPH during prolonged running in the heat is a novel finding that could have important clinical outcomes. For example, increased intestinal injury and permeability is a distinct feature of many acute and chronic health conditions, and has been implicated as a primary mechanism for endotoxaemia and gastrointestinal-originated exertional heat stroke.(6, 14, 32, 36, 47) Our findings indicate that frequent macronutrient intake reduces small intestine permeability compared to WATER. These findings are supported by previous research showing ingestion of carbohydrates every 10 min during 1 h running at $70\% \dot{V}O_{2\text{max}}$

reduces aspirin-induced gastroduodenal permeability.(69) However, small intestine permeability was not reduced and there was no further benefit obtained from the addition of glutamine at a similar rate to the WPH.(69) In the current study, energy-matched WPH was more effective than GLUC at maintaining tight junction integrity and regulation during EHS, despite similar I-FABP profiles. Indeed, longer-term (7-28 days) supplementation of whey-based proteins and amino acids (i.e., glutamine) have been associated with improvements in gastrointestinal permeability with proposed mechanisms that include increased heat shock protein expression (e.g., HSP70), stabilised tight junction protein complexes, and thus reducing localised epithelial and systemic inflammatory responses.(57, 174, 175) It is therefore, plausible that WPH may have further reduced small intestine permeability *via* increased heat shock protein expression.(46, 96) Further research is required to elucidate the exact mechanisms that explain the observed differences in small intestine permeability between GLUC and WPH.

Systemic endotoxin and cytokine profile

In the current study, circulating endotoxin concentrations increased in all trials, despite a lower small intestine permeability with GLUC and WPH. The lack of a consistent relationship between intestinal permeability and circulatory endotoxin concentrations is supported by other studies, indicating factors beyond the extent of intestinal permeability such as lymphatic translocation, hepatic clearance rates, and (or) exercise-associated depression in immune function (e.g., phagocyte elastase degranulation) may contribute to mild exercise-associated endotoxaemia.(58, 137, 163, 176) For example, gram-negative endotoxins may enter the systemic circulation through the lymphatic system, rather than the paracellular route, and be bound to lipoproteins, soluble/membrane CD14 and (or) appear as outer membrane vesicles with differing clearance capacities and rates.(72-74, 151, 177)

Changes in endotoxin flux by measuring anti-endotoxin antibodies may, therefore, be a useful indicator of circulatory endotoxin clearance and partly predict neutralization capacity.(178) In the

current study, anti-endotoxin antibodies were increased post-EHS with GLUC and reduced with WATER, suggesting that carbohydrate intake may support endotoxin clearance, potentially through maintenance of immune function.(163, 176) The higher intestinal permeability and reduced anti-endotoxin antibodies with WATER, indicate increased endotoxin translocation and clearance may have occurred, despite similar post-exercise circulatory endotoxin concentration to GLUC and WPH. Indeed, endurance training has been proposed to improve endotoxin tolerance arising from repeated endotoxin exposure.(74, 77) Therefore, the commonly observed mild endotoxaemia might be a normal physiological response to strenuous exercise and pose no additional health risk if endotoxin clearance capacity (i.e., anti-endotoxin antibodies, liver function, phagocytic immune cell function) and recovery time is adequate. For example, anti-endotoxin antibodies are often reduced following endurance and ultra-endurance events, followed by a super-compensation in antibodies during recovery, suggesting a beneficial adaptation.(22, 137) In addition to higher anti-endotoxin antibodies with GLUC, lower post-exercise plasma cortisol and IL-6 concentrations were observed, compared to WATER, which may be attributed to the increased blood glucose levels.(112, 179, 180) Similar observations of increased blood glucose combined with reduced plasma cortisol and pro-inflammatory cytokine concentrations have previously been reported with carbohydrate intake during prolonged exercise, suggesting a reduced stress response with carbohydrate consumption compared to WATER.(112, 179) These findings may have significant gastrointestinal-immune health implications, whereby repeated carbohydrate consumption during exercise may ameliorate localised gastrointestinal injury, cytokine signalling, local and systemic inflammatory responses. Moreover, active individuals adhering to low-carbohydrate diets and (or) exercising fasted in an attempt to increase fat oxidation and (or) avoid gastrointestinal issues may be at increased risk of exercise-associated gastrointestinal disturbances and related health implications.

Gastrointestinal symptoms

Gastrointestinal symptoms are a major medical complaint consistently affecting >60% of runners during endurance events, especially when conducted in hot ambient conditions.(15, 16, 22, 23) Indeed, we have recently shown that exposure to hot ambient conditions during running directly contributes to the development of gastrointestinal symptoms, compared with temperate conditions.(173) Moreover, a recent study has demonstrated that large doses (90 g·h⁻¹) of multiple-transportable carbohydrates during prolonged running, a period when the gastrointestinal tract is compromised, results in malabsorption and the onset of gastrointestinal symptoms.(25) Reductions in splanchnic blood flow during high intensity exercise, or arising from heat exposure, can impair carbohydrate absorption.(91, 143) Unabsorbed carbohydrates may contribute to the development of upper-gastrointestinal symptoms *via* the ileal brake mechanism that slows gastric emptying, or lower-gastrointestinal symptoms due to increased colonic content and pressure.(169, 181) In the current study, GLUC was provided at a similar rate (i.e., 45 g·h⁻¹), in a readily absorbed concentration, to that reported during competitive endurance running events.(23, 85) No observable differences were found in gastrointestinal symptoms between GLUC and WATER, suggesting that prescribed provision of moderate carbohydrate intake does not increase gastrointestinal symptoms over *ad libitum* water intake, and thus is well tolerated.

While GLUC did not appear to exacerbate gastrointestinal symptoms, similar energy and volume intake of WPH increased the incidence and severity of symptoms, particularly upper-gastrointestinal symptoms, compared to GLUC and WATER. The increased prevalence of symptoms such as bloating and urge to regurgitate, suggest a possible delay in gastric emptying and (or) altered gastrointestinal motility with WPH.(146, 147) Several other studies have also found increased reports of gastrointestinal symptoms with consumption of moderate to large protein and (or) amino acid intake before or during exercise.(97, 99, 182) However, hydrolysed proteins have been reported to be digested and absorbed at a faster rate than complete proteins(172), which may have contributed to the

lack of symptoms observed in a small proportion of participants in the current study. Consistent with previous research, our findings highlight large individual variability in tolerance to the type and quantity of nutrition intake during running.(23, 25) It is plausible that smaller doses of WPH than provided during this study may be beneficial in maintaining gastrointestinal integrity while avoiding the occurrence of gastrointestinal symptoms however, this requires further investigation. Indeed, the occurrence of gastrointestinal symptoms during prolonged running likely arises due to the additional burden of nutrient digestion and absorption during a period of compromised gastrointestinal integrity and (or) function. Therefore, it is suggested that individual tolerance to nutrient intake during exercise be established and (or) trained for increased tolerance to optimise gastrointestinal integrity, health, and exercise performance outcomes.(25, 183)

Due to the aforementioned differences in tolerance to nutrient intake during exercise, WATER was provided *ad libitum* in the current study to act as a control for gastrointestinal symptoms. Despite advising participants to drink sufficient amounts of water to maintain euhydration, a limitation of the current study is the additional body mass loss (~0.9%) that occurred with WATER compared to GLUC and WPH. However, body mass losses were below values considered as modest dehydration ($\geq 2\%$ exercise-induced body mass loss) and values shown to contribute to the development of gastrointestinal symptoms.(84, 105) Moreover, additional body mass losses are expected to have occurred with WATER compared to GLUC and WPH due to substrate mass loss.(184, 185) Considering T_{re} (i.e., thermoregulatory strain) and plasma osmolality (i.e, hydration status) were not significantly different between trials, it is likely that such minimal differences in body mass loss would have had any impact on gastrointestinal outcomes.(29, 55)

Conclusion and future perspectives

Regular provision of glucose during EHS reduces intestinal injury and permeability, supports endotoxin clearance, and potentially lessens the stress response, compared to when only water is

ingested. Frequent intake of hydrolysed whey protein during EHS also reduces intestinal injury and permeability compared to water intake. However, protein appears to have minimal impact on systemic responses and such large intakes appear to increase gastrointestinal symptoms compared to water and carbohydrate intake. Therefore, frequent ingestion of carbohydrates may be more appropriate to recommend for supporting gastrointestinal integrity during EHS. Future research should aim to identify the minimum carbohydrate dose and frequency required to ameliorate gastrointestinal perturbations and if these benefits exist at higher exercise intensities and (or) over longer durations.

6.7 Conclusion

In summary, the findings from this experimental study demonstrate that frequent nutrient ingestion, in the form of glucose or an energy-matched hydrolysed whey protein, can attenuate damage to the gastrointestinal tract and assist in maintaining the gastrointestinal barrier. Frequent carbohydrate ingestion is likely to be more suitable than protein due to the provision of an exogenous glucose supply for oxidation and poor tolerance to large frequent doses of protein. While the findings of this experimental study appear to mediate gastrointestinal perturbations via splanchnic hypoperfusion, no reduction in gastrointestinal symptoms was observed with carbohydrate intake compared to water, despite a reduction in stress hormones. Considering elevated core body temperatures during exercise can delay gastric emptying, it is plausible that cooling strategies designed to attenuate thermoregulatory strain may improve gastric emptying and reduce the occurrence of upper-gastrointestinal symptoms.(88) Indeed, in study one of this thesis (**Chapter 4**) a relationship between exercise-induced increases in core body temperature and symptoms of urge to regurgitate and nausea were observed. Additionally, we observed positive correlations between intestinal epithelial injury, cytokine responses and stress hormone responses with exercise-induced increases in core body temperature. Further research is therefore warranted to determine if reducing thermoregulatory strain through a cooling strategy during exertional-heat stress can attenuate upper-gastrointestinal symptoms, intestinal injury, cytokinaemia and stress hormone responses.

CHAPTER SEVEN

Does the temperature of water ingested during exertional-heat stress influence gastrointestinal injury, symptoms and inflammatory cytokine profile?

7.1 Background

A significant amount of research has recently been conducted on a variety of cooling strategies that are applied before and/or during exercise in the heat to reduce thermoregulatory strain and improve exercise performance.(121) These cooling strategies may therefore, play a role in attenuating components of exercise-induced gastrointestinal syndrome that are exacerbated by thermoregulatory strain. However, to date no research has been conducted to explore the effects of cooling prior to or during exercise on gastrointestinal outcomes. Considering the prolonged duration of exertional-heat stress and the practical and logistical considerations associated with various cooling methods that are discussed within this chapter the experimental study aimed to explore the effectiveness of repeated ingestion of cold fluids within a range of palatable temperatures on intestinal injury, gastrointestinal symptoms and systemic cytokine responses.

7.2 Abstract

Objectives: The study aimed to determine the effects of temperature of ingested water during exertional-heat stress on gastrointestinal injury, symptoms and systemic inflammatory responses.

Design: Randomised cross-over study.

Method: Twelve endurance runners completed 2 h running at 60% $\dot{V}O_{2max}$ in 35°C ambient temperature on three separate occasions, consuming 250 ± 40 mL water at either 0.4 ± 0.4°C (COLD), 7.3 ± 0.8°C (COOL), or 22.1 ± 1.2°C (TEMP) before and every 15 min during running. Rectal
[132]

temperature and gastrointestinal symptoms were recorded every 10 min during exercise. Blood was collected pre, immediately and 1 h post-exercise to determine plasma intestinal fatty-acid binding protein (I-FABP), cortisol, and inflammatory cytokine concentrations.

Results: COLD and COOL blunted the rise in rectal temperature compared to TEMP ($1.6 \pm 0.4^{\circ}\text{C}$ and $1.7 \pm 0.4^{\circ}\text{C}$ vs. $2.0 \pm 0.5^{\circ}\text{C}$, respectively; trial x time, $p= 0.033$). I-FABP increased post-exercise (419%, $p< 0.001$), with a trend for reduced I-FABP with COLD and COOL (mean reduction 460 $\text{pg}\cdot\text{mL}^{-1}$ and 430 $\text{pg}\cdot\text{mL}^{-1}$, respectively), compared to TEMP ($p= 0.066$). No differences were observed among trials for gastrointestinal symptoms, but a trend for increased upper-gastrointestinal symptoms with TEMP ($p= 0.087$) compared to COLD and COOL was observed. IL-6, IL-1 β , IL-8, IL-10 and IL-1ra increased post-exercise ($p< 0.05$); however no differences were observed among trials.

Conclusions: COLD and COOL water ingestion during exertional-heat stress ameliorates thermoregulatory strain compared to TEMP. However, this appears to have no effect on cytokine profile and minimal effect on intestinal epithelial injury and gastrointestinal symptoms.

7.3 Introduction

Increased thermoregulatory strain during exertional-heat stress exacerbates splanchnic hypoperfusion, potentially compromising intestinal integrity (i.e., increased epithelial injury and permeability), which may lead to endotoxaemia, cytokinaemia, and gastrointestinal symptoms.(1, 2, 186) Elevations in core body temperature appear to account for 63% of the variance in intestinal permeability in response to different exertional loads(29) suggesting increased core body temperature is a major contributor to exercise-induced gastrointestinal syndrome.(2) We recently demonstrated (**Chapter 4**) that increased rectal temperature (T_{re}) in response to 2 h running at 60% maximal oxygen uptake ($\dot{V}O_{2max}$) in 35°C ambient temperature (T_{amb}) was associated with greater intestinal epithelial injury, plasma cortisol concentration, systemic inflammatory responses, and gastrointestinal

symptoms compared with running in temperate conditions ($22^{\circ}\text{C T}_{\text{amb}}$).⁽¹⁷³⁾ Field-based research supports these findings, consistently showing endotoxaemia, cytokinaemia, and gastrointestinal symptoms during prolonged exercise with heat exposure.^(22, 23, 70) The implications of these gastrointestinal perturbations can range from reduced nutrient intake, impaired exercise performance and sub-optimal post-exercise recovery processes, through to impaired nutrient absorption.^(23, 24, 38) Furthermore, such gastrointestinal perturbations during prolonged strenuous exercise may lead to acute health conditions, such as fatal septic shock, ischaemic bowel disease, gastritis and gastrointestinal paralysis, although such severe consequences have been infrequently reported.⁽²⁾

Ingestion of cold fluids or ice (e.g., ice slurry) before (i.e., pre-cooling) and (or) during (i.e., per-cooling) exertional-heat stress is a practical strategy shown to reduce core body temperature, and improve perceived exertion, thermal comfort, and endurance exercise performance.^(121, 187, 188) It is therefore plausible that these cooling strategies may ameliorate gastrointestinal perturbations associated with increased core body temperature during exertional-heat stress. However, this novel concept remains to be investigated. Pre-cooling, with ingestion of large volumes (0.5-1.0 L) of cold fluids at rest has been shown to lower core body temperature for up to 30 min during short duration (≤ 1 h) exercise in the heat.^(126, 189) Additionally, cold water immersion as a pre-cooling strategy before exercise in the heat has been shown to reduce core body temperature for 30 min and subsequently attenuate increases in systemic IL-6 and IL-10.⁽¹³⁰⁾ However, such a short reduction in thermoregulatory strain is unlikely to be of benefit during prolonged (≥ 2 h) exertional-heat stress where the greatest gastrointestinal perturbations, symptoms and systemic cytokinaemia have previously been reported.^(2, 82, 186) Therefore, a per-cooling strategy may be required to alleviate such prolonged thermoregulatory strain and subsequent development of exercise-associated gastrointestinal perturbations and symptoms.

Ingestion of ice (e.g. $\leq 0^{\circ}\text{C}$) at rest prior to exercise is more effective than the same volume of cold fluid (e.g. $4\text{-}10^{\circ}\text{C}$) at reducing core body temperature.(121, 125) However, the benefits of ice ingestion during exercise (i.e., as a per-cooling strategy) appear to be limited due to issues tolerating large volumes, which may ultimately compromise hydration status and lead to further gastrointestinal perturbations.(2, 127, 128) Limitations in tolerance to ice during exertion and the practicalities of obtaining ice in the field setting, suggest cold fluids may be a more suitable per-cooling method. Whereas ice may be difficult to ingest in large volumes, fluids ranging from 0°C to 22°C have been shown to increase palatability, consumption, and hydration status during exercise.(127) Considering dehydration can exacerbate intestinal permeability and gastrointestinal symptoms(2), an appropriate cooling strategy should aim to find a balance between palatability, practicality, hydration status, and cooling in order to effectively ameliorate exertional-heat stress associated gastrointestinal perturbations and symptoms.

The current study, therefore, aimed to determine the effects of different water temperatures, within the optimised palatability and hydration range of 0°C to 22°C , consumed before and frequently during prolonged exertional-heat stress on intestinal epithelial injury, gastrointestinal symptoms, and systemic inflammatory cytokine profile. It was hypothesised that cold (0°C) and cool (7°C) water ingestion before and during exertional-heat stress would reduce thermoregulatory strain, intestinal epithelial injury, gastrointestinal symptoms, and result in lower perturbations to systemic inflammatory responses compared to temperate (22°C) water consumption.

7.4 Methods

Twelve (male $n= 6$, female $n= 6$) non-heat acclimatised endurance-trained runners [mean \pm SD: age 37 ± 8 years, nude body mass 66.7 ± 10.7 kg, height 1.74 ± 0.10 m, % body fat mass $19 \pm 6\%$, $\dot{V}O_{2\max}$ 56 ± 6 mL \cdot kg \cdot min $^{-1}$] volunteered to participate in the study. All participants gave written informed consent. The study protocol received approval from the local ethics committee and conformed to the 2008 Helsinki Declaration for Human Research Ethics.

One week before the first experimental trial, height and nude body mass (Seca 515 MBCA, Seca Group, Hamburg, Germany) were recorded. $\dot{V}O_{2\max}$ (Vmax Encore Metabolic Cart, Carefusion, San Diego, California, US) was estimated by means of a continuous incremental exercise test to volitional exhaustion on a motorized treadmill (Forma Run 500, Technogym, Seattle, Washington, US).(163) To determine running speed for the exercise trials, the speed at 60% $\dot{V}O_{2\max}$ and 1% gradient was extrapolated from the $\dot{V}O_2$ -work rate relationship and then verified (9.4 ± 1.2 km \cdot h $^{-1}$).

Participants were provided with a diet low in fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) for the 24 h period before each experimental trial (9.9 ± 0.7 MJ, 420 ± 66 g carbohydrate, 106 ± 6 g protein, 54 ± 3 g fat) to reduce potential gastrointestinal symptoms arising from pre-exercise food and fluid intake.(101) Participants were asked to refrain from consuming high FODMAP foods, alcohol, and caffeinated beverages during the diet controlled period, and strenuous exercise during the 48 h period before each experimental trial. Compliance was determined by a dietary and exercise log. Participants reported to the laboratory at 0800h after consuming the standardised low FODMAP breakfast (2.5 MJ, 125 g carbohydrate, 14 g protein, and 4 g fat) with 400 mL of water (consumed at 0700h). Participants were asked to void before nude body mass measurements and completion of a self-reported gastrointestinal symptom assessment tool.(25) Blood was then collected by venepuncture from an antecubital vein into a vacutainer (6 mL, 1.5 IU \cdot mL $^{-1}$

heparin). To monitor T_{re} during running, participants inserted a thermocouple 12 cm beyond the external anal sphincter (Grant REC soft insertion probe thermocouple; Grant 2010 Squirrel data logger, Shepreth, UK).

In a randomised counterbalanced order, participants completed three experimental trials separated by one-week, consisting of 2 h (initiated at 0900h) running exercise on a motorised treadmill at the previously determined speed that elicited 60% $\dot{V}O_{2max}$ within an environmental chamber set at $35.1 \pm 0.5^{\circ}C$ and $25 \pm 3\%$ relative humidity. Participants were provided with a total of $30 \text{ mL}\cdot\text{kg}^{-1}$ body mass water, at either $0.4 \pm 0.4^{\circ}C$ (COLD), or $7.3 \pm 0.8^{\circ}C$ (COOL) or $22.1 \pm 1.2^{\circ}C$ (TEMP), provided in equal amounts ($250 \pm 40 \text{ mL}$) immediately before and every 15 min during exercise. COLD and COOL water was chilled using a domestic portable fridge-freezer (MT45FP, Engle, NZ) and water temperature was measured using a portable thermometer (Acurite, Lake Geneva, WI, USA) immediately prior to participant consumption. All water was provided in a thermal insulated water bottle (CamelBak, Petaluma CA, USA) for consumption within a 3 min period. Heart rate, rating of perceived exertion (RPE)(141), thermal comfort rating(142), T_{re} , and gastrointestinal symptoms were measured and recorded every 10 min during exercise. Immediately after exercise, blood was collected and nude body mass was recorded. Participants remained seated during the recovery period and consumed water at room temperature ($22^{\circ}C$) *ad libitum*, until a final blood sample was collected 1 h post-exercise. To reduce any seasonal heat acclimatisation, the experimental procedures were conducted over the cooler seasonal periods (T_{amb} consistently $\leq 20^{\circ}C$).

Whole blood haemoglobin and haematocrit values were used to estimate changes in plasma volume relative to baseline, and used to correct plasma variables(166). Blood glucose concentration was determined pre- and post-exercise using a portable glucose monitor (Accu-Chek Proforma, Roche Diagnostics, Basel, Switzerland). The remaining blood samples were centrifuged at 4000 rpm (1500 g) for 10 min within 15 min of sample collection. Plasma was aliquoted into eppendorfs and frozen

at -80°C until analysis, except for 50 µL that were used to determine plasma osmolality (coefficient of variation (CV): 1.6%) by freeze-point osmometry (Osmomat 030, Gonotec, Berlin, Germany). Plasma concentrations of interleukin (IL)-6, IL-1β, tumour necrosis factor (TNF)-α, IL-8, IL-10, and IL-1 receptor antagonist (ra) were determined by multiplex enzyme-linked immunosorbent assay (ELISA; HCYTOMAG-60K, EMD Millipore, Darmstadt, Germany). Plasma concentrations of I-FABP (HK406, Hycult Biotech, Uden, Netherlands) and cortisol (RE52061, IBL International, Hamburg, Germany) were determined by ELISA. All variables were analysed in duplicate as per manufacturer's instructions on the same day, with standards and controls on each plate, and each participant assayed on the same plate. The CVs for I-FABP, IL-10, IL-1ra, IL-1β, IL-6, IL-8, TNF-α and cortisol were 3.1%, 6.9%, 7.7%, 11.9%, 7.5%, 4.7%, 8.9% and 3.3%, respectively.

Statistical analysis

Data in the text and tables are presented as mean and 95% confidence interval (CI), and accumulative score and individual participant range for gastrointestinal symptoms. For clarity, data in figures are presented as mean ± standard error of the mean (SEM). Standardised data management, pre-analysis diagnostic tests, and statistical analysis was performed as previously reported(25). In short, a two-way repeated measures ANOVA with post hoc Tukey's HSD test was used to analyse variables, except gastrointestinal symptoms which were examined using Friedman's test. Statistics were analysed using SPSS statistical software (V.23.0, Chicago, Illinois, USA) with significance accepted at $p \leq 0.05$.

7.5 Results

No differences were observed between trials for total water intake during exercise (COLD: 1.8 (1.6-2.1) L; COOL (1.8 (1.6-2.1) L; and TEMP (1.8 (1.5-2.1) L) and exercise-induced loss of body mass (COLD: 0.9 (0.6-1.4)%; COOL (1.1 (0.7-1.5)%; and TEMP (1.1 (0.6-1.6)%). A main effect of time was observed for plasma osmolality ($p= 0.028$), which was lower post-exercise (291 (290-293) mOsmol·kg⁻¹) compared to pre-exercise (295 (293-297) mOsmol·kg⁻¹), with no difference between trials observed.

A trial x time interaction was observed for T_{re} ($p= 0.033$), which was lower with COLD from 40 min and COOL from 50 min exercise onwards compared to TEMP (**Figure 7.1**). The exercise-induced rise in T_{re} was lower with COLD (1.6 (1.4-1.9)°C) and COOL (1.7 (1.5-1.9)°C compared to TEMP (2.0 (1.7-2.3)°C, $p= 0.017$). Main effects of time ($p < 0.001$) and trial ($p= 0.006$) were observed for heart rate (**Figure 7.1**), which was significantly elevated from 30 min exercise onwards, compared to 10 min ($p < 0.01$), and higher with TEMP (160 (148-171) beats per minute (bpm)) compared to COLD (155 (144-165) bpm) and COOL (153 (142-165) bpm). Main effects of time were observed for RPE ($p < 0.001$) and thermal comfort rating ($p < 0.001$), which were significantly elevated from 40 min and 50 min exercise onwards, respectively, compared to 10 min exercise (**Figure 7.1**).

Main effects of time ($p < 0.001$) and trial ($p= 0.029$) were observed for plasma cortisol concentration, which was elevated immediately (524 (361-688) nmol·mL⁻¹) and 1 h post-exercise (518 (357-678) nmol·mL⁻¹) compared to pre-exercise (254 (211-297) nmol·mL⁻¹, $p < 0.01$), and lower with COLD (354 (240-468) nmol·mL⁻¹) compared to TEMP (507 (344-670) nmol·mL⁻¹, $p < 0.05$), but not different to COOL (435 (334-536) nmol·mL⁻¹). A main effect of time was observed for blood glucose concentration, which was higher post-exercise (5.3 (4.8-5.6) mmol·L⁻¹), compared to pre-exercise (4.8 (4.5-5.1) mmol·L⁻¹, $p= 0.035$), with no difference between trials.

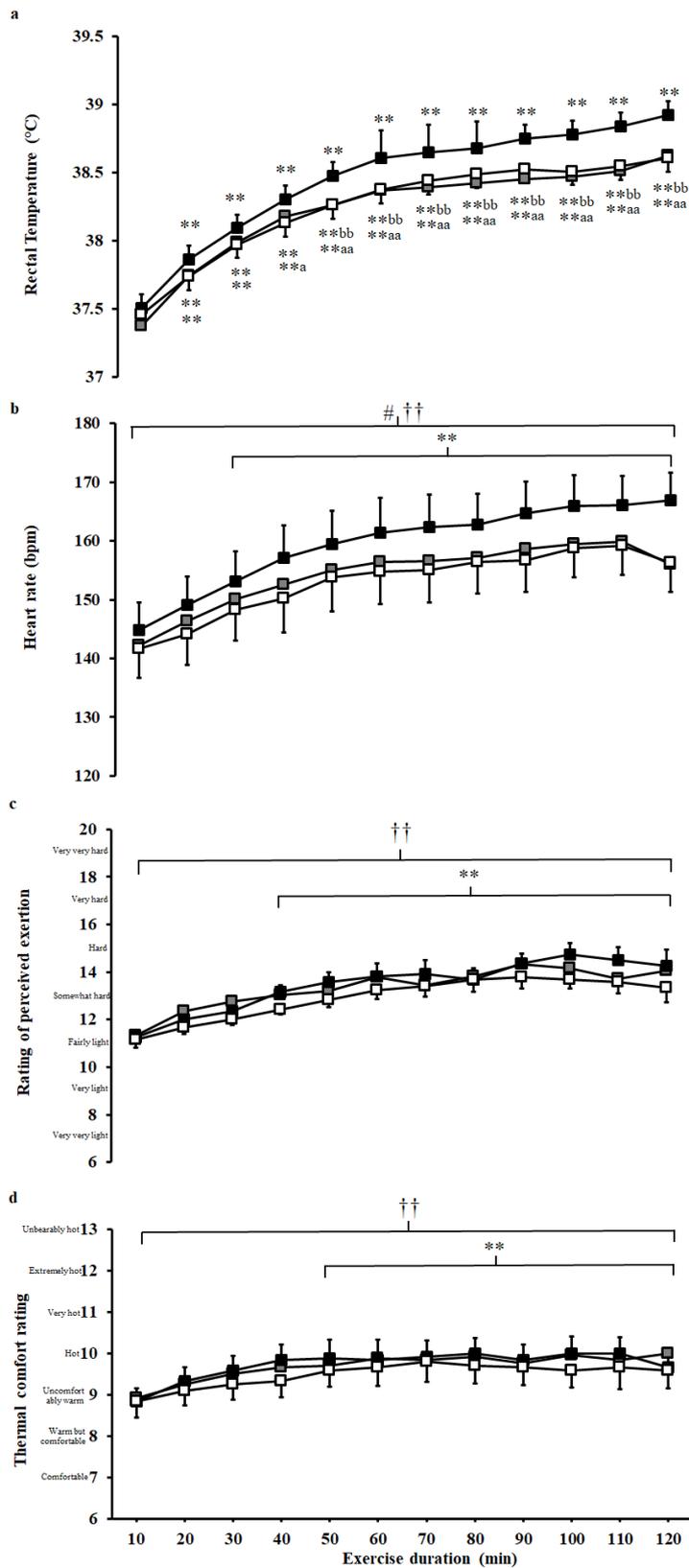


Figure 7.1 Rectal temperature (a), heart rate (b), rating of perceived exertion (c), and thermal comfort rating (d) responses during 2 h running at 60% $\dot{V}O_{2max}$ in 35°C with water ingestion at 0°C (COLD: white square), 7°C (COOL: grey square) and 22°C (TEMP: black square). Mean \pm SEM (n= 12): †† main effect of time $p < 0.01$, # main effect of trial $p < 0.05$, ** $p < 0.01$ vs. 10 min exercise, ^{aa} $p < 0.01$ and ^a $p < 0.05$ COLD vs. TEMP, ^{bb} $p < 0.01$ COOL vs. TEMP.

A main effect of time was observed for plasma I-FABP concentration ($p < 0.001$), which was significantly elevated immediately and 1 h post-exercise, compared with pre-exercise (518%, $p < 0.01$ and 299%, $p < 0.05$, respectively) (**Figure 7.2**). No interaction effect was observed for plasma I-FABP concentration however, a trend for a main effect of trial was observed ($p = 0.066$), whereby there was a tendency for lower I-FABP concentration with COLD and COOL, compared to TEMP (**Figure 7.2**). No differences in the incidence and severity of gastrointestinal symptoms were observed between trials (all $p > 0.05$). However, trends were observed for increased upper-gastrointestinal symptoms ($p = 0.087$) and urge to regurgitate ($p = 0.061$) with TEMP compared to COLD and COOL (**Table 7.1**).

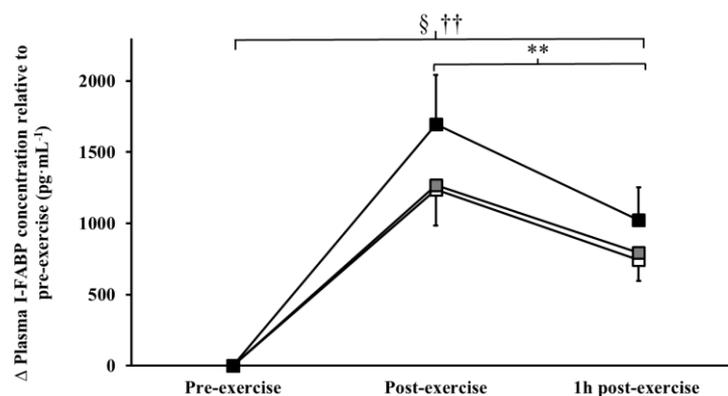


Figure 7.2. Change in plasma intestinal fatty acid binding protein (I-FABP) in response to 2 h running at 60% $\dot{V}O_{2max}$ in 35°C with water ingestion at 0°C (COLD: white square), 7°C (COOL: grey square) and 22°C (TEMP: black square). Mean \pm SEM (n= 12): †† main effect of time $p < 0.01$, § main effect of trial $p = 0.066$, ** $p < 0.01$ vs. pre-exercise.

Table 7.1. Incidence and severity of gut discomfort, total, upper-, and lower-gastrointestinal symptoms in response to 2 h running at 60% $\dot{V}O_{2max}$ in 35°C T_{amb} with ingestion of cold (COLD: 0°C), cool (COOL: 7°C) and temperate water (TEMP: 22°C).

	COLD		COOL		TEMP		Friedman <i>p</i> -value
	Incidence	Total (range)	Incidence	Total (range)	Incidence	Total (range)	
Gut Discomfort	NA	157 (0-32)	NA	187 (0-51)	NA	283 (0-48)	0.159
Total gastrointestinal symptoms	75%	238 (0-49)	92%	320 (0-97)	92%	398 (0-78)	0.138
Upper-gastrointestinal symptoms	67%	129 (0-38)	75%	119 (0-30)	83%	235 (0-63)	0.087
Belching	50%	66 (0-30)	58%	72 (0-21)	75%	118 (0-41)	0.157
Heartburn	8%	14 (0-14)	16%	7 (0-4)	25%	20 (0-15)	0.150
Bloating	16%	13 (0-9)	25%	22 (0-10)	16%	28 (0-22)	0.607
Upper abdominal pain	25%	26 (0-15)	25%	15 (0-6)	16%	23 (0-16)	0.607
Urge to regurgitate	0%	0 (0-0)	8%	3 (0-3)	25%	26 (0-10)	0.061
Regurgitation	8%	10 (0-10)	0%	0 (0-0)	16%	20 (0-10)	0.368
Lower-gastrointestinal symptoms	33%	68 (0-27)	58%	134 (0-36)	58%	98 (0-46)	0.307
Flatulence	25%	32 (0-16)	50%	24 (0-12)	42%	45 (0-20)	0.619
Urge to defecate	8%	3 (0-3)	25%	37 (0-22)	50%	57 (0-26)	0.119
Abdominal pain/s	16%	33 (0-24)	42%	77 (0-39)	25%	57 (0-30)	1.000
Abnormal defecation ¹	0%	0 (0-0)	0%	0 (0-0)	8%	10 (0-10)	0.368
Others							
Nausea	16%	30 (0-15)	25%	50 (0-38)	25%	35 (0-19)	0.807
Dizziness	8%	11 (0-11)	16%	17 (0-9)	25%	30 (0-15)	0.584
Abdominal stitch	8%	3 (0-3)	16%	8 (0-4)	16%	4 (0-3)	0.584
Appetite	NA	420 (0-104)	NA	414 (0-97)	NA	383 (0-85)	0.274
Thirst	NA	778 (0-109)	NA	905 (26-106)	NA	849 (22-102)	0.274
Drink tolerance	NA	1129 (51-128)	NA	1243 (60-127)	NA	1140 (50-121)	0.083

Overall participant summative accumulation of rating scale point score of measured time periods and individual participant range (n=12). ¹ abnormal defecation including loose watery stools, diarrhoea and blood in stools. NA: not applicable.

A main effect of time was observed for plasma IL-6 ($p= 0.029$, peaking post-exercise), IL-1 β ($p= 0.010$, peaking post-exercise), IL-8 ($p= 0.035$, peaking post-exercise), IL-10 ($p< 0.001$, peaking 1 h post-exercise) and IL-1ra ($p= 0.025$, peaking 1 h post-exercise) concentrations, with a trend for a main effect of time for plasma TNF- α ($p= 0.073$, peaking post-exercise) (**Figure 7.3**). However, no interaction effect or differences in measured cytokines were observed among trials.

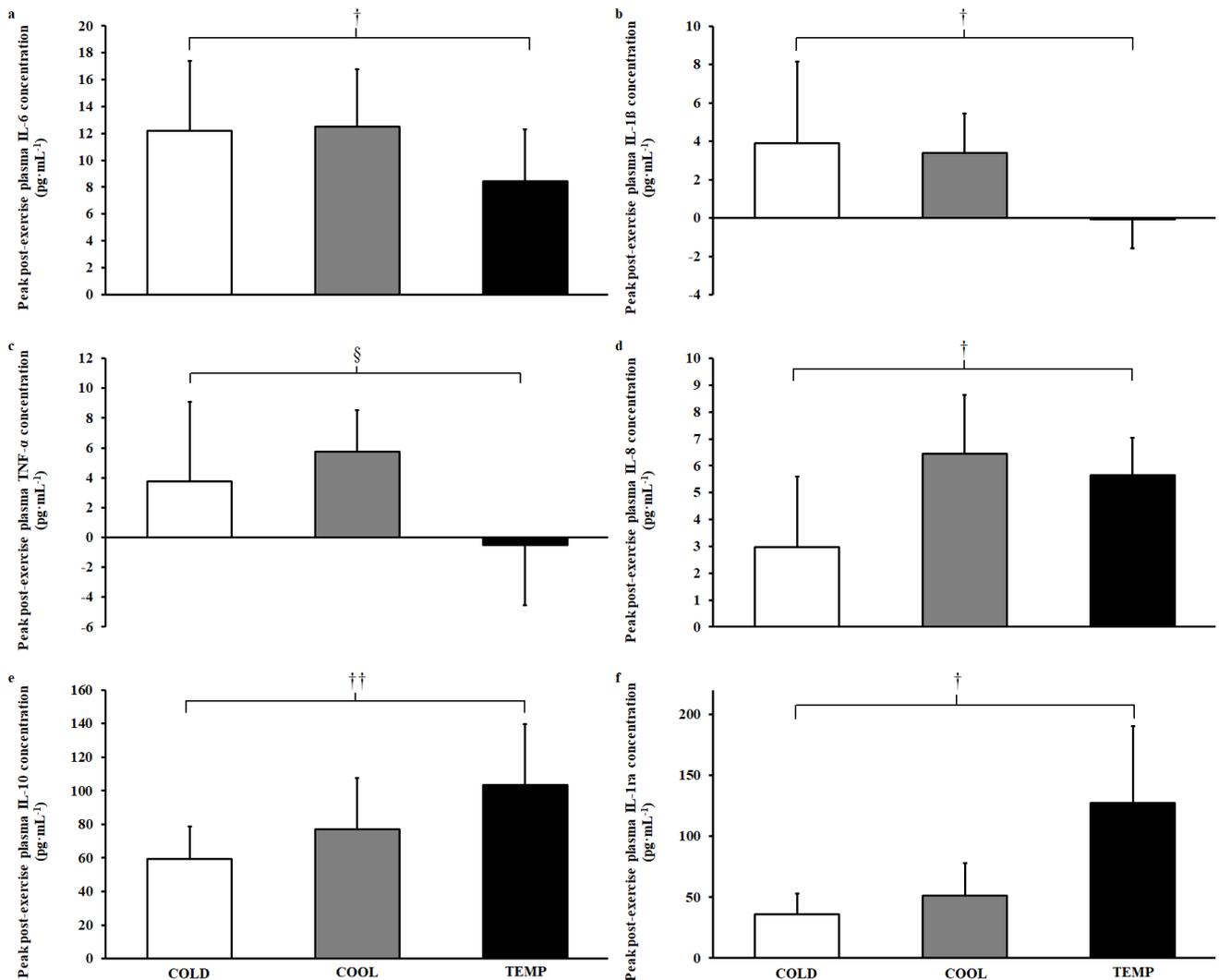


Figure 7.3: Peak post-exercise plasma IL-6 (a), IL-1 β (b), TNF- α (c), IL-8 (d), IL-10 (e), and IL-1ra (f) concentrations during recovery from 2 h running exercise at 60% $\dot{V}O_{2max}$ in 35°C with water ingestion at 0°C (COLD: white), 7°C (COOL: grey) and 22°C (TEMP: black). Mean \pm SEM (n= 11): †† main effect of time $p< 0.01$, † main effect of time $p< 0.05$ and § main effect of time $p= 0.073$.

7.6 Discussion

To our knowledge, this is the first study to investigate the effects of different water temperatures consumed before and during exertional-heat stress on gastrointestinal injury, symptoms, and systemic inflammatory cytokine profile. Despite a diminished thermoregulatory strain with COLD and COOL, no differences in systemic inflammatory responses, and only trends for modest reductions in intestinal epithelial injury and upper-gastrointestinal symptoms, were observed with COLD and COOL compared to TEMP. These findings suggest that ingestion of moderate amounts of water at 0°C to 7°C during exertional-heat stress does not substantially ameliorate gastrointestinal perturbations and systemic inflammatory responses; possibly due to insufficient reductions in thermoregulatory strain and (or) other contributing factors (e.g., splanchnic hypoperfusion).(2)

The investigation of cold fluids as a strategy for ameliorating gastrointestinal perturbations associated with thermoregulatory strain is a novel area of research. In the current study, a trend for reduced intestinal epithelial injury with COLD and COOL (I-FABP reduction of 460 pg·mL⁻¹ and 430 pg·mL⁻¹, respectively) was observed compared to TEMP, suggesting low water temperatures may provide a small benefit to the maintenance of gastrointestinal integrity during exertional-heat stress. Large individual variations in T_{re} and post-exercise plasma I-FABP highlights that COLD and COOL water ingestion may only be beneficial in a proportion of athletes, and (or) greater attenuation of T_{re} rise, through more varied and aggressive cooling strategies, may be required to elicit a substantial amelioration in exercise-associated intestinal injury. For example, differences in factors that can affect thermoregulation (e.g., sex, fitness, body fat mass, metabolic heat production and (or) heat loss mechanisms) may have contributed to the individual variation in both thermoregulatory and I-FABP responses observed. Indeed, previous studies investigating per-cooling methods have shown mixed results in the ability to lower core body temperature during exercise.(121, 125) Moreover, studies failing to observe a difference in core body temperature have been of short duration (i.e., ≤ 60 min)

and (or) conducted in milder ambient conditions (i.e., $\leq 30^{\circ}\text{C}$) than the current study.(121) From a practical perspective, similar T_{re} , heart rate, and I-FABP responses were observed with COLD and COOL, suggesting that water consumption at 7°C during exertional-heat stress may be as effective in reducing thermoregulatory strain and intestinal epithelial injury as water at 0°C . This outcome is of translational significance, since the overall logistical provision of COOL water is more practical and achievable than the provision of COLD water to individuals and large groups in the field setting. Further, provision of cool carbohydrates (i.e., sports drink) may be a practical strategy that can be used to blunt thermoregulatory strain and provide greater attenuation of intestinal epithelial injury via maintenance of splanchnic perfusion.(186) Moreover, considering injury to the intestinal epithelium impairs nutrient absorption(38), ingestion of cold fluids combined with carbohydrates during exertional-heat stress, may limit this impairment which has implications for exogenous fuel provisions for skeletal muscle and subsequent exercise performance(83).

To the author's knowledge, the current study is the first to investigate the effects of water temperature ingested during exertional-heat stress on systemic cytokine responses. Increases in circulating pro- and anti-inflammatory cytokines are commonly observed after exertional-heat stress, and have been linked to exercise-associated endotoxaemia in response to compromised intestinal epithelial integrity.(22, 70) Our findings indicate that repeated water ingestion at 0°C and 7°C during prolonged exertional-heat stress does not ameliorate cytokinaemia, despite a smaller rise in T_{re} , compared with 22°C . It is possible that COLD and COOL did not attenuate the rise in T_{re} sufficiently to influence systemic inflammatory responses. For example, clamping of core body temperature during exercise has been shown to attenuate pre- to post-exercise increases in IL-6, IL-12, TNF- α and IL-1ra, with significant correlations observed between increases in core body temperature and cytokines.(80) Previous pre-cooling studies have shown mixed results in the ability to attenuate exertional-heat stress associated systemic cytokine responses. Whole body pre-cooling (-0.4°C core body temperature) with cold water immersion (20.3°C) before 90 min running at $65\% \dot{V}O_{2\max}$ in 32°C showed a modest

attenuation of IL-6 and IL-10 (mean decrease 2.1 pg·mL⁻¹ and 3.8 pg·mL⁻¹, respectively) compared with exercise without pre-cooling, with no differences in TNF- α or IL-1ra observed.(130) Pre-cooling via ice-vest and cold towels before 30 min intermittent sprint exercise in the heat showed no effect on IL-6 compared to no cooling.(131) However, the additional workload performed with pre-cooling may have confounded results.(131) Together these findings suggest that cooling strategies may have limited effects on systemic cytokine profile, particularly when improvements in exercise performance are observed.

Gastrointestinal symptoms consistently affect $\geq 60\%$ endurance runners and have been shown to occur more frequently during prolonged exercise in the heat.(15, 23, 70) While the underlying cause of these symptoms is multifactorial, the additional thermoregulatory strain imposed by heat exposure may exacerbate splanchnic hypoperfusion and sympathetic drive, which are believed to be primary contributors to the development of gastrointestinal symptoms.(2) We observed no significant differences in gastrointestinal symptoms between trials, thereby suggesting that COLD and COOL may not have sufficiently attenuated the thermoregulatory strain and (or) stress response to the extent required to reduce gastrointestinal symptoms. Trends for a reduction in urge to regurgitate and the incidence and severity of upper-gastrointestinal symptoms, which are most common during endurance exercise in the heat, suggest that colder fluids may be beneficial for some athletes during exertional-heat stress. Moreover, these benefits were likely mediated by reductions in core body temperature and stress hormone responses (i.e., cortisol) which have previously been linked to gastrointestinal motility (e.g., gastric emptying).(88) Considering these findings, future research should aim to investigate the effects of more aggressive cooling strategies on the prevention or attenuation of gastrointestinal symptoms during prolonged exertional-heat stress.

7.7 Conclusion

COLD and COOL water ingestion before and during exertional-heat stress attenuates thermoregulatory strain compared to TEMP, which appears to have small non-significant reductions in intestinal epithelial injury, and incidence and severity of upper-gastrointestinal symptoms. However, neither COLD or COOL appears to have any effect on systemic inflammatory profile. Future research is warranted to establish if more aggressive cooling regimes (i.e., combined internal and external cooling) can prevent gastrointestinal perturbations and the associated systemic responses induced by prolonged exertional-heat stress.

Practical implications

- Frequent water consumption at 0°C and 7°C during prolonged exertional-heat stress can blunt the thermoregulatory strain to a similar extent, compared to water at 22°C.
- Ingestion of cold water at 0°C to 7°C every 15 min during prolonged running in the heat has the potential to modestly reduce intestinal injury and upper-gastrointestinal symptoms in some athletes, which may have implications for nutrient uptake, exercise performance, and recovery nutrition.
- Despite attenuating thermoregulatory strain, frequent ingestion of cold water at 0°C and 7°C during prolonged running in the heat has no effect on systemic inflammatory cytokine.
- More aggressive cooling strategies (i.e., internal and external cooling) during prolonged running in the heat may be required to further diminish thermoregulatory strain and the associated gastrointestinal perturbations, symptoms, and systemic responses.

7.8 Chapter conclusion

In summary, findings from this study highlight the large individual variability in thermoregulatory responses, and subsequently gastrointestinal response to cold fluids, despite providing fluids in proportion to body mass. Trends for a modest reduction in intestinal epithelial injury and upper-gastrointestinal symptoms suggest cooling strategies may be useful if larger reductions in thermoregulatory strain can be obtained and/or in individuals who respond well to cooling strategies. The practical and logistical considerations of implementing more aggressive cooling strategies during endurance running events may limit the use of these strategies in the prevention of exercise-induced gastrointestinal syndrome. However, it is possible that combining frequent carbohydrate intake (**Chapter 6**) with a cooling strategy may be more beneficial than implementing a cooling strategy in isolation.

CHAPTER EIGHT

General discussion

8.1 Background

Regular physical activity or exercise of a low to moderate intensity and modest duration (e.g. <1 h) is widely acknowledged as being beneficial for health and prevention of chronic disease.(4) In contrast, moderate to high intensity exercise of a prolonged nature (e.g. ≥ 1 h) may damage the gastrointestinal tract, with extreme exercise (i.e., ultramarathon running) and/or insufficient recovery between exercise bouts potentially predisposing the active individual to a range of acute and chronic health implications and/or symptomatic manifestations.(2) Indeed, perturbations to gastrointestinal integrity likely arise due to repetitive and/or substantial acute exercise stress and the associated pro-inflammatory systemic responses.(2, 70, 71) The term “exercise-induced gastrointestinal syndrome” has been developed to describe changes to circulatory and endocrine systems during prolonged moderate to high intensity exercise, resulting in a cascade of perturbations to gastrointestinal integrity and function that may lead to gastrointestinal symptoms and adverse health outcomes (**Figure 2.1**).(2) Such gastrointestinal disturbances appear to occur most frequently during prolonged (i.e., ≥ 2 h) running with $\geq 60\%$ of athletes consistently reporting gastrointestinal symptoms which can impair nutrient intake, performance/workload, post-exercise recovery processes and result in withdrawal from competition.(22-24) In the absence of a reduction in intensity or workload, prolonged physical exertion in the heat challenges thermoregulation, which may exacerbate the primary underlying mechanisms of exercise-induced gastrointestinal syndrome and increase the risk of active individuals experiencing symptomatic manifestations, and in rare cases, more severe health complications (i.e., ischaemic colitis).(6, 14, 23, 24, 32) The purpose of this thesis was therefore to investigate the effects of exertional-heat stress on gastrointestinal integrity, symptoms and associated systemic responses [149]

and subsequently determine if acute nutrition strategies, including frequent carbohydrate, protein and cold water ingestion, could attenuate the gastrointestinal perturbations induced by exertional-heat stress.

The major findings from this thesis were that: 1) Prolonged submaximal running in 35°C increased intestinal epithelial injury, gastrointestinal symptoms, reduced endotoxin clearance capacity and increased systemic cytokine responses when compared to running in temperate (22°C) ambient conditions, however heat exposure during exertion did not increase small intestine permeability or intestinal inflammation. 2) Prolonged submaximal running in 30°C increased intestinal epithelial injury, upper-gastrointestinal symptoms and systemic cytokine responses compared to running in temperate conditions, but mild heat stress did not increase small intestine permeability or systemic endotoxin profile. 3) The exercise-induced increase in rectal temperature was positively associated with injury to the intestinal epithelium, gastrointestinal symptoms of nausea and urge to regurgitate, the compensatory anti-inflammatory cytokine response (i.e., IL-10) and stress hormone response (i.e., cortisol), suggesting thermoregulatory strain may be a contributor to these gastrointestinal and systemic perturbations. 4) The extent and duration of perturbations to gastrointestinal integrity, symptoms and systemic responses appear to increase with the magnitude of thermoregulatory strain during prolonged running in the heat. 5) Compared with water intake, frequent ingestion of a glucose-based carbohydrate and hydrolysed-whey protein solution during prolonged exertional-heat stress abolished intestinal epithelial injury and attenuated small intestine permeability. Only carbohydrate influenced the systemic and stress response (i.e., reduces IL-6 and cortisol and increases anti-endotoxin antibodies) compared to water, whereas protein increased the occurrence and severity of gastrointestinal symptoms compared to both water and carbohydrate, thereby suggesting carbohydrate ingestion may be a more appropriate strategy than protein. 6) Frequent ingestion of cold or cool water at 0°C and 7°C during prolonged running in the heat modestly attenuated thermoregulatory strain, intestinal epithelial injury and upper-gastrointestinal symptoms compared to

water at 22°C. However, the modest reduction in thermoregulatory strain and large individual variation, limited the ability to detect a statistical difference in gastrointestinal responses to different water temperatures. Additionally, ingestion of colder water temperatures had no effect on systemic cytokine responses.

These major findings in relation to the influence of exertional-heat stress and prevention and management strategies on exercise induced gastrointestinal syndrome are shown in **Figure 8.1** and **8.2**, respectively. In comparison to prolonged running in temperate ambient conditions, exertional-heat stress (35°C T_{amb}) increases thermoregulatory strain, stress hormones, sympathetic drive and exacerbates splanchnic hypoperfusion that subsequently injures the intestinal epithelium, perturbs systemic endotoxin and cytokine profiles and increases gastrointestinal symptoms (**Figure 8.1**). Running for 2 h at 60% $\dot{V}O_{2max}$ in 35°C does not increase intestinal permeability above equivalent running in 22°C. Moreover, it is likely that gastrointestinal motility and transit are influenced by exertional-heat stress(88) however, these outcomes were not the focus of this thesis. Frequent carbohydrate and protein intake during exertional-heat stress abolished injury to the intestinal epithelium and reduced small intestine permeability, likely due to attenuation of splanchnic hypoperfusion (**Figure 8.2**). Cold fluids may attenuate injury to the intestinal epithelium and upper-gastrointestinal symptoms via attenuation of thermoregulatory strain, and stress hormones for water at 0°C only. Frequent carbohydrate intake during exertional-heat stress can reduce stress hormones, attenuate IL-6 and support endotoxin clearance, likely due to increased blood glucose levels that support carbohydrate oxidation during prolonged exercise in the heat. Large protein intakes should be avoided due to increased gastrointestinal symptoms which likely arise due to delayed gastric emptying and/or altered motility.

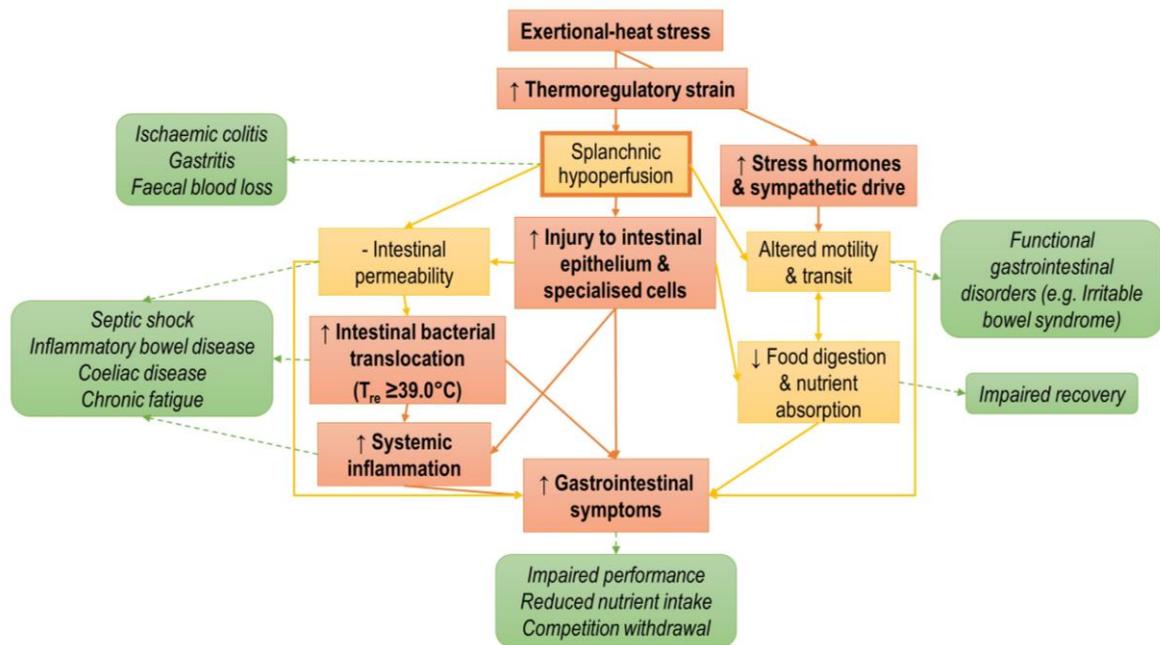


Figure 8.1: The exacerbation of exercise-induced gastrointestinal syndrome by prolonged exertional-heat stress (2 h running at 60% $\dot{V}O_{2max}$ in 35°C T_{amb}).

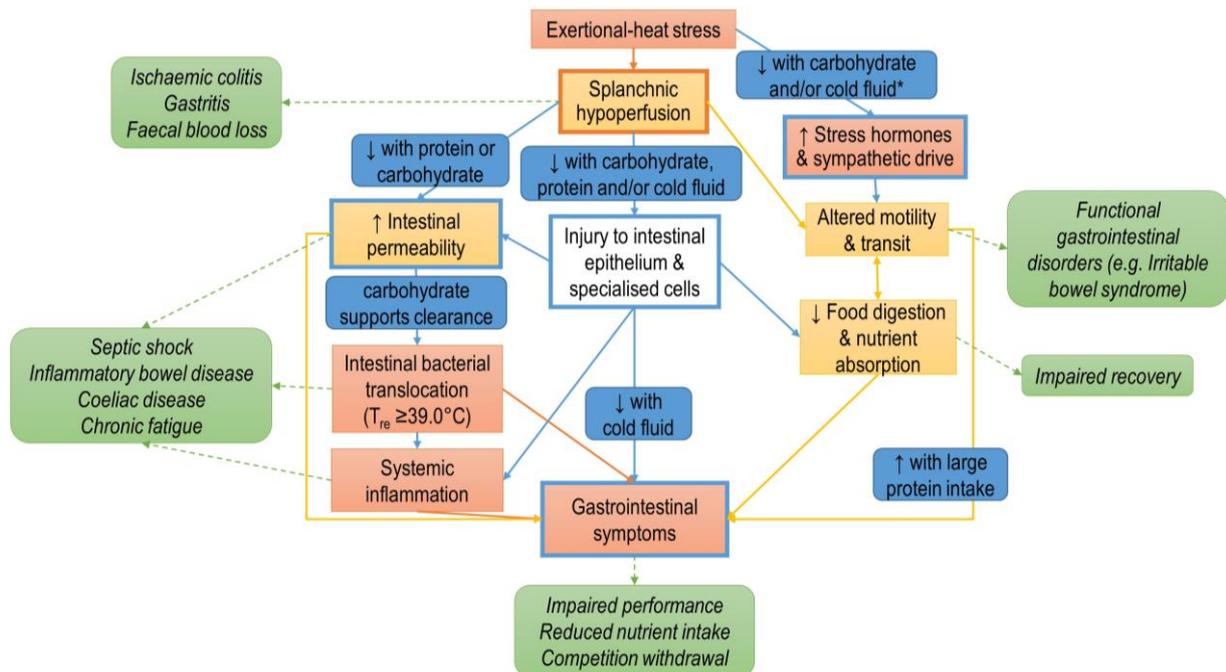


Figure 8.2: The influence of carbohydrate, protein and cold fluids consumed frequently during prolonged exertional-heat stress (2 h running at 60% $\dot{V}O_{2max}$ in 35°C T_{amb}) on exercise-induced gastrointestinal syndrome. *Cold fluid at 0°C.

8.2 The effect of exertional-heat stress on gastrointestinal integrity, symptoms, systemic endotoxin and cytokine profile.

This thesis includes two complimentary studies that investigated the effects of moderate ($35^{\circ}\text{C T}_{\text{amb}}$) and mild ($30^{\circ}\text{C T}_{\text{amb}}$) heat exposure during prolonged submaximal running on gastrointestinal and systemic perturbations (**Chapter 4** and **5**, respectively). The novel aspects of these exertional-heat stress studies included 1) prolonged duration running exercise; 2) comprehensive analysis of gastrointestinal integrity as a primary outcome measure; and 3) the investigation of gastrointestinal symptoms in response to heat stress and in conjunction with perturbations to gastrointestinal integrity. In contrast, previous exertional-heat stress studies have primarily included higher intensity exercise of a shorter duration (i.e., ≤ 1 h at $\geq 70\% \dot{V}\text{O}_{2\text{max}}$) and/or focused on secondary measures of gastrointestinal perturbations, including endotoxaemia and systemic cytokine responses.(44, 58) Moreover, previous studies have not concurrently explored the effects of heat exposure, or the associated thermoregulatory strain, on gastrointestinal symptoms(58, 74), which are amongst the most commonly reported medical complaints in endurance running. The studies within this thesis are therefore the first to identify that heat exposure during prolonged exercise substantially increases injury to the intestine and predisposes individuals to gastrointestinal symptoms. These findings suggest that development of these gastrointestinal disturbances during prolonged running in the heat has the potential to reduce nutrient intake and absorption of nutrients, impair exercise performance and may increase the number of athlete withdrawals from competitive endurance events.(23, 24, 190)

Combined findings from the exertional-heat stress studies within this thesis propose that small intestine permeability and intestinal inflammation are not exacerbated by heat exposure during prolonged submaximal running. A previous study by Yeh et al.(58) came to the same conclusion by measuring plasma claudin-3 as marker of gastrointestinal permeability. However, the validity of plasma claudin-3 as marker of gastrointestinal permeability is questionable, since this tight junction

protein is found within other bodily organs and is one of more than twenty tight junction proteins that can simultaneously interact to maintain or alter tight junction homeostasis.(59, 191) In contrast to our findings and those of Yeh et al.(58), previous studies have proposed an increased gastrointestinal permeability arising from heat stress and/or hyperthermia due to an increase in circulatory endotoxin concentration.(29) Indeed, we observed elevated circulatory endotoxin and a reduction in anti-endotoxin antibodies after running in 35°C compared to temperate conditions. However, the lactulose to L-rhamnose ratio, a direct measure of small intestine permeability was not different between conditions, and was not associated with the magnitude of increase in rectal temperature during exercise. The findings from this thesis, therefore, refute an increased gastrointestinal permeability arising from heat exposure and/or hyperthermia during prolonged submaximal running and caution the use of circulatory endotoxin as a secondary marker of gastrointestinal permeability particularly given the aforementioned limitations of this marker (**Chapter 2.1.3**). It is however, possible that higher thermal strain (i.e., ambient and core body temperatures) elicited by exposure to hotter ambient temperatures and/or more strenuous or prolonged exercise may increase gastrointestinal permeability, although this requires further investigation.

The minor increase in faecal calprotectin observed post-exercise in the first exertional-heat stress study within this thesis suggests a very negligible rise in intestinal inflammation, that is well-below the reference values used to identify chronic intestinal inflammation in inflammatory diseases of the bowel.(60) Only one study to date has reported the use of faecal calprotectin as marker of acute intestinal inflammation in response to exercise stress, with similar findings.(40) It is, therefore, likely that exercise, with or without heat stress, is unlikely to increase intestinal inflammation to a level that is of any clinical significance in a healthy athletic population group. Additionally, it may be difficult to capture the stool corresponding with acute transient intestinal inflammation, making it possible that the observed results may have been either underestimated or that acute exercise has minimal impact on intestinal inflammation. Indeed, faecal calprotectin did not increase in response to chronic

exercise stress during a case-study simulated multi-day ultramarathon run in our laboratory (unpublished data) suggesting that multiple days of exercise stress does not elicit significant intestinal inflammation. Together, these findings suggest faecal calprotectin may not be a useful marker of gastrointestinal inflammation in response to exercise. Faecal calprotectin may be useful in monitoring intestinal inflammation in response to exercise stress in populations with pre-existing inflammatory bowel disease where a more chronic level of intestinal inflammation may occur. Moreover, findings from this thesis support the use of I-FABP as a sensitive marker of intestinal injury that may also be useful in assessing exercise-induced gastrointestinal perturbations that are associated with splanchnic hypoperfusion and intestinal ischaemia.(36, 40)

The secondary outcomes of this thesis were to explore systemic endotoxin and cytokine responses to exertional-heat stress, both of which have been previously explored due to potential links to acute and chronic health conditions.(44, 58, 74, 77) Findings from this thesis support previous observations of perturbed endotoxin profile (i.e., increased circulatory endotoxin and reduced anti-endotoxin antibodies) during moderate exertional-heat stress that elicit core body temperatures $\geq 39.0^{\circ}\text{C}$.(44, 58, 74, 77, 82) The minor (additional 0.4°C) increase in core body temperature observed between running in 30°C vs. 22°C in this thesis did not influence circulatory endotoxin profile. Our findings therefore, suggest that perturbations to the endotoxin profile occur only during substantial exertional-heat stress with a threshold core body temperature of $\geq 39.0^{\circ}\text{C}$.

In addition, our findings suggest the systemic inflammatory cytokine responses may occur at a lower magnitude of thermoregulatory strain. This is possibly due to a lower compensatory anti-inflammatory cytokine response, suggesting that mild exertional-heat stress drives a more pro-inflammatory state; or more likely, the level of inflammation was not sufficient enough to elicit a substantial anti-inflammatory response. Indeed, running in 35°C showed a lower IL- 1β :IL-10 and TNF- α :IL-10 ratio compared to running in 22°C , indicating a greater compensatory anti-

inflammatory response. No difference in the IL-1 β :IL-10 and TNF- α :IL-10 ratio was observed after running in 30°C or between 30°C and 22°C. Moreover, in this thesis IL-10, a potent anti-inflammatory cytokine, was positively correlated with the magnitude of increase in rectal temperature during exercise, suggesting a possible link between thermoregulatory strain and the compensatory anti-inflammatory response. Indeed, substantial increases in the anti-inflammatory cytokines IL-10 and IL-1ra have been observed during multi-stage ultramarathon competition in the heat, along with smaller increases in the pro-inflammatory cytokines IL-1 β and TNF- α .(70) Together, these findings suggest the inflammatory cytokine response induced by exertional-heat stress appear to be adequately attenuated by a compensatory anti-inflammatory cytokine response in healthy trained athletes, thereby attenuating the risk of adverse health implications.(70, 74) However, the safety of exertional-heat stress in individuals pre-disposed to and/or with pre-existing inflammatory conditions is currently unknown and requires further research.(2)

The two exertional-heat stress studies contained within this thesis were conducted separately to provide a clear picture on the effects of mild heat stress in isolation so that results were not confounded by the slightly higher temperatures in the exertional-heat stress study. Conducting the two studies together may have strengthened the findings from this thesis however, this would likely have required a larger participant cohort, greater participant burden and made the study completion within the cooler months difficult to achieve. Conducting the $\dot{V}O_{2max}$ in temperate ambient conditions is a limitation of the exertional-heat stress studies within this thesis due to the effects of cardiovascular drift (i.e., increased heart rate and reduced stroke volume) which would have equated to participant's running at a higher % of their respective $\dot{V}O_{2max}$ in the WARM and HOT trials than in the TEMP trial.(192-194) Cardiovascular drift is however, a normal phenomenon that would occur during outdoor running and/or competitive endurance events in the heat, but may be modified by slower pacing strategies. While the effect of cardiovascular drift is an acknowledged limitation, frequent variations in running speed and the invasive nature of collecting frequent oxygen uptake data during

prolonged running in the heat may have negatively affected the participant's ability to consume fluids and impacted the reporting of gastrointestinal symptoms.

The use of recreationally trained athletes from both sexes in this thesis may have contributed to a large variability in physiological, and possibly gastrointestinal, responses to exertional-heat stress and the prevention and management strategies. For example, sex differences, including the use of oral contraceptives and different phases of menstrual cycle in the female sex (i.e., hormone variations) and variability in body size, body fat and fitness may have affected thermoregulation and therefore, subsequent primary and secondary outcome measures.(195, 196) However, aside from limiting the age to ≤ 45 years (1, 197), the population groups used throughout the studies in this thesis were representative of the vast majority of athletes competing in endurance running events. In recent years there has been a rapid increase in the number of females competing in endurance running events, with female runners occasionally exceeding male competitors.(133) The inclusion of both sexes within this thesis therefore, enhances the translational aspects of this research to the broader running population. However, the influence of biological sex and associated differences in thermoregulation during exercise(198) on gastrointestinal responses is an area that requires further research.

In addition, the healthy recreationally trained athletes recruited throughout the studies of this thesis may also limit the direct application of findings to elite athletes and individuals with a pre-existing gastrointestinal condition. It could be speculated that compared to athletes with less training, the higher metabolic rate and heat production of elite athletes (i.e., competing at higher exercise intensities) may exhibit greater injury to the intestinal epithelium and increased intestinal permeability.(43, 54) However, elite athletes may exhibit lower circulatory endotoxin, higher anti-endotoxin antibodies, lower pro-inflammatory cytokines with concomitant elevated anti-inflammatory cytokines resulting from training adaptations.(74) Therefore, it is likely that some of the health implications associated with systemic endotoxaemia and cytokinaemia may be reduced in

elite or highly trained athletes.(74) However, these athletes may still benefit from strategies that maintain the integrity of the gastrointestinal tract, such as frequent consumption of carbohydrates (**Chapter 6**) and the addition of cooling strategies (**Chapter 7**) to aid thermoregulation.

The direct translation of laboratory-based findings from the exertional-heat stress studies of this thesis to the field setting requires validation. Variation in heat exchange mechanisms (i.e., environmental conditions, pacing, terrain, pack running etc.) in the field setting may confound findings.(199, 200) It is, however, speculated that the magnitude of thermoregulatory strain and stress hormone responses during prolonged running in the heat, which appear to be key exacerbating factors, may be more important in determining the extent of gastrointestinal perturbations and symptoms than factors associated with indoor vs. outdoor running (i.e., treadmill vs. overground and consistent vs. variable pace/terrain/weather, respectively). For example, findings from this thesis suggest the extent of intestinal injury and gastrointestinal symptoms may increase in conjunction with rectal temperature and stress hormone responses during prolonged running. Indeed, positive correlations were observed between the exercise-induced increase in rectal temperature with I-FABP, gastrointestinal symptoms and cortisol responses. In comparison, the muscle activation pattern and energy cost of running at a 1% inclination on a treadmill has been proposed as negligible, with treadmill running kinematics from this thesis therefore likely to be deemed generalisable to outdoor running.(164, 201-203) Any translational differences between the laboratory and field setting are therefore, likely to be related to differences in metabolic heat production and/or heat exchange (i.e., environmental conditions).(199, 200) Fans were not used during the exertional-heat experimental trials in this thesis due to issues maintaining the temperature of the custom-made environmental chamber. This is an acknowledged limitation which may have altered heat exchange mechanisms during exercise in the laboratory which may reduce the translational aspect of the findings from this thesis.

In line with these translational aspects, the findings from the exertional-heat stress studies within this thesis may not be directly applicable to other modes of exercise such as cycling or swimming, where heat exchange mechanisms are substantially altered, and physical load bearing and abdominal vibrations are reduced in comparison to running.(91, 204, 205) Indeed, findings from previous studies indicate that intestinal injury, permeability and symptoms are lower during cycling compared to running exercise.(2, 53) Our results may however, translate to active occupational workers, such as the military, who have been shown to reach high core body temperatures (39.5-41.2°C) during load-bearing exertion in the heat and display elements of exercise induced gastrointestinal syndrome during combat training.(12, 155) Moreover, findings from this thesis may translate to several summer sports, such as triathlon, tennis or soccer which include prolonged bouts of continuous or intermittent running with heat exposure. Indeed, thermoregulatory strain and gastrointestinal perturbations may be higher during intermittent high intensity exercise compared to continuous submaximal exercise of the same duration and energy cost.(45, 206) However, rest periods during summer sports such as tennis and soccer allow a temporary reduction in core temperature and provide an increased opportunity to implement cooling strategies (e.g. cold fluids, fanning, ice vests etc.) which may attenuate the overall thermal load.(207) The occurrence of gastrointestinal symptoms and perturbations over multiple days of heat exposure in sports such as tennis and certain active occupations (i.e., military) have the potential to impair performance, recovery process and increase the risk of chronic inflammation and associated health conditions.(70) Future research is therefore warranted to determine the extent of gastrointestinal perturbations and symptoms arising during sports such as tennis and occupational heat exposure such as the military.

8.3 The effect of carbohydrate and protein intake during exertional-heat stress on gastrointestinal integrity, symptoms, systemic endotoxin and cytokine profile.

The third study within this thesis (**Chapter 6**) is the first to demonstrate that frequent intake of a glucose-based carbohydrate and hydrolysed whey protein during exertional-heat stress prevents perturbations to gastrointestinal integrity. Indeed, glucose and hydrolysed whey protein intake resulted in a complete abolishment of the pre- to post-exercise increase in I-FABP, which was subsequently maintained throughout the post-exercise recovery period. The effectiveness of frequent carbohydrate and protein intake in attenuating exercise-induced intestinal injury exceeds that of previous research, where nutrients (i.e., carbohydrate and amino acids) were less frequently administered.(39, 90, 95, 96) These findings, therefore, suggest the frequency of nutrient intake may be an important factor in ameliorating intestinal injury. Additionally, no difference in I-FABP was observed between the energy-matched nutrients, suggesting that digestion and absorption processes associated with frequent energy intake may maintain splanchnic perfusion and therefore, be more important than the type of nutrient ingested in preventing intestinal injury. However, this is an area requiring further clarification, particularly in light of current sports nutrition guidelines advising the ingestion of multiple transportable carbohydrates that use different intestinal nutrient transporters, which may subsequently alter post-prandial hyperaemia.(170) Moreover, previous literature suggests that certain nutrients, such as glucose, may be more effective in enhancing post-prandial hyperaemia than other nutrients such as protein.(94)

The observed reduction in small intestine permeability with frequent intake of a glucose-based carbohydrate and hydrolysed whey protein during prolonged running in the heat is another important novel finding from this thesis. Previous studies including 1 h running at 70% $\dot{V}O_{2\max}$ and 1.5 h cycling at 70% have not observed a reduction in gastrointestinal permeability with frequent carbohydrate intake, except when aspirin was administered prior to exercise.(69, 95) Differences in findings may

be attributed to methodological differences, including nutrient composition, and/or the extent of intestinal permeability when the lactulose and L-rhamnose sugar probe was ingested. For example, previous studies investigating the effect of carbohydrate intake on permeability have administered the sugar probes at the commencement of exercise when little or no damage to the intestine had occurred, except when aspirin was ingested prior to exercise.(69, 95) Therefore, any possible benefit of the carbohydrate solution over a placebo may have been missed. Whereas, in our study the dual sugar probes were administered at 90 min during exercise, providing exposure of the sugar probes to the intestine towards the end of the exercise bout, when the most damage had occurred.(37)

The nutrient composition of the carbohydrate solution may also account for differences between our findings and those of other studies. Previous studies have used a combination of sucrose and glucose(69, 95), whereas the study within this thesis used a glucose solution only, which is proposed to be a potent stimulator of post-prandial hyperaemia. Currently, the majority of commercially-available sports nutrition products contain a combination of glucose and fructose due to recent sports nutrition guidelines recommending a 2:1 ratio of glucose to fructose with intakes of 60-90 g/h carbohydrate for prolonged exercise. It could be hypothesised that fructose may not be as effective as glucose at stimulating post-prandial hyperaemia (i.e., improve splanchnic blood flow) and the potential associated benefits to gastrointestinal integrity during exercise due to passive transport of fructose across the apical membrane. However, the effects of fructose supplementation in isolation or with glucose on gastrointestinal integrity is currently unknown and warrants further investigation.

The additional reductions in small intestine permeability with consumption of a hydrolysed whey protein compared to a glucose-based carbohydrate solution during study 3 of this thesis (**Chapter 6**), support the notion that nutrient composition, rather than energy content, may be an important factor in attenuating small intestine permeability during exertional-heat stress. The precise mechanism responsible for the additional benefits of a hydrolysed whey protein over a glucose-based

carbohydrate requires further investigation. However, it is possible that the hydrolysed whey protein exerted beneficial effects through multiple pathways, including the provision of amino acids that stimulate post-prandial hyperaemia, and provision of glutamine which may stabilise tight junction protein complexes through upregulation of heat shock proteins and/or a reduction in localised inflammation.(90, 94, 96)

Despite the additional reduction in small intestine permeability with hydrolysed whey protein compared to glucose-based carbohydrate intake, the increased incidence and severity of gastrointestinal symptoms limits the use of such large protein doses during prolonged exercise. In contrast, the glucose-based carbohydrate did not increase gastrointestinal symptoms compared to water intake, and showed a lower stress response (e.g., cortisol and IL-6 and increased anti-endotoxin antibodies) which may be beneficial for post-exercise recovery. Interestingly, the attenuation of cortisol with a glucose-based carbohydrate compared to water did not reduce gastrointestinal symptoms. This reduction in the stress hormone cortisol with carbohydrate ingestion may be attributed to increased blood glucose levels providing an exogenous fuel supply.(115) However, the attenuation of stress responses may not have been sufficient to influence gastrointestinal motility due to similar thermoregulatory strain observed with carbohydrate and water. Together these findings suggest that attenuation of thermoregulatory strain may be important in reducing gastrointestinal symptoms during exertional-heat stress via effects on gastrointestinal motility. For example, attenuation of splanchnic hypoperfusion and stress hormones, which are believed to be primary contributors to the development of gastrointestinal symptoms, did not reduce gastrointestinal symptoms during the 2 h running period in this study. It is therefore, possible that alterations in gastrointestinal motility may be a primary causal pathway in the development of early exercise-induced gastrointestinal symptoms and precede significant perturbations to gastrointestinal integrity. It is however, acknowledged that more prolonged or severe perturbations to gastrointestinal integrity

likely contribute to the development of gastrointestinal symptoms during longer (≥ 3 h) duration exercise.

The provision of exogenous glucose for oxidation during prolonged exercise has consistently been shown to improve endurance exercise performance.(83) Therefore, frequent ingestion of carbohydrates containing glucose may be a suitable strategy to recommend for maintenance of gastrointestinal integrity during prolonged exertional-heat stress. Determining the minimum dose and frequency of carbohydrate consumption and/or if small amounts of a whey-based protein combined with carbohydrate can be tolerated, will be an important step towards developing more specific guidelines for athletes, coaches and practitioners in the prevention of exercise-induced gastrointestinal syndrome. The combination of protein with a glucose-based carbohydrate may be advantageous in attenuating exercise-induced gastrointestinal permeability, since the hydrolysed whey protein was more effective than glucose in attenuating intestinal permeability, whilst providing an exogenous fuel source. Future research is therefore, warranted to determine the minimal dose of protein required to obtain benefits to gastrointestinal integrity without inducing gastrointestinal symptoms, and if a combination of protein and glucose-based carbohydrate is more effective than either nutrient in isolation. Current sports nutrition guidelines for carbohydrate intake during prolonged exercise recommend consuming up to 90 g/h of multiple transportable carbohydrates (e.g. 2:1 glucose to fructose ratio).(83, 170) However, these guidelines have been based on prolonged cycling exercise, where the occurrence of gastrointestinal disturbances are much lower than in running-based sports.(53, 83) Indeed, recent research shows that consumption of 90 g/h multiple transportable carbohydrates results in carbohydrate malabsorption and gastrointestinal symptoms in the majority of athletes during prolonged running in temperate ambient conditions.(25, 165) In contrast, the 45g/h of a glucose-based carbohydrate in this thesis appeared to be well tolerated, with no additional symptoms observed in comparison to *ad libitum* water. Moreover, observations from competitive events suggest marathon and ultramarathon runners commonly consume 35-45 g/h

carbohydrates, whereas cyclist and triathletes tend to consume ≥ 60 g/h providing further support for the revision of carbohydrate intake guidelines for running-based sports.(16, 85)

As a starting point for future sports nutrition guidelines, findings from this thesis suggest the ingestion of 15 g of a glucose-based carbohydrate solution every 20 min during prolonged running in the heat is tolerable and increases the provision of exogenous glucose for oxidation. A period of ‘gut training’ may be used to enhance tolerance to higher carbohydrate intakes, but the optimal carbohydrate intake rate during running exercise is currently unknown and may vary between individuals.(25) Findings from this thesis also suggest the frequency of carbohydrate ingestion appears to be an important factor in gastrointestinal health and maintenance of nutrient absorption. Small doses consumed more frequently may be more beneficial to the maintenance of gastrointestinal integrity than consuming larger doses of carbohydrate less frequently, which is commonly observed in training and/or sporting competition. Although the constraints of obtaining frequent (i.e., every 20 min) access to carbohydrates in some sports may limit the practical translation of these findings. However, the minimum frequency and dose of carbohydrates that is required to attenuate perturbations to gastrointestinal integrity remains to be elucidated.

8.4 The effect of temperature of ingested water during exertional-heat stress on gastrointestinal integrity, symptoms and systemic cytokine profile.

The fourth and final study within this thesis (**Chapter 7**) is the first to explore the effect of a cooling strategy during exertional-heat stress on gastrointestinal perturbations. Findings from this study suggest that frequent ingestion of cold fluids during exertional-heat stress may play a role in reducing intestinal injury and possibly upper-gastrointestinal symptoms, but appears to have no effect on systemic cytokinaemia. Variability in thermoregulatory responses and gastrointestinal outcomes in response to cooling during prolonged exercise may limit the ability to draw firm conclusions on the effectiveness of cooling strategies. Differences in anthropometry (i.e., body mass, body surface area, and body fat), fitness and sex in the participant cohort used in this study may explain the large variation, despite providing fluids in proportion to body mass and exercising at the same relative intensity.(195, 196) Future cooling studies, should therefore endeavour to control for these variables however, the interpretation of findings may then be limited to a specific population subgroup rather than generalisable to the larger running community.

In addition to the previously mentioned variation, we observed only a minor reduction in thermoregulatory strain (i.e., 0.3-0.4°C reduction in rectal temperature) with cold fluids, primarily during the second hour of exercise. However, even such a small blunting of thermoregulatory strain attenuated the mean increase in post-exercise I-FABP by >400 pg/mL, but only a trend for statistical significance was observed. While there may be limitations associated with direct comparison between studies, a mean reduction of >400 pg/mL I-FABP exceeds the pre- to post exercise increases reported after 1 h of cycling or running.(39-42) This suggests cooling strategies have the potential to induce a moderate attenuation of intestinal injury. Further, greater differences in core body temperature and I-FABP may have been observed if the temperature of the control fluid was provided at 37°C (i.e., as opposed to 22°C), which has commonly been used by previous studies to align with resting core body

temperature.(121, 187, 208, 209) However, the palatability of water at 37°C may have influenced the perception and/or reporting of gastrointestinal symptoms, therefore, limiting the practical and translational aspects of the study findings.(127)

The use of a more aggressive cooling strategy, for example ice slurry ingestion and/or combined with external cooling, is hypothesised to have resulted in greater reductions in core body temperature and subsequently may have had a greater impact on gastrointestinal outcomes.(126) Nevertheless, the practical application of these cooling methods, including tolerance issues, during running would be expected to limit the translation of findings. However, more aggressive cooling strategies may be easier to implement in certain occupational settings and sports, such as tennis, that include frequent rest breaks. Moreover, from a mechanistic perspective it would be interesting to investigate the use of external cooling methods (i.e., cold water immersion, ice vests or ice packs), compared to internal cooling methods (i.e., cold water/ice ingestion) on gastrointestinal outcomes. For example, reducing skin temperature through external cooling methods will increase the core to skin temperature gradient(210), thereby reducing skin blood flow which may have beneficial effects on splanchnic perfusion and gastrointestinal integrity. In contrast, the ingestion of cold fluids has the potential to reduce gastric temperature which may also be beneficial to gastrointestinal integrity.

Heat acclimatisation is a useful strategy for attenuating thermoregulatory strain during exertional-heat stress and may therefore, have the potential to enhance the effectiveness of cooling strategies on gastrointestinal outcomes. The use of heat acclimatisation on intestinal injury and circulatory endotoxaemia has previously been explored(44), but not in conjunction with cooling strategies. A 5-day period of heat acclimatisation was not sufficient enough to attenuate the increase in I-FABP and circulatory endotoxin concentration after a short bout of intense exercise, despite observing small reductions in resting and final core body temperatures.(44) Although, smaller pre- to post-exercise increase in I-FABP were observed on day 5 of the heat acclimatisation protocol, suggesting the

potential for further attenuation with the addition of a cooling strategy. Additionally, there may be potential for greater differences to be observed during prolonged submaximal running, rather than short duration high intensity exercise.(44)

The combination of frequent carbohydrates (**Chapter 6**) with cold fluids (**Chapter 7**) is also a combined strategy that has the potential to reduce gastrointestinal perturbations through different mechanisms, which has the potential for additive effects. In this thesis, we have shown that frequent carbohydrate ingestion during exertional-heat stress can ameliorate intestinal injury and permeability and reduce the stress hormone cortisol (**Chapter 6**). However, carbohydrates did not reduce the thermoregulatory strain and gastrointestinal symptoms compared to water intake at the same temperature. Therefore, the ingestion of cold water with carbohydrates during exertional-heat stress has the potential to add to these existing benefits by blunting the thermoregulatory strain. Further, reducing thermoregulatory strain may aid gastric emptying which has the potential to reduce the occurrence of upper-gastrointestinal symptoms, which have been most commonly reported throughout the studies in this thesis and previous field-based studies.(23, 70, 88)

8.5 Directions for future research

The novel findings from this thesis make a significant contribution to the current literature regarding exertional-heat stress induced gastrointestinal perturbations, and prevention and management strategies. However, the findings from this thesis highlight several possible directions for future research. One such area of research that has not been explored throughout this thesis is perturbations to gastrointestinal motility, which appears to be a major contributor to the development of upper-gastrointestinal symptoms; and were most commonly observed throughout the studies in this thesis. Gastric emptying rate and oro-caecal transit time determined by gastric aspiration, ¹³C-acetate breath test and lactulose challenge breath test, respectively, have been the most commonly reported measures

of gastrointestinal motility in response to exercise. However, the recent development of a wireless pH and motility capsule may prove to be a useful non-invasive method of measuring gastrointestinal motility during exercise and provide further insight on the area of exercise stress and nutrition interventions on gastrointestinal motility and symptoms.(211) Previous research has identified a strong correlation between elevated core body temperature during exercise in the heat and impairment in gastric emptying.(88) Additionally, higher exercise intensity has also been implicated in impaired gastric emptying, suggesting similar mechanisms to which perturbations to gastrointestinal integrity occur (i.e., thermoregulatory/cardiovascular strain and stress hormone response).(146, 147) Findings from study 4 (**Chapter 7**) suggest cooling strategies may play a role in reducing upper-gastrointestinal symptoms during prolonged exertional-heat stress, likely through maintenance of gastric emptying rate. However, a more aggressive cooling strategy resulting in a large reduction in core body temperature may be required to observe consistent benefits across all athletes. Therefore, future studies should aim to explore the effects of cooling strategies during exertional-heat stress on gastric emptying rate and determine if more substantial cooling can attenuate the incidence and severity of upper-gastrointestinal symptoms.

In addition to investigating gastrointestinal motility, a possible direction of future research is to explore the effects of exercise and heat stress on individuals with pre-existing gastrointestinal conditions. The limited research to date suggests that low to moderate intensity short duration exercise is likely to be safe, may aid in symptomatic management and quality of life in conditions such as irritable bowel syndrome.(2, 212, 213) However, it is plausible that more strenuous and prolonged exercise may exacerbate disturbances to gastrointestinal integrity, motility and systemic inflammation, thereby aggravating the gastrointestinal disease and/or disorder and associated symptomatic manifestations.(2) Such research is therefore, important in establishing practitioner guidelines for the management of gastrointestinal conditions and reducing the social and economic impacts associated with an increased gastrointestinal disease burden.

As previously highlighted within this discussion, future research is warranted to determine the minimum nutrient intake required to maintain gastrointestinal integrity during exertional-heat stress and determine if findings from this thesis can translate into the field setting, including active occupations (i.e., military) and running-based sports (i.e., tennis and soccer) with heat exposure. Moreover, it would be prudent to determine if the benefits of frequent carbohydrate intake on gastrointestinal integrity remain at higher exercise intensities. Indeed, it is unknown if the combination of a delay in gastric emptying and reduction in nutrient absorption previously observed during running at exercise intensities of $\geq 70\% \dot{V}O_{2\max}$ may impair the beneficial effects of glucose ingestion on gastrointestinal integrity.(143, 146) However, from a practical perspective the requirement for exogenous glucose as an energy substrate during short duration high intensity exercise is either non-existent or minimal in comparison to endurance-based events which are conducted at a lower exercise intensity.

8.6 Implications for athletes, coaches and sports practitioners

The findings from this thesis builds on the existing literature that has identified causal pathways and prevention and management strategies for exercise-induced perturbations to gastrointestinal integrity and symptoms. The findings from this thesis and previous research have important translational implications for athletes, coaches and sports practitioners to support health and performance outcomes of athletes and active individuals. Previous literature suggests that symptomatic athletes may have a higher pre-disposition to the occurrence of exercise-induced gastrointestinal syndrome due to exaggerated primary causal pathways including exaggerated splanchnic hypoperfusion, stress hormone responses and sympathetic drive.(26, 53) Determining the underlying cause and appropriate management of symptomatic athletes is currently challenging due to the complexity and multifactorial causes of these gastrointestinal perturbations. However, **Table 8.1** provides a synthesis of findings from this thesis and previous research that could be used by sports practitioners and health professionals in practice to support and optimise gastrointestinal health and physical performance.

Table 8.1. Prevention strategies and hypothesised mechanisms for exercise-induced perturbations to gastrointestinal integrity and symptoms.

Prevention strategy		Mechanism
Gastrointestinal integrity		
Intestinal epithelial injury	<ol style="list-style-type: none"> 1. Fluid intake / maintaining euhydration 2. Frequent carbohydrate / protein intake 3. L-citrulline intake pre-exercise and possibly during prolonged exercise 4. Cold fluids / cooling strategy 	<ol style="list-style-type: none"> 1. Maintenance of plasma volume aids thermoregulation and splanchnic perfusion 2. Nutrient absorption maintains splanchnic perfusion 3. Increased nitric oxide production and localised vasodilation of intestinal villi 4. Attenuates thermoregulatory strain and aids splanchnic perfusion
Intestinal permeability	<ol style="list-style-type: none"> 1. Fluid intake / maintaining euhydration 2. Frequent carbohydrate / protein intake 	<ol style="list-style-type: none"> 1. Maintenance of plasma volume aids thermoregulation and splanchnic perfusion 2. Nutrient absorption maintains splanchnic perfusion
Gastrointestinal symptoms		
Upper-gastrointestinal Symptoms (Belching, heartburn, bloating, upper abdominal pain, urge to regurgitate, regurgitation/vomit, nausea)	<ol style="list-style-type: none"> 1. Fluid intake / maintaining euhydration 2. Cold fluids / cooling strategies 3. Avoid excessive carbohydrate intake during exercise 4. Avoid highly concentrated carbohydrates during exercise 5. Avoid high intakes of protein and fat pre-exercise and during exercise 6. Gut training with carbohydrates 	<ol style="list-style-type: none"> 1. Maintenance of plasma volume aids thermoregulation, splanchnic perfusion, attenuates stress hormone response 2. Attenuates thermoregulatory strain and stress hormone response 3. Delays gastric emptying, may contribute to malabsorption and further delays in gastric emptying 4. Delays gastric emptying 5. Delays gastric emptying 6. Improves tolerance and nutrient absorption to carbohydrate intake
Lower-gastrointestinal Symptoms (Flatulence, urge to defecate, lower abdominal pain/s, abnormal defecation)	<ol style="list-style-type: none"> 1. Fluid intake / maintaining euhydration 2. Low FODMAP diet pre-exercise 3. Avoid excessive fibre intake pre-exercise 4. Avoid excessive carbohydrate intake during exercise 5. Gut training with carbohydrates 6. Avoid gastric stimulants (i.e., caffeine) 7. Defecation prior to exercise 	<ol style="list-style-type: none"> 1. Maintenance of plasma volume aids thermoregulation, splanchnic perfusion, attenuates stress hormone response 2. Reduces gas production, bloating, colonic pressure 3. Reduces stool volume and colonic pressure 4. Avoids malabsorption which can lead to gas production, bloating, osmotic diarrhoea 5. Avoids malabsorption which can lead to gas production, bloating, osmotic diarrhoea 6. Avoids an increase in colonic motility 7. Reduces the risk of defecation during exercise
Exercise-related prevention and management strategies		
Influences gastrointestinal integrity and symptoms	<ol style="list-style-type: none"> 1. Avoid high exercise intensity / pacing too fast (determine heart rate for symptom threshold) 2. Increase fitness 3. Heat acclimatisation 4. Avoid non-steroidal anti-inflammatory drugs 5. Avoid heat exposure and/or clothing that increases thermoregulatory strain 	<ol style="list-style-type: none"> 1. Avoid excessive thermoregulatory strain, splanchnic hypoperfusion, stress hormone response and sympathetic drive 2. Reduction in stress hormones, sympathetic drive and systemic responses 3. Increase in plasma volume, lower core body temperature, may aid splanchnic perfusion 4. Irritates the gastrointestinal system causing dysfunction and damage 5. Avoid excessive thermoregulatory strain, splanchnic hypoperfusion, stress hormone response and sympathetic drive

In summary, the novel studies within this thesis establish the effects of prolonged exertional-heat stress on gastrointestinal integrity and symptoms, and provide new evidence for practical nutrition prevention and management strategies. While the studies from this thesis have been explored in a laboratory-controlled environment, it is likely that findings will directly translate to recreationally trained endurance runners in the field under similar conditions of thermoregulatory strain. Further, findings have the potential to be transferrable to active occupations with heat exposure and running-based summer sports, but further research on gastrointestinal perturbations in these population groups is required.

CONCLUSIONS

The studies contained within this thesis have investigated the effects of exertional-heat stress on gastrointestinal perturbations and subsequent prevention and management strategies. The general purpose of this thesis was to firstly, establish the effects of exertional-heat stress on gastrointestinal integrity, symptoms and associated systemic responses and determine if increases in body temperature during exercise were related to these perturbations. Secondly, this thesis explored the effectiveness of frequent carbohydrate, protein and cold fluid intake on the prevention and management of exertional-heat stress induced perturbations to gastrointestinal integrity, symptoms and systemic responses. The main findings from this thesis were that: 1) Prolonged submaximal running in 35°C T_{amb} injures the intestinal epithelium, contributes to the development of gastrointestinal symptoms, perturbs systemic endotoxin and cytokine profile, but does not increase small intestine permeability or intestinal inflammation. 2) Prolonged submaximal running in 30°C T_{amb} injures the intestinal epithelium, contributes to the development of upper-gastrointestinal symptoms, perturbs systemic cytokine profile, but does not increase small intestine permeability or systemic endotoxin profile. 3) Exposure to hotter ambient conditions during prolonged running induces greater and more prolonged damage to the intestinal epithelium, greater gastrointestinal symptoms, perturbations to systemic endotoxin profile and a more substantial compensatory anti-inflammatory cytokine response, likely due to the greater thermoregulatory strain and physiological stress. 4) Intestinal epithelial injury, gastrointestinal symptoms, the compensatory anti-inflammatory cytokine response and stress hormone responses are positively associated with exercise-induced thermoregulatory strain during prolonged running. 5) Frequent intake of a glucose-based carbohydrate and hydrolysed whey protein during prolonged running in the heat can prevent injury to the intestinal epithelium and attenuate the increase in small intestine permeability; but, poor tolerance to large doses of the hydrolysed whey protein, and a reduction in stress responses and provision of glucose as an energy substrate for oxidation make carbohydrate ingestion a more

practical and suitable strategy. 6) Frequent ingestion of cold water (15 mL/kg body mass/h) at 0-7°C during exertional-heat stress mildly blunts thermoregulatory strain, modestly attenuates injury to the intestinal epithelium and may reduce the occurrence of upper-gastrointestinal symptoms in some individuals, but does not affect systemic cytokine responses.

Together the studies within this thesis highlight that thermoregulatory strain and the increased stress response during running in the heat appear to be key contributing factors to intestinal injury and gastrointestinal symptoms. The thermoregulatory strain and stress response during running in the heat likely exacerbate splanchnic hypoperfusion, perturbing the integrity of the gastrointestinal tract, which can be attenuated by frequent carbohydrate and protein intake. Moreover, ingestion of cold water during exertional-heat stress may attenuate injury to the intestinal epithelium and development of upper-gastrointestinal symptoms by blunting the thermoregulatory strain. The maintenance of intestinal integrity and attenuation of gastrointestinal symptoms through frequent ingestion of carbohydrates and/or with a cooling strategy, has the potential to improve nutrient intake during exercise, nutrient absorption, physical performance, recovery from exercise, reduce the risk of withdrawal from competition and the development of acute and chronic health conditions. These findings and the use of practical nutrition strategies are of importance to athletes, coaches, sports medicine practitioners, sports dietitians, gastroenterologists and have the potential to translate to active occupations such as the military. Future research is warranted to determine the effects of exercise, heat stress and prevention and management strategies on individuals with gastrointestinal conditions, and to investigate the effectiveness of carbohydrates combined with cooling as a prevention and management strategy in the field setting.

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Appendix A



Monash University Human Research Ethics Committee (MUHREC)
Research Office

Human Ethics Certificate of Approval

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

Project Number: CF13/3647 - 2013001878

Project Title: Impact of exertional heat stress on gastrointestinal permeability and markers of appetite regulation

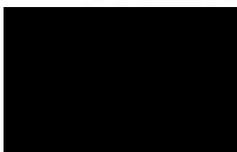
Chief Investigator: Dr Ricardo Costa

Approved: From: 2 May 2014

To: 2 May 2019

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

1. The Chief investigator is responsible for ensuring that permission letters are obtained, if relevant, before any data collection can occur at the specified organisation.
2. Approval is only valid whilst you hold a position at Monash University.
3. It is the responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval and to ensure the project is conducted as approved by MUHREC.
4. You should notify MUHREC immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.
5. The Explanatory Statement must be on Monash University letterhead and the Monash University complaints clause must include your project number.
6. **Amendments to the approved project (including changes in personnel):** Require the submission of a Request for Amendment form to MUHREC and must not begin without written approval from MUHREC. Substantial variations may require a new application.
7. **Future correspondence:** Please quote the project number and project title above in any further correspondence.
8. **Annual reports:** Continued approval of this project is dependent on the submission of an Annual Report. This is determined by the date of your letter of approval.
9. **Final report:** A Final Report should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected date of completion.
10. **Monitoring:** Projects may be subject to an audit or any other form of monitoring by MUHREC at any time.
11. **Retention and storage of data:** The Chief Investigator is responsible for the storage and retention of original data pertaining to a project for a minimum period of five years.



Professor Nip Thomson
Chair, MUHREC

cc: Dr Lisa Ryan

MUHREC – Approved Amendments - CF13/3647 - 2013001878 - Impact of exertional heat stress on gastrointestinal permeability and markers of appetite regulation

MRO Human Ethics Team [REDACTED]

10 September 2014 14:56

PLEASE NOTE: To ensure speedy turnaround time, this correspondence is being sent by email only. MUHREC will endeavour to copy all investigators on correspondence relating to this project, but it is the responsibility of the first-named investigator to ensure that their co-investigators are aware of the content of the correspondence.

Dear Researchers

Thank you for submitting a Request for Amendment to the above named project.

This is to advise that the following amendments have been approved:

1. Change to Title: Impact of exertional heat stress on gastrointestinal integrity and markers of appetite regulation.
2. Change to Personnel: add Mrs Rhiannon Snipe
3. Change to procedures involving participants
 - Trial 4: 35 degrees with restricted fluid intake (3ml/kg/BM/h) changed to ad libitum fluid intake split design with blinded nutrition intake of either 45g/h carbohydrate solution or 45g/h whey protein hydrolysate solution to determine the effects of nutrient intake on gastrointestinal integrity.
 - Sugars drink to determine intestinal permeability changed to include markers of nutrient absorption (previously 100ml deionised water + 5g lactulose, 5g rhamnose, and 5g sucrose 15 minute before commencing the running exercise bout in each trial) to 250ml deionised water + 5g lactulose + 5g D-xylose + 2.5g 3-0-Methyl glucose + 1g L- rhamnose consumed at 90mins of running exercise bout in trials 1-3 and 100ml deionised water +5g lactulose + 1g L-rhamnose in trial 4 (due to interference of nutrients with absorption sugars).
 - Addition of breath-by-breath sample every 20mins to determine differences in substrate utilisation
 - Addition of breath hydrogen samples every 30mins from pre-sugars test to 4h post exertional heat stress to measure oro-caecal transit time from lactulose fermentation in large bowel

Approved Documents:

1. Explanatory Statement date 1 September 2014
2. Consent Form
3. Advertisement
4. Protocol

Thank you for keeping the Committee informed.

Professor Nip Thomson
Chair, MUHREC

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Human Ethics
Monash Research Office

Human Ethics Certificate of Approval

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

Project Number: CF15/3845 - 2015001683

Project Title: Internal cooling study

Chief Investigator: Dr Ricardo Da Costa

Approved: From: 30 November 2015 To: 30 November 2020

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

1. The Chief investigator is responsible for ensuring that permission letters are obtained, if relevant, before any data collection can occur at the specified organisation.
2. Approval is only valid whilst you hold a position at Monash University.
3. It is the responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval and to ensure the project is conducted as approved by MUHREC.
4. You should notify MUHREC immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.
5. The Explanatory Statement must be on Monash University letterhead and the Monash University complaints clause must include your project number.
6. **Amendments to the approved project (including changes in personnel):** Require the submission of a Request for Amendment form to MUHREC and must not begin without written approval from MUHREC. Substantial variations may require a new application.
7. **Future correspondence:** Please quote the project number and project title above in any further correspondence.
8. **Annual reports:** Continued approval of this project is dependent on the submission of an Annual Report. This is determined by the date of your letter of approval.
9. **Final report:** A Final Report should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected date of completion.
10. **Monitoring:** Projects may be subject to an audit or any other form of monitoring by MUHREC at any time.
11. **Retention and storage of data:** The Chief Investigator is responsible for the storage and retention of original data pertaining to a project for a minimum period of five years.



Professor Nip Thomson
Chair, MUHREC

cc: Mrs Rhiannon Snipe

Human Ethics Office
Monash University



ABN 12 377 614 012 CRICOS Provider #00008C

Appendix B



Monash University
Department of Nutrition & Dietetics



PARTICIPANT INFORMATION SHEET - 1st September 2014 Explanatory Statement

Impact of exertional heat stress on gastrointestinal integrity and markers of appetite regulation.

Research Team Contact: Rhiannon Snipe [REDACTED]
Dr Ricardo Costa [REDACTED]

Thank you for taking the time to read this research study participant information sheet. The information contained within and consent form is 4 pages long. Please ensure you have all the pages. This form explains what you will be asked to do. If you have any questions about this research please ask one of the research team.

Invitation to take part

Since you are a recreational, amateur and/or elite level endurance athlete, with experience in marathon, ultra-marathon and long-distance triathlon training and competition you have been invited to take part in this research investigation. Before you do so, it is important for you to understand why the research is being conducted and what you will be required to do once you agree to be involved. Please read the following information carefully. You should ask the research team if there is anything that is not clear or if you would like more information.

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

Background: Exposure to hot ambient conditions during physical exertion is common amongst many activities within health care, security, industry and sport. Such exertional-heat stress has consistently been associated with gut distress and loss of appetite, resulting in an inability to maintain work-output and/or withdrawal from activity; constituting both a productivity and financial burden. The mechanism responsible for exertional-heat stress induced gut distress and appetite suppression appears to be multi-factorial in origin, but ultimately leads to gut surface damage and leakage, and an overall reduction in food and fluid intake. Increased gut leakage during exertional-heat stress has also previously been linked to discomfort symptoms, heat illnesses and other medical manifestations. To date it is still unknown the degree to which exercise duration/intensity and ambient temperature impacts on intestinal integrity.

What is the purpose of this research study?

The study aims to examine the impact of prolonged strenuous exercise in different levels of hot ambient temperatures with and without nutrient intake on intestinal permeability, perceptible gastrointestinal symptoms, markers of appetite regulation and perceptible appetite sensation.

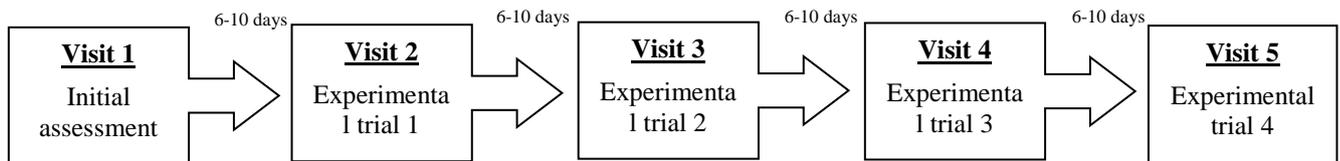
What does participation in this research project involve?

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

- Complete an initial assessment that will include anthropometrical measures (height, weight, and 7-site skinfold (triceps, biceps, subscapular, suprailiac, abdominal, thigh and calf) and a maximal aerobic exercise test (~15-20 minutes) on an electric treadmill. The initial assessment will be conducted at the BASE Facility- Sport & Exercise Dietetics Clinic.

Completing the experimental trials

- You will only be required to take part in **four experimental trials** in a random order differing in ambient temperatures and nutrient intake, which will be at least 6 days apart.



Trials: Run on an electric treadmill at 60% of maximal effort for 2 hours at 20°C, 30°C, and 35°C ambient temperature with water provided as desired, and 35°C ambient temperature with randomized nutrient intake of a 250ml (6%) carbohydrate or protein solution provided every 20mins.

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

- Consume a standard breakfast 2 hours before the exercise trials.
- Provide a urine and faecal sample before and during recovery from the exercise trials.
- To have blood samples taken from the antecubital vein by a trained researcher. These will occur immediately before, after and during recovery from the exercise trials.
- Consume 100-250ml of a sugar drink at 90 minutes during the running exercise and provide a breath sample every 30 minutes thereafter.
- Complete a food and fluid diary during the study.
- Provide a breath-by-breath sample every 20 minutes during each exercise trial.
- Have body mass measured before, after and every 30 minutes during each exercise trial.
- Wear a heart rate monitor during each trial, in which heart will be constantly monitored and recorded every 10 minutes. Answer questions relating to exertion and gastrointestinal symptoms every 10 minutes during running exercise.
- To constantly monitor and recorded core body temperature every 10 minutes by researchers, for health and safety reasons, self-insert a 2.5mm thick thermal coupling probe 12cm above the anal sphincter. The procedure of thermal coupling self-insertion is extremely easy, not harmful, and standard practice in thermoregulatory measurements. If your body temperature increases too much (2.5°C over resting levels), you will be asked to stop exercising and cooled immediately by the research team using cool wet towels and fans until your body temperature reduces back to general resting values.
- After each experimental trial, you will be provided with a recovery meal and drink that meets the recovery nutrition and hydration guidelines and recommendation.

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

- You will be asked to follow a low FODMAP meal plan for 24h prior to each exercise trial and refrain from strenuous exercise and using non-steroidal anti-inflammatories for 48 hours prior to each exercise trial.
- You will be asked to refrain from consuming any dietary supplements one month prior to and during the study.

Advantages of taking part

As part of the experimental design that will be conducted at the BASE Facility- Sports & Exercise Dietetic Clinic, participants will be provided with a full fitness and dietary assessments with report. Such services comprise a substantial consultation value (\$340). Individual results will be sent within one week after the completion of the experimental design.

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

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

Possible discomforts and risks of taking part

The discomforts and risks of taking part in this study, which you will probably be most concerned about are: blood sampling, physical exertion, gastrointestinal distress, heat stress and time commitment.

1. Anthropometrical measures

Height will be measured by a stadiometer and body mass by calibrated weighing scales. These measures are similar to those consistently measured for health monitoring in the GP setting, which pose no direct risk. Four site skinfold (triceps, biceps, subscapular, and suprailiac) measurements in triplicate will be conducted by a trained researcher. This technique poses no risk and will consist of only a mild cutaneous pressure for approximately three seconds at each site. Some participants may find this procedure similar to a tight pinch.

2. Blood sampling

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

3. Physical exertion

The physical components of this study include; the incremental aerobic fitness test and multiple 2 hour steady state running components. The incremental aerobic fitness test will only require you to run at your maximal capacity for approximately 1 minute. You most probably feel the same type of exhaustion and fatigue for longer periods during your normal training and competition habits. The 2 hours running exercise bouts at 60% of maximal effort may promote post-exercise fatigue. However, they are of a similar volume and intensity of effort to that experienced during your training and competitions. As such, the 2 hours running exercise components can also be seen as useful intensive and prolonged training sessions. Identical to exercise during training and competition, some participants may find the exercise element results in a variety of common symptoms: dizziness, headache, thirst, nausea, light-headedness, fainting, muscle and joint soreness.

4. Gastrointestinal distress and low appetite

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

5. Exercising in the heat

Identical to exercise during training and competition in the heat, some participants may find the exercise in the heat element results in a variety of common symptoms: dizziness, headache, thirst, nausea, light-headedness, fainting, mild dehydration and thermal discomfort. To monitor your body temperature for safety reasons, you will be asked to self-insert a thermal coupling 12cm in the rectal sphincter. The procedure of thermal coupling self-insertion is extremely easy, not harmful, and standard practice in thermoregulatory measurements. If your body temperature increases too much (2.5°C over resting levels) you will be asked to stop exercising and cooled immediately by the research team. In addition, we will also monitor your level of thermal comfort during exercise.

6. Time commitment

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

- One occasion for the initial assessment (approx. 1 hour).
- 4 occasions for the exercise trials (approx. 7 hours each).

Total time required: 29 hours

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

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

What will happen to your data?

To ensure protection of participants' right to privacy to the best of our ability, confidentiality will be maintained throughout data collection. All information collected during the study will be coded and treated confidentially. All data is anonymous as soon as it is collected and will be stored electronically using participant codes so that individuals cannot be identified. Any data from your participation in the study will be used by the research team. It may also be disseminated

externally at scientific conferences and publication in scientific journals to inform academic and professional practice; however your name or identity will remain anonymous. Data will be retained in the Department for at least five years and destroyed thereafter.

Complaints

Should you have any concerns or complaints about the conduct of the project, you are welcome to contact the Executive Officer, Monash University Human Research Ethics (MUHREC): Executive Officer, Monash University Human Research Ethics Committee (MUHREC) [REDACTED] Research Office Monash University VIC 3800. Tel: [REDACTED] [REDACTED] Email: [REDACTED] Fax: + [REDACTED]

For any further questions or specific research information please feel free to contact the research team.

Rhiannon Snipe: [REDACTED]
Dr Ricardo Costa: [REDACTED]

EXPLANATORY STATEMENT**Project Title: Internal Cooling Study****Project Number: CF15/3845 - 2015001683****Dr Ricardo Costa**

Department of Nutrition and Dietetics

Phone: [REDACTED]

email: [REDACTED]

Rhiannon Snipe

(PhD Candidate)

Phone: [REDACTED]

email: [REDACTED]

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

Background: Exposure to hot temperatures during physical exertion is common amongst many activities within health care, security, industry and sport. Such exertional-heat stress has consistently been associated with gut distress and loss of appetite, resulting in an inability to maintain work-output and/or withdrawal from activity; constituting both a productivity and financial burden. The underlying causes appear to be multi-factorial with increased core temperature likely contributing to gut distress and an overall reduction in food and fluid intake. Gut distress during exertion in the heat has also been linked to heat illnesses and other medical conditions. Preliminary laboratory-based research has shown that increased ambient temperatures result in increased core temperatures and gut distress. To date it is unknown if lowering core body temperature through consumption of cold beverages can reduce gut distress, the risk of heat illness and further medical complications.

What is the purpose of this research study?

The study aims to examine the impact of internal cooling through consumption of ice and cold water during running in hot ambient temperatures on markers of gastrointestinal damage, perceptible gastrointestinal symptoms and heat illness risk.

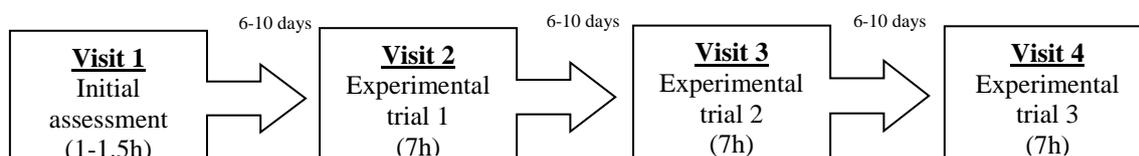
What does participation in this research project involve?

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

- Complete an initial assessment (approximately 1-1.5h in duration) that will include anthropometrical measures (height, weight, and 7-site skinfold [triceps, biceps, subscapular, suprailiac, abdominal, thigh and calf]) and a maximal aerobic exercise test (~15-25 minutes) on an electric treadmill. The initial assessment and all experimental trials be conducted at the BASE Facility- Sport & Exercise Dietetics Clinic.

Completing the experimental trials

- You will only be required to take part in **three experimental trials** in a random order usually conducted one week apart (or every 6-10 days).



All Trials: Involve a 2 hour run on an electric treadmill at 60% of maximal effort (e.g. easy long-run pace) in 35°C ambient temperature with water intake as follows:

- 650ml thermoneutral (19-21°C) water pre-exercise & 230ml every 20mins during exercise.
- 650ml cold (3-4°C) water pre-exercise & 230ml every 20mins during exercise.
- 650ml ice cold (-1°C) water pre-exercise & 230ml every 20mins during exercise.

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

- Consume the study food provided for 24 hours prior to each experimental trial. The study food provided are low in fermentable carbohydrates and will be individually tailored to meet your pre-exercise requirements. As part of this meal plan a standardised breakfast will need to be consumed at home 2 hours before the exercise trials. All study food will be provided in advance and can be consumed at home or as part of your normal daily routine.
- Complete a basic food and exercise diary during the study.
- Provide a urine and faecal sample before and during recovery from the exercise trials.
- To have blood samples taken from the antecubital vein by a trained researcher. These will occur immediately before, after and during recovery from the exercise trials.
- Consume 100ml of a sugar drink at 90 minutes during the running exercise and provide a breath sample every 30 minutes thereafter.
- Have body mass measured before, after and every 30 minutes during each exercise trial.
- Wear a heart rate monitor during each trial, in which heart will be constantly monitored and recorded every 10 minutes. Answer questions relating to exertion and gastrointestinal symptoms every 10 minutes during running exercise.
- To constantly monitor and record core body temperature every 5 minutes by researchers, for health and safety reasons, self-insert a 2.5mm thick thermal coupling probe 12cm above the anal sphincter. The procedure of thermal coupling self-insertion is extremely easy, not harmful, and standard practice in thermoregulatory measurements. If your body temperature increases too much (2.5°C over resting levels), you will be asked to stop exercising and cooled immediately by the research team using cool wet towels and fans until your body temperature reduces back to general resting values.
- After each experimental trial, you will be provided with a recovery meal and drink that meets the recovery nutrition and hydration guidelines and recommendation.

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

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

Why were you chosen for this research?

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

Consenting to participate in the project and withdrawing from the research

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

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

Advantages of taking part

As part of the experimental design that will be conducted at the BASE Facility- Sports & Exercise Dietetic Clinic, participants will be provided with a report of the full fitness assessment and physiological responses on completion of the study. Such services comprise a substantial consultation value (\$340). Individual results will be sent within two weeks after the completion of the experimental design.

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

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

Possible discomforts and risks of taking part

The discomforts and risks of taking part in this study, which you will probably be most concerned about are: blood sampling, physical exertion, gastrointestinal distress, heat stress and time commitment.

1. Anthropometrical measures

Height will be measured by a stadiometer and body mass by calibrated weighing scales. These measures are similar to those consistently measured for health monitoring in the GP setting, which pose no direct risk. Seven site skinfold measurements in duplicate will be conducted by a trained researcher. This technique poses no risk and will consist of only a mild cutaneous pressure for approximately three seconds at each site. Some participants may find this procedure similar to a tight pinch.

2. Blood sampling

Only qualified phlebotomy trained researchers with experience at performing this procedure will collect blood samples. To ensure you are completely comfortable with giving blood samples, we will familiarise you with the blood sample collection procedure during the initial assessment. The amount of blood collected during each trial (6ml) will not impact upon normal physiological functioning. Some participants may find this procedure may create a bruise around the puncture site. Fainting may also occur in 'at risk' participants. Familiarising you with the blood sample collection procedure during the initial assessment will determine if you are an 'at risk' participant for fainting during blood sampling.

3. Physical exertion

The physical components of this study include; the incremental aerobic fitness test and multiple 2 hour steady state running components. The incremental aerobic fitness test will only require you to run at your maximal capacity for approximately 1 minute. You most probably feel the same type of exhaustion and fatigue for longer periods during your normal training and competition habits. The 2 hours running exercise bouts at 60% of maximal effort may promote post-exercise fatigue. However, they are of a similar volume and intensity of effort to that experienced during your training and competitions. As such, the 2 hours running exercise components can also be seen as useful intensive and prolonged training sessions. Identical to exercise during training and competition, some participants may find the exercise element results in a variety of common symptoms: dizziness, headache, thirst, nausea, light-headedness, fainting, muscle and joint soreness.

4. Gastrointestinal distress

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

5. Exercising in the heat

Identical to exercise during training and competition in the heat, some participants may find the exercise in the heat element results in a variety of common symptoms: dizziness, headache, thirst, nausea, light-headedness, fainting, mild dehydration and thermal discomfort. To monitor your body temperature for safety reasons, you will be asked to self-insert a thermal coupling 12cm in the rectal sphincter. The procedure of thermal coupling self-insertion is extremely easy, not harmful, and standard practice in thermoregulatory measurements. If your body temperature increases too much (2.5°C over resting levels) you will be asked to stop exercising and cooled immediately by the research team. In addition, we will also monitor your level of thermal comfort during exercise.

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

6. Time commitment

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

- One occasion for the initial assessment (approx. 1-1.5 hour).
- 3 occasions for the exercise trials (approx. 7 hours each).

Total time required: 22 hours

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

Confidentiality & data storage

To ensure protection of participants' right to privacy to the best of our ability, confidentiality will be maintained throughout data collection. All information collected during the study will be coded and treated confidentially. All data is anonymous as soon as it is collected and will be stored electronically using participant codes so that individuals cannot be identified. Any data from your participation in the study will be used by the research team. It may also be disseminated externally at scientific conferences and publication in scientific journals to inform academic and professional practice; however your name or identity will remain anonymous. Data will be retained in the Department for at least five years and destroyed thereafter.

Complaints

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

Executive Officer

Monash University Human Research Ethics Committee (MUHREC)

[REDACTED]

[REDACTED]

Monash University VIC 3800

Tel: + [REDACTED]

Email: [REDACTED]

Fax: [REDACTED]

[REDACTED]

Thank you,

[REDACTED]

Dr Ricardo Costa

Appendix C



MONASH University

CONSENT FORM

Project: Impact of exertional heat stress on gastrointestinal integrity and markers of appetite regulation

Chief Investigator: Dr Ricardo Costa

Student Researcher: Rhiannon Snipe

I have been asked to take part in the Monash University research project specified above. I have read and understood the Explanatory Statement and I hereby consent to participate in this project.

I consent to the following:	Yes	No
1. I confirm that I have read and understand the Participant Information Sheet dated 1 st September 2014 for the above study. The nature, demands, and risks of the project have been explained to me. I have had the opportunity to consider the information, ask questions, and have had these answered satisfactorily by researchers.	<input type="checkbox"/>	<input type="checkbox"/>
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason, without penalty, and without my medical care or legal right being affected.	<input type="checkbox"/>	<input type="checkbox"/>
3. I understand that I may register any complaint I might have about this study with the Chief Investigator, and that I will be offered the opportunity of providing feedback on the study using the standard report forms.	<input type="checkbox"/>	<input type="checkbox"/>
4. I fully understand the risks involved in participating in the project and agree to these risks. I agree to take part in the above study and understand that Monash University and the research team have implemented strategies to manage these risks. I however take responsibility for participating in the above study.	<input type="checkbox"/>	<input type="checkbox"/>
5. I agree to allow my data to be used by the researchers and other academic for the advancement of scientific knowledge and disseminated within the scientific and academic arena with confidentiality being maintained throughout.	<input type="checkbox"/>	<input type="checkbox"/>
6. I agree to allow my data to be used in future research.	<input type="checkbox"/>	<input type="checkbox"/>

Name of Participant _____

Participant Signature _____ Date _____

CONSENT FORM

Project: Internal Cooling Study

Project Number: CF15/3845 - 2015001683

Chief Investigator: Dr Ricardo Costa

Student Researcher: Rhiannon Snipe

I have been asked to take part in the Monash University research project specified above. I have read and understood the Explanatory Statement and on signing this document I am consenting to participate in this project.

	Yes	No
1. I confirm that I have read and understand the Explanatory Statement dated 12 th November 2015 for the above study. The nature, demands, and risks of the project have been explained to me. I have had the opportunity to consider the information, ask questions, and have had these answered satisfactorily by researchers.	<input type="checkbox"/>	<input type="checkbox"/>
2. I agree to providing blood, breath, urine and faecal samples in accordance with the requirements of the project as detailed in the Explanatory Statement and explained by the research team.	<input type="checkbox"/>	<input type="checkbox"/>
3. I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason, without penalty, and without my medical care or legal right being affected.	<input type="checkbox"/>	<input type="checkbox"/>
4. I understand that I may register any complaint I might have about this study with the Chief Investigator, and that I will be offered the opportunity of providing feedback on the study using the standard report forms.	<input type="checkbox"/>	<input type="checkbox"/>
5. I fully understand the risks involved in participating in the project and agree to these risks. I agree to take part in the above study and understand that Monash University and the research team have implemented strategies to manage these risks. I however take responsibility for participating in the above study.	<input type="checkbox"/>	<input type="checkbox"/>
6. I agree to allow my data to be used by the researchers and other academic for the advancement of scientific knowledge and disseminated within the scientific and academic arena with confidentiality being maintained throughout.	<input type="checkbox"/>	<input type="checkbox"/>
7. I agree to allow my data to be used in future research.	<input type="checkbox"/>	<input type="checkbox"/>

Name of Participant _____

Participant Signature _____ Date _____

Appendix D



Monash University
Department of Nutrition & Dietetics



PRE-EXERCISE QUESTIONNAIRE

Name..... Date.....

Date of Birth..... Gender (please circle): M / F

1. Are you in good health? Yes No If no, explain:

2a. How would you describe your present level of activity? (Tick as appropriate.)

Vigorous		Moderate		Light	
----------	--	----------	--	-------	--

2b. How often do you exercise? (Tick as appropriate)

< once per month		1-3 times per month		Once per week	
2-3 times per week		4-5 times per week		≥6 times per week	

2c. Exercise type(s).....

2d. Approximately how many hours per week do you exercise?.....

3. Do you suffer, or have you ever suffered from the following: (Tick as appropriate.)

Asthma		Epilepsy		Gastrointestinal surgery	
Diabetes		High blood pressure		Food allergy	
Bronchitis		Heart conditions		Coeliac disease	
Anxiety		Low Vitamin D levels		Food intolerances	
Depression		Heat-related illness'		Irritable bowel syndrome	

4. Have you suffered from a serious illness or accident? Yes No
If yes, explain:

5. Do you avoid any particular foods? Yes No
If yes, explain (what food & reason):

6. Are you currently taking any medication or supplements? Yes No
If yes, explain:

7. Have you taken probiotic supplements or used laxatives in the last three months? Yes No

8. Are you currently attending the GP for any condition or have you consulted your doctor in the last three months? If yes, explain: Yes No

9. Have you, or are you, presently taking part in any other laboratory experiment? Yes No
If yes, explain:

15.. Have you travelled (interstate or international) in the past 2 weeks and/or are you planning to travel during the next 6-8 weeks? **Yes** **No** If yes, explain:

16. ***Females only:***

Length of menstrual cycle (days)..... Date of last menstruation.....

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to do the experimental/testing exercise task if they:

- Have a fever, cough, cold, or suffer from fainting spells or dizziness.
- Have suspended training due to a joint or muscle injury.
- Have a known history of medical disorders, i.e. high blood pressure, heart or lung disease.
- Have a hyper/hypothermia, heat exhaustion, or any other heat or cold disorder.
- Have anaphylactic shock symptoms with needles, probes, or other medical-type equipment.
- Have chronic or acute symptoms of gastrointestinal bacterial infections.
- Have a history of infectious disease (e.g. HIV, Hepatitis B), and if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other conditions of the rectum.

DECLARATION

I agree that I have none of the above conditions and I hereby volunteer to be a participant in experiments / tests during (date/s).

My replies to the above questions are correct to the best of my knowledge and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment/test and possible risks involved.

I understand that I may withdraw from the experiment/test at any time and that I am under no obligation to give reasons for withdrawal or to a return for experimentation. I also understand that if I fail to complete an experiment/test, I will not be able to obtain or have access to the results due to absence of results.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment/test, will neither be detrimental to, or further, my position as a student.

I undertake to obey laboratory/study regulations and the instructions of the experimenter/tester regarding safety, subject only to my right to withdraw declared above.

Participant signature.....

Name of experimenter/tester.....

Signature..... Date.....

WHEN COMPLETED- ONE COPY TO RESEARCHER FILE

Appendix E

Likert-Type Rating Scale of Gastrointestinal Symptoms

Period: _____

	No symptoms						Extremely Bad Symptoms				
	0	1	2	3	4	5	6	7	8	9	10
Overall Gut Distress											
Upper Gastrointestinal Symptoms											
Belching	0	1	2	3	4	5	6	7	8	9	10
Heartburn	0	1	2	3	4	5	6	7	8	9	10
Bloating (stomach fullness)	0	1	2	3	4	5	6	7	8	9	10
Stomach cramps	0	1	2	3	4	5	6	7	8	9	10
Urge to vomit	0	1	2	3	4	5	6	7	8	9	10
Vomit	0										10
Lower Gastrointestinal Symptoms											
Flatulence (lower abdominal bloating)	0	1	2	3	4	5	6	7	8	9	10
Urge to defecate	0	1	2	3	4	5	6	7	8	9	10
Left intestinal cramps	0	1	2	3	4	5	6	7	8	9	10
Right intestinal cramps	0	1	2	3	4	5	6	7	8	9	10
Loose stools	0										10
Diarrhoea	0										10
Bleeding	0										10
Other Gastrointestinal Symptoms											
Nausea	0	1	2	3	4	5	6	7	8	9	10
Dizziness	0	1	2	3	4	5	6	7	8	9	10
Stitch	0	1	2	3	4	5	6	7	8	9	10
Low High											
Taste fatigue (cannot face the same food/fluid)	0	1	2	3	4	5	6	7	8	9	10
Interest in food (I want to eat)	0	1	2	3	4	5	6	7	8	9	10
Interest in drink (I want to drink)	0	1	2	3	4	5	6	7	8	9	10
Tolerance to food (I could eat)	0	1	2	3	4	5	6	7	8	9	10
Tolerance to drink (I could drink)	0	1	2	3	4	5	6	7	8	9	10
Low hunger High hunger											
Appetite (hunger scale)	0	1	2	3	4	5	6	7	8	9	10
Thirst	0	1	2	3	4	5	6	7	8	9	10

Borg Scale

6	
7	Very very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very very hard
20	

Borg, G.A.V., (1982). Psychophysical bases of perceived exertion. MSSE, 14(5):377-381.

Thermal Comfort Rating

McGinnis 13-point Thermal Rating Scale

13	Unbearably hot
12	Extremely hot
11	Very hot
10	Hot
9	Uncomfortably warm
8	Warm but comfortable
7	Comfortable
6	Warm

Hollies, N. R. S., & Goldman, R. F. (1977). Psychological scaling in comfort assessment. In N. R. S. Hollies, & R. F. Goldman (Eds.), *Clothing comfort: Interaction of thermal, ventilation, construction, and assessment factors* (pp. 107_120). Michigan, USA: Ann Arbor Science Publishers Inc.