

**Effects of Exercise Intensity, Modality and Environment on Gastrointestinal
Permeability, Damage and Symptomology in Healthy Males.**

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Executive summary.

It is becoming accepted that exercise increases gastrointestinal (GI) symptomology and permeability potentially effecting performance and recovery. This thesis addresses the issues of how exercise intensity, modality and pattern of exercise will impact upon GI permeability, damage and symptomology expression. Further it will examine how these factors may be modified by the environmental conditions under which they take place and by the use of pharmacological agents. In study 1 chapter 4, six male soccer players undertook both a 90-min rest or soccer specific intermittent exercise protocol (SSIE) under two environmental conditions (Hot 32°C or Cold 12°C) to evaluate how GI permeability and symptomology was affected by simulated soccer match play activity. SSIE elevated GI permeability relative to rest in both hot and cold conditions but these changes were only significant in the protocols undertaken in the heat. Such differences potentially reflecting the attenuated exercise intensity elicited by the protocol. However, exercise and rest in the heat relative to cold was associated with significantly higher GI permeability and wider array of subjective gastrointestinal symptomology.

In study 2 chapter 5 the activity patterns typically experienced in soccer i.e. continuous and intermittent running were compared when 10 male participants undertook a series of protocol (s); rest, continuous steady state and intermittent exercise performed at the same 'relative intensity' of 70% $\dot{V}O_2$ peak. Interaction with environmental stressors in the Hot 32°C or Cold 12°C on GI permeability and subjective GI symptomology was determined. GI permeability increased under both continuous and intermittent exercise compared to rest. No differences between continuous and intermittent exercise patterns were observed when undertaken in the cold. However, a stepwise increase in permeability was noted in the heat: Rest < SS < HIIT. Minimal expression of GI symptoms was noted and these were unrelated to the objective GI permeability markers. When relative exercise intensity is controlled for at 70% of a velocity associated with $\dot{V}O_2$ peak no difference in GI permeability occur between HIIT and steady state exercise when this is undertaken in the cold. This response is abolished when exercise is undertaken in under HOT conditions but does not attain significance.

Study 3 chapter 6 using a double blind repeated measures design examined the effects of HIIT exercise and the co-administration of Non-Steroidal Anti Inflammatory Drugs (NSAIDs) upon GI permeability and symptomology. Twelve trained intermittent games players participated. It was observed that HIIT exercise consisting off, repeated sprint activity [4 sets x 6 x 35 m (< 6s)] does not increase GI permeability relative to rest. Further when NSAIDS

(2 x 400 mg Ibuprofen) are added to this model no further changes in gut permeability and symptoms are observed above that of the relevant control. These data suggest that following the present dosing regimen in trained male games players GI permeability and symptoms are unaffected by a single bout of HIIT exercise. Taken together the HIIT exercise model undertaken here and the co administration of Ibuprofen do not increase GI permeability seen with longer duration exercise.

Finally, study 4 chapter 7 addressed whether exercise modality running vs cycling may be important in the development of GI disturbances given the epidemiological data that reports higher GI symptomology during and after running. Six male triathletes undertook three separate trials; a steady state 1000 kilojoule (KJ) cycling work test at 70% $\dot{V}O_2$ peak, an equivalent treadmill running protocol matched on total energy expenditure and equivalent period of non-exercise. Under these conditions GI permeability, as expressed by L:R ratio and GI symptoms were examined. Data indicate relative to rest an increase in GI permeability but indicate no modality specific differences in GI permeability and symptom expression between running and cycling. Running relative to cycling is associated with higher albeit still relatively limited subjective GI symptoms contrasting the equivalence seen in GI permeability L:R ratios. This disassociation in subjective symptoms and objective GI permeability in triathletes requires further consideration as regards mechanism of action and causality between these markers.

This thesis has considered the effect of exercise intensity, modality and exercise patterning and their interactions with environmental stress upon objective and subjective markers of GI Function. Data suggest that exercise induced increases in GI permeability relative to rest occur when the exercise intensity and duration exceed a critical threshold of ~70 % peak aerobic capacity for at least 50 minutes. Manipulation of exercise patterning i.e. HIIT vs continuous undertaken at the same relative intensity shows no difference in GI permeability when undertaken in the cold relative to the heat. Undertaking exercise in a Hot (32°C) environment accentuates permeability. Subjective GI symptomology does not mirror changes in the objective GI permeability markers with all subjective data indicating registering limited symptomology. It was further observed that HIIT exercise consisting of supra-maximal, short duration repeated sprints (<6s) performed on a repeated basis does not alter GI permeability. When NSAIDs are co-ingested with this model no further changes in gut permeability and symptoms are observed. Finally, exercise modality does not impact alter GI permeability and the relationship to GI symptomology.

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List of Abbreviations

CHO	Carbohydrate
EE	Energy Expenditure
FC	Faecal calprotectin
GI	Gastrointestinal
HB	Haemoglobin
HCT	Haematocrit
HIIT	High Intensity Interval Training
HIIT _{HOT}	High Intensity Interval Training in hot condition
HIIT _{COLD}	High Intensity Interval Training in cold condition
HPLC	High-performance liquid chromatography
HR	Heart Rate
I-FABP	Intestinal Fatty Acid Binding Protein
I-BPF	Ibuprofen
L:R	Lactulose/L-rhamnose
LPS	Lipopolysaccharide
LBP	Lipopolysaccharide-Binding Protein
NSAIDs	Non-Steroidal Anti Inflammatory Drugs
PVC	Plasma volume
RHOT	Rest in Hot condition
RCOLD	Rest In cold condition
RPE	Rate Perceived Exertion
SAT	Sugar Absorption Test
SS	Steady State
SSHOT	Steady State in hot condition
SSCOLD	Steady State In cold condition
SSIE	Soccer Specific Intermittent Exercise

TC	Thermal Comfort
$\dot{V}O_2 \text{ max}$	Maximal Oxygen Uptake
$\dot{V}O_2 \text{ peak}$	Peak Oxygen Uptake

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Presented abstracts arising from this thesis:

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Hamzah,N., Matt Malone, Mark Scott Neil Chester, Simon Fleming and Dominic A. Doran. Influence Of Environmental Temperature On Biomarkers Of Gastrointestinal (GI) Permeability, And GI Symptomology, Following Soccer Specific Exercise. Presented at *Conference on Movement, Health and Exercise (MoHE 2017)*, Kuala Lumpur, September 2017.

Chapter 1 – General Introduction

Introduction

1.1 Background

Physical stress in the form exercise activity represents a challenge to the maintenance of homeostasis. It is through the repeated imposition and manipulation of this stress that positive and negative exercise related changes in health and performance occur principally through the manipulation of frequency, intensity, duration and type of exercise as well as recovery super-compensation and adaptation will occur (Cunanan et al., 2018). Whilst, much is understood about these adaptive responses at both the whole body and molecular level in cardiovascular, muscular and, immunological systems the effects of exercise performed acutely and chronically on the gastrointestinal tract/barrier (GI) has been subject to limited evaluation (Barberio et al., 2015; Costa et al., 2017a; Gil et al., 1998). Given the central role of the gut in nutrient absorption (Janssen Duijghuisen et al., 2016; Pfeiffer et al., 2009) and emerging roles in regulation of immunity (Valdés-Ramos et al., 2010), inflammation (Somsouk et al., 2015), host-microbiota interactions (Marques et al., 2014; Yu, 2012), fatigue (Shukla et al., 2015) and the regulation of heat related injury (Avinash et al., 2017; Vargas & Marino, 2016) further understanding of how the gastrointestinal system is affected by exercise is warranted.

The anatomy and functionality of the gastrointestinal system has been well described; essentially in its basic form consisting of a long tube extending from the oral cavity to the anus with sections adapted to digesting extracting and expelling ingested materials. However, this simplicity of concept is underpinned by a highly complex interrelated physical, immunological, hormonal, central and peripheral neural axis and microbiota regulated pathway(s) that are still been elucidated; pathways whose effects impact across metabolic, physiological, immunological psychological and behavioral constructs (Bischoff, 2011; Stewart et al., 2017) . The "Gastrointestinal Barrier" is a generic term used to describe the combination of physical, cellular and humoral properties of the gastric and intestinal mucosa; a barrier that plays a critical role in regulating the selective transfer of nutrients, whilst excluding potentially harmful substances passing into systemic circulation (Bischoff, 2011; Camilleri et al., 2012; Fasano, 2011; Lambert, 2008; Stewart et al., 2017). However, the integrity of the GI barrier may be compromised by a range of stressors i.e. illness, exercise, heat stress, bacteria, and pharmacological agents (Ashton et al., 2003; Costa et al., 2017b; Grootjans et al., 2013a; Shulman et al., 2014; Vieth & Montgomery, 2017). Loss of GI barrier integrity leads to an increase in gastrointestinal permeability, i.e. the non-mediated transfer of luminal antigenic agents from gut to systemic circulation effecting mild to severe local and systemic inflammatory reactions and adverse gastrointestinal symptoms (Fasano & Shea-

Donohue, 2005; Fehrenbach & Schneider, 2006; Lambert, et al., 2002b; Selkirk, et al., 2008b).

Epidemiological and experimental data report increased symptomology of gastrointestinal disturbance in both the upper and lower GI tract (nausea, regurgitation, wind, vomiting, diarrhoea, cramps, abdominal pain and bloating) in both male and female athletes (Haaf, et al., 2014; Lambert et al., 1999; Peters et al., 1999; Riddoch & Trinick, 1988; ter Steege, et al., 2008; ter Steege, et al., 2012). In particular, athletes that take part in endurance events across a range of duration/distance's seem susceptible to this symptomology and frequently express gastrointestinal symptoms in a range between 4-96% (Costa et al., 2017; Haaf et al., 2014; ter Steege et al., 2008). Interestingly, Worobetz et al. (1985) and Riddoch & Trinick, (1988) have indicated lower frequencies of GI symptomology in cycling and swimming activities relative to running. It may be that the modality of exercise and the relative mechano-physiological stimuli elicited induce changes in GI function, although the mechanism and magnitude of any resultant dysfunction are unquantified.

It has been suggested that the training status of the individual/athlete, the exercise duration and intensity undertaken (Lambert et al., 1999; Pals et al., 1997), as well environmental temperature (Pires et al., 2016) and hydration status (Costa et al., 2016b; Rehrer et al., 1990) may be critical factors predisposing towards the expression of subjective GI symptomology and objective markers of GI dysfunction (Pals et al., 1997; Selkirk et al., 2008). Mechanistic links between the presentation of gastrointestinal symptoms, and disruption to gut mucosa have been ascribed to tissue hyperthermia, reductions in splanchnic blood flow of 50-80 %, due to sympathetic mediated vasoconstriction of the splanchnic vascular bed in order to meet blood flow requirements of the active muscles and cutaneous blood flow to support the transfer of heat from the core to the body surface (Crandall & Gonzalez-Alonso, 2010). This reduction in blood flow being inversely proportional to the percentage of maximal oxygen consumption ($\dot{V}O_{2 \max}$) achieved during exercise performance (Ashton et al., 2003; de Oliveira et al., 2014; Gutekunst et al., 2013; van Wijck, et al., 2011). Impairments in splanchnic blood flow may further predispose toward heat mediated disruption to epithelial, mucus and smooth muscle barriers in the GI wall (Lambert, 2009; Selkirk et al., 2008). Secondary to acute reductions in splanchnic blood flow tissue hypoxia may be expressed due to an ischemia reperfusion cycle and associated oxidative and nitrosative stress (Hayashi et al., 2012; Kannan et al., 2011; Lambert et al., 2002; Mensink et al., 2011; Rowell, 2004; Wu et al., 2017). As a consequence the magnitude of the paracellular penetration of GI luminal antigens may increase, leading to immune activation and inflammation (Fasano, 2012). The mechanism of this 'leaky gut' as it

has been termed, has been associated with disruption to enterocyte tight junction protein expression and impairment of basement membrane function subsequent to up-regulation of pro-inflammatory pathways in humans (Fasano, 2012; Fasano & Shea-Donohue, 2005; Zuhl, et al., 2014b).

Given the importance of maintaining GI splanchnic blood flow and dissipating heat accumulation, knowledge of the impact of exercise patterning such as how the exercise intensity and duration are modulated to alter the training load may have an important bearing upon GI symptomology and permeability. In particular experimental and epidemiological data on both objective and subjective markers of GI dysfunction has generally reported upon a continuum of steady state activities from short [5 k-21k], marathon, ultra-marathon to multi day endurance activities (Gill et al., 2015a; Haaf et al., 2014; Keeffe et al., 1984; Roberts et al., 2016a; Wilson, 2017). The physiological and metabolic responses to intermittent exercise are well established, recent work has considered high intensity intermittent exercise to convey performance and health related benefits in a more time efficient and enjoyable manner than longer duration steady state activity (Burgomaster et al., 2008; García-Pinillos et al., 2017; Jiménez-Pavón & Lavie, 2017) , however the nature of the exercise results in a cyclical pattern of cardiovascular strain and gut perfusion/reperfusion that could impact gut function. Comparison of the relative effects of constant intensity exercise to intermittent exercise with regard to GI symptomology and permeability remains unknown, the effects of very high intensity exercise remains unknown, the effects of these activities under differing environmental stress remains unknown. The combined interaction between steady state and intermittent exercise, similar to that expressed during invasion field games remains unknown. Further work to resolve these considerations are warranted.

Whilst exercise may mediate changes in GI function and contribute to subjective symptom expression its combination with pharmaceutical agents and impact on the gut has come to prominence (McAnulty et al., 2007; Nieman et al., 2006; Tscholl & Dvorak, 2012). Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used over the counter agents used in the acute and chronic treatment and management of soft-tissue injuries and for analgesic purposes in athletes (Da Silva et al., 2015; Holgado et al., 2017; Tscholl, et al., 2016; Vaso et al., 2015). Clinically, NSAID induced GI mucosal damage is a well described adverse effect of their usage (Marlicz et al., 2014). Significantly, NSAIDs such as ibuprofen have previously been found to increase gastrointestinal GI permeability at rest and following exercise particularly after prolonged, sub-maximal endurance events such as marathon and triathlons (Jeukendrup et al., 2000; Küster, et al., 2013; McAnulty et al., 2007; Nieman et al., 2006; Smetanka et al., 1999; Whatmough, et al., 2017). Since the use of NSAIDs in a variety

of sports and individual events is widespread, it is important to determine the effect it has on the GI barrier function especially when they are combined with exercise particularly High Intensity Interval Training (HIIT).

As a result of physical activity whether undertaken for health or performance related outcomes the gastrointestinal system is subject to a range of stressors that are likely to impact upon individual health and wellbeing. Whilst several possible mechanisms of action and pathways to explain these effects are postulated there remains a paucity of data as relates to the effects of exercise intensity, duration, patterning and their environmental interactions. There is a clear need to characterise the impact of these factors upon GI subjective and objective functioning.

1.2 Aims and objectives

The aim of the thesis is to examine the role of exercise, environment and pharmacological agents upon on gastrointestinal permeability, damage and symptomology.

Objectives:

1. To examine the impact of combined continuous and intermittent exercise in the form of soccer specific intermittent exercise (SSIE) under Cold (12 °C) and Hot (32 °C) environmental conditions upon GI permeability and GI symptomology.
2. To examine the individual role of continuous and intermittent exercise performed under Cold (12 °C) and Hot (32 °C) environmental conditions upon GI permeability and symptomology.
3. To examine the effects of high intensity repeated sprint exercise upon GI permeability and secondly to determine the effects of acute NSAID [ibuprofen] ingestion preceding such activity upon GI permeability and symptomology.
4. To examine the role of exercise modality i.e. cycling vs. running upon GI permeability, damage and symptomology.

1.4 Hypothesis

Each research chapter will involve its own hypothesis to be tested.

- Chapter 4: Soccer specific intermittent exercise [SSIE] in the heat will increase GI permeability and symptoms relative to rest and cold condition. It will also be expected that exposure to heat will increase passive GI permeability and symptomology relative to cold.
- Chapter 5: High intensity intermittent (HIIT) and continuous steady state exercise will increase GI permeability and symptomology relative to rest. High intensity intermittent relative to steady state exercise in the Heat (32° C) relative to Cold (12° C) will express higher GI permeability and GI symptomology.
- Chapter 6: GI permeability and symptomology will increase following supramaximal High Intensity Intermittent Exercise (HIIT) relative to rest. HIIT exercise and NSAID ingestion will act synergistically to augment this increase in GI permeability and symptoms relative to placebo and rest conditions.
- Chapter 7: Indices of GI permeability and symptomology will be higher during running relative to cycling when matched for absolute work load and relative exercise intensity.

Chapter 2 – Literature Review

2.0 The Gastrointestinal System.

The gastrointestinal system in its simplest form consists of a hollow muscular tube arising in the oral cavity, continuing through the pharynx, oesophagus, stomach and intestines to the rectum and anus, where food is eventually expelled. A variety of other accessory organs (salivary glands, liver, pancreas and gall bladder) contribute to this digestive and absorption process through the secretion of a different enzymes to break down the food into its component nutrients. Through the action of the muscular walls of the gut facilitating a peristaltic action, the ingested materials are moved along the length of the GI tract.

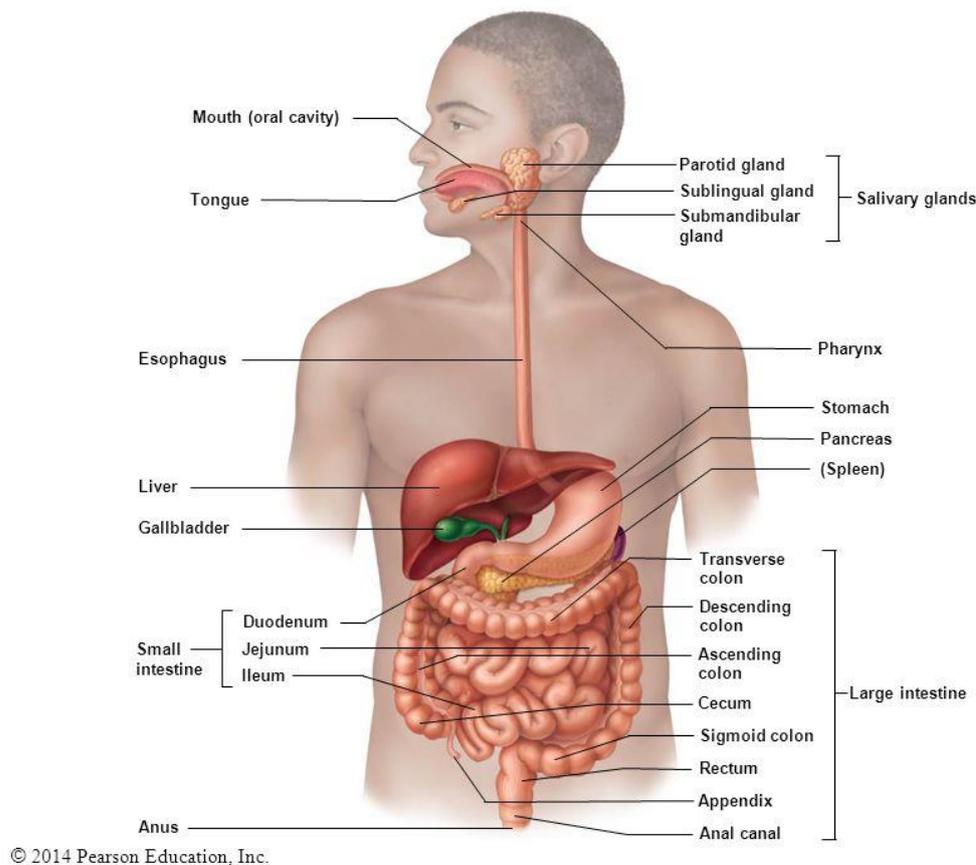


Figure 2.1 Schematic representation of the Human GI system. (Pearson Education 2009)

The primary purpose of the gastrointestinal system is to facilitate the digestion of ingested material and absorption of ingested fluids in addition to excretion and immunological regulation (Cheng et al., 2010). In its simplest form food must be ingested into the mouth to be mechanically processed and moistened via mastication. Secondly, digestion occurs mainly in the stomach and small intestine where proteins, fats and carbohydrates are chemically broken down into their basic building blocks. Smaller food derived molecules are then absorbed across the epithelium of the small intestine and subsequently enter the

circulation. The large intestine (colon) plays a key role in reabsorbing water and the compaction and storage of waste in the sigmoid colon and rectum prior to elimination. In relation to the questions under consideration in this thesis the primary portion of the GI tract under consideration is the small intestines. It this structure which will be briefly reviewed in relation to its anatomy and functions.

2.1 The Small intestine.

The small intestine has three major components; the duodenum, jejunum, and ileum. Structurally it is approximately 6m in length, extending from the pyloric sphincter of the stomach to the ileo-caecal valve that separates the ileum from the caecum. The small intestine is compressed into numerous folds and occupies a large proportion of the abdominal cavity (Figure 2.1). The small intestine performs the majority of digestion and absorption of nutrients. The duodenum is the proximal C-shaped section that facilitates the mixing of digestive enzymatic secretions from the pancreas and liver with the contents expelled from the stomach. The start of the jejunum is defined by the duodeno-jejunal flexure, it is in the jejunum where the majority of digestion and absorption of nutrients occurs. The final portion, the ileum, is the longest part of the small intestine and empties into the caecum at the ileocaecal junction, prior to entry into the large intestine. The small intestine is lined with specialist cells arranged into permanent folds called plicae circulares. Each plica has numerous villi (folds of mucosa) and each villus is covered by epithelium with projecting microvilli (brush border), the primary functional role being to increase the surface area for absorption (Figure 2.2).

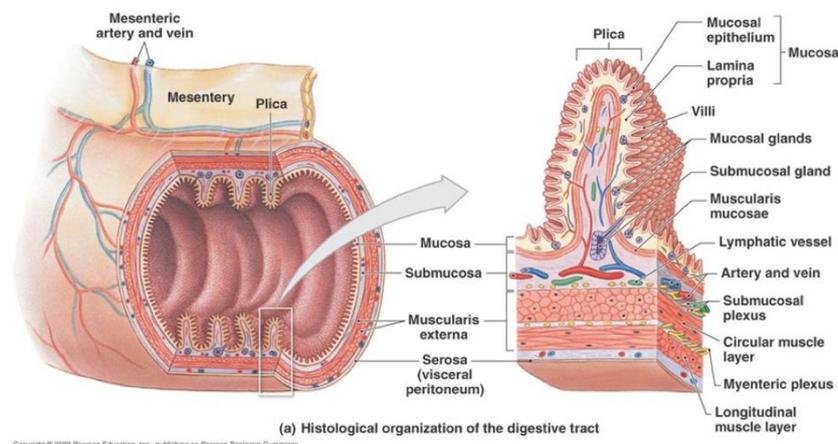


Figure 2.2 Schematic cross sectional representation of the small intestine Human GI system. (Pearson Education 2009).

The mucosa of the small intestine contains several specialized cells. Some are responsible for absorption, whilst others secrete digestive enzymes and mucous to protect the intestinal lining from digestive actions (Grootjans et al., 2016). In addition, the gut provides a barrier between the systemic circulation and potentially toxic intra-luminal antigens (Grootjans et al., 2016). In order to facilitate this function, the GI tract is equipped with several barriers. It is this element of the GI tract that this review will now consider.

2.2 The Gastrointestinal Barrier: Structure and Function

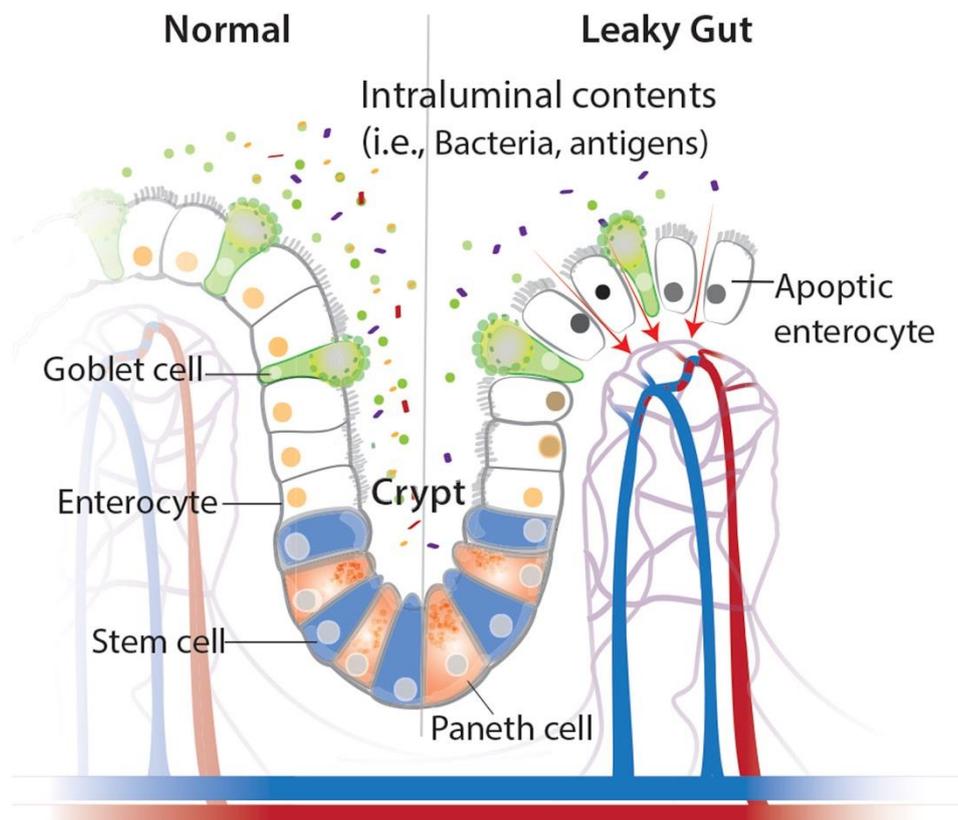


Figure 2.3 Schematic cross sectional representation of the small intestine Human GI system. (Taken from Stewart et al., 2016).

The gastrointestinal (GI) barrier from a structural perspective can be described in terms of two key components: (1) the intrinsic barrier comprising a monolayers of absorptive enterocyte cells and lining epithelial cells with associated tight junction proteins between them; and (2) the extrinsic barrier; comprising specialised secretions (mucins), immunological cells in close contact with both the epithelial cells and external environment of

the lumen (Bischoff et al., 2014; Fasano et al., 2011; Stewart et al., 2016; Zuhl et al., 2014). In terms of its function, the intestinal barrier serves two main purposes: (1) to act as a filter to allow the absorption of essential nutrients from the intestinal lumen into the circulation and (2) a preventative barrier/mechanism inhibiting the translocation of antigenic agents and endotoxin i.e. lipopolysaccharide from the lumen of the GI tract (Camilleri et al., 2012; Farhadi et al., 2003). Maintaining an intact and effective GI barrier is critical in the maintenance of general health, the prevention of oxidative stress related tissue damage and disease through implementing a selective transport process (Camilleri et al., 2012; Farhadi et al., 2003). A range of specialised membrane proteins (tight junction proteins) of the intrinsic barrier connect adjacent cells on the apical and lateral membranes, to form an extracellular selectively permeable barrier or 'Tight Junction' (Figure 2.4). These proteins include a wide range of protein families that include claudin, occludin and junctional adhesion molecules (JAM) (González-Mariscal et al., 2003; Fasano et al., 2011). Whilst a large number of these proteins are now identified it is likely that those of the Claudin family and its distinct primary isoforms 1, 2 and 3 are functionally responsible for cell adherence, whilst those of the occludin family act to partially regulate tight junction integrity (Doklandy et al., 2016; Zuhl et al., 2014). However, the complexity of tight junctions is such that their integrity and function are also associated with other peripheral scaffolding proteins (e.g. Zonulin) which are in turned linked to actin and microtubules scaffolds (Van Itallie & Anderson et al., 2014). This complex network of regulatory proteins is also associated with other signaling proteins that affect the barrier and broader cell functions through mediating alterations in their phosphorylation states (Gonzalez-Mariscal et al., 2008). These tight junctions are estimated to exclude substances with a radius exceeding 15 Å (~3.5 kDa) passing via a paracellular pathway (Vojdani et al., 2013). Functionally, through these structural and signalling pathways this barrier regulates selective movement of ions, water and nutrients, and helps protect against leakage of luminal related antigens (Camilleri et al., 2012). It is this leaky gut that has been associated with the widespread reports of GI symptoms during and following exercise activity. In the following section this aspect of exercise and the GI system will be explored

2.3 Epidemiology and Symptomology of Gastrointestinal Dysfunction.

Epidemiological and experimental data report increased expression of GI symptomology and disturbance in both the upper and lower GI tract (nausea, regurgitation, wind, vomiting, diarrhoea, cramps, abdominal pain and bloating) in both male and female athletes (Haaf et al., 2014; Lambert et al., 1999; Peters et al., 1999; Riddoch & Trinick, 1988; ter Steege, et al., 2008; ter Steege et al., 2012). In particular, athletes that take part in endurance events across a range of duration/distance's seem susceptible to this symptomology and frequently

express gastrointestinal symptoms in a range between 4-96% (Costa et al., 2017; Haaf et al., 2014; Peters et al., 1999; ter Steege et al., 2008). Symptom severity presented in such events range from mild (wind, bloating) to severe (acute colitis, faecal occult blood, chronic ischemia) the latter symptoms being expressed particularly at the extreme endurance event end of the scale (Cohen et al., 2009; Costa et al., 2016; Gill et al 2016; Grames & Berry-Cabán, 2012; Jeukendrup et al., 2000; Pfeiffer et al., 2012; Roberts et al., 2016a; Stuempfle & Hoffman, 2015; Stuempfle et al., 2016). Consequently, a graded response across literature is evident in symptom frequency and severity which may be considered as a function of distance covered (Costa et al., 2016b; Haaf et al., 2014; Riddoch & Trinick, 1988). It is evident that as athletes move from shorter distances such a marathon to ultra-distances and multistage races there is a more consistent rise in symptom expression and severity (Gill et al., 2015; Stuempfle & Hoffman, 2015). A predominance towards either upper or lower gastrointestinal symptom expression is also not consistently apparent across endurance activities (Snipe, et al., 2017; ter Steege et al., 2008). Interestingly, a dichotomy exists in that most experimental studies measure small intestine function (Gill et al., 2015; Lambert et al., 2008; March et al., 2017; McKenna et al., 2017; Playford et al., 2001; Pugh et al., 2017; Smetanka et al., 1999) whilst the data still indicate significant symptomology in the large intestine which has been largely ignored experimentally (Lambert, 2004).

In terms of the general aetiology of such factors; age, gender, environment, nutrition both pre and in-event as well as clinical predisposition to GI issues may be contributory factors (Lambert et al., 1999; Packer & Hoffman-Goetz, 2012; Pfeiffer et al., 2012; Wright et al., 2011). The complex nature of the GI system and its functions, most likely mean that there is probably no single causal factor that predisposes to GI dysfunction. Several recent narrative and systematic reviews suggest there are complex pathways involved which may cause GI dysfunction and symptomology (Costa et al., 2017; van Wijck et al., 2012; ter Steege et al., 2012).

2.4 Gastrointestinal permeability.

Gastrointestinal permeability refers to the non-mediated translocation of low molecular weight particles through the mucosal and endothelial membranes via paracellular and/or transcellular pathways (Figure 2.4) (Arrieta et al., 1996; Camilleri et al., 2012). As such 'GI permeability' is a description of the functional status of the GI barrier and can be assessed by measuring the rate of movement of 'measurement probes' across the GI barrier. In defining permeability, it must be remembered that permeability is a normal function of the GI barrier and essential to maintaining health and function (Vojdani et al., 2013). It is only when this GI barrier is subject to a breach that facilitates the transfer of substances that should

be exclude mediates adverse physiological responses or pathology development (Bischoff et al., 2014; Fasano et al., 2011). The following sections shall explore the assessment of GI permeability.

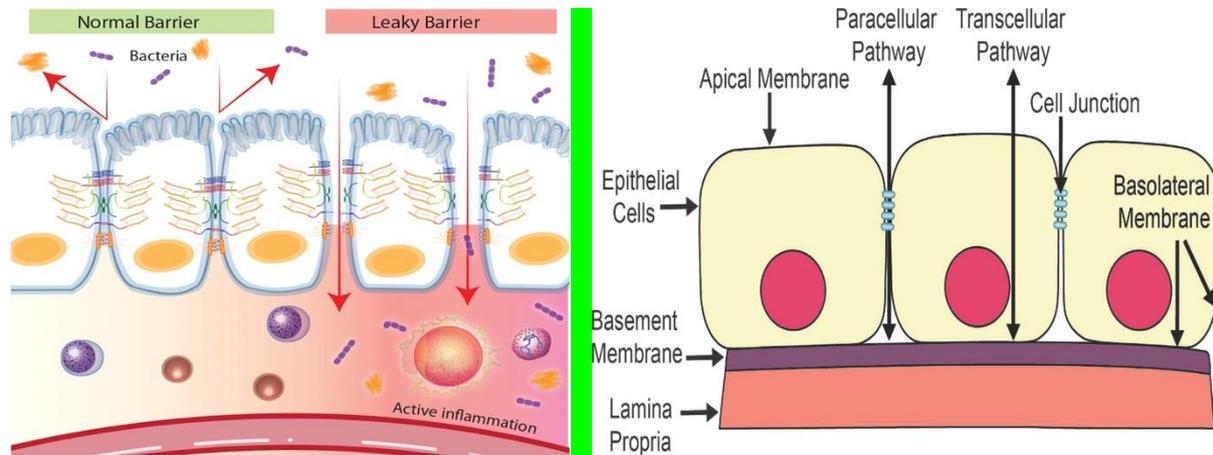


Figure 2.4 Schematic representation of the GI Barrier with barrier integrity maintained and disrupted with pathways of translocation outlined (After Stewart et al., 2016).

2.5 Gastrointestinal Permeability Assessment.

Gastrointestinal permeability has been evaluated using a number of techniques in both human and animal models (Lambert et al., 2009; Rao et al., 2011). Determination of GI permeability is commonly applied in clinical and research settings and is usually determined by the differential urinary excretion or serum appearance of orally administered non-digestible, sugar probes sometimes termed the ‘Sugar Absorption Test’ [SAT] (Camilleri et al., 2012; Fleming et al., 1996; Haase et al., 2000; van Wijck et al., 2012; van Wijck et al., 2013) (Table 2.1a). Using a combination of inert ‘sugar’ probes that vary in molecular weight provides an indication of the different regions of the gut subject to intestinal permeability changes may be determined (Lambert, 2008; van Wijck et al., 2013). In selecting the appropriate sugar probes the primary method of translocation across the GI barrier should be considered so as to allow direct quantification on the mechanism by which they cross the intestinal epithelium i.e. specific pathway of mediated or non-mediated transport enabling a probe for a specific function or pathway for investigation to be selected (Arrieta et al., 2006; Lambert, 2008). The sugar probes to be utilised it is suggested should resist metabolic degradation and be fully excreted by the kidney after reaching the circulation to ensure a reliable quantitative relationship between uptake from the intestine and recovery in the urine and/or blood (Arrieta et al., 2006).

The type of sugars previously used in 'SAT' assessment are diverse although a disaccharide (usually lactulose) and a monosaccharide (L-rhamnose, mannitol or D-xylose) are typically combined to assess small intestine non-mediated permeability i.e. simple diffusion (van Wijck et al., 2013) (Table 2.1). Lactulose (molecular weight = 342 Da & 0.42nm diameter) the larger of the probes is assumed to only be absorbed through low-incidence, large aqueous mucosal pores but mainly by paracellular pathways when intestinal barrier function is compromised whereas L-rhamnose, mannitol and D-xylose (molecular weight = 164 Da, 182 Da & 150 Da respectively <0.4 nm) the smaller, are considered to cross the intestinal barrier freely by simple diffusion through high incidence, small aqueous mucosal pores (Arrieta et al., 2006; Bischoff et al., 2014; Stewart et al., 2017). As such the different size molecules follow different routes through the intestinal barrier: the larger molecules are assumed to permeate via paracellular pathways, and the smaller molecules are assumed to translocate via a mixture of paracellular and transcellular route. Varying the number of sugars in the test solution to assess intestinal absorption and permeability reduces the variation due to non-mucosal factors; all sugars are likely to be affected to a similar extent. This multi-sugar approach facilitates region specific permeability measurements of the GI tract (Arrieta et al., 2006; Lambert, 2009). In using this approach for example sucrose is destroyed once it leaves the stomach and so sucrose permeability is a reflection of gastroduodenal disease whilst lactulose and mannitol/l-rhamnose are metabolised in the caecum and provide information regarding the small intestinal epithelium. Finally, probes such as sucralose and Cr-EDTA are stable throughout the gut and can therefore provide estimates as to the permeability of the colonic epithelium (Arrieta et al., 2006; Fassano et al., 2011; Lambert, 2009).

Intestinal permeability can therefore be determined as a ratio of urinary/serum/plasma recovery of the large molecules divided by the small molecules normally over a 5 hour period or other chosen time frame (Karaeren et al., 2002; Mattiol et al., 2010). Whilst the urinary recovery of these 'sugar probes' has been the clinical gold standard more recently others have sought to modify the assay process in order to improve logistics and reduce the burden of the assessment method. Blood derived permeability analysis can also be used to assess intestinal damage over a shorter time period usually 2 hr (Fleming et al., 1996; van Wijck et al., 2012; van Wijck et al., 2013). Generally, serum or plasma derived measured have been validated as an alternate protocol although this validation protocol has been utilised only in passive situations. As such it may be impacted by several factors including the pharmacokinetics (Van wijck et al., 2013) and dynamics of the sugar probes in the blood following exercise (Lenz et al., 2010). It is known that when exercise is undertaken the

pharmacokinetics i.e. the rate at which a substance(s) is/are absorbed, appear and are eliminated from circulation can be altered due to modifications in GI pH, GI motility, splanchnic blood flow (Lenz et al., 2010, Khazaeinia & Ramsey, 2000; Ylitalo,1991) no data currently exists to support a similar time course during exercise as has been reported during the rest validation work for SAT tests during exercise related studies. This is key limitation on numerous exercise model studies as the peak determination of L:R ratio may be delayed beyond the time frame previously reported. In addition to potential difference in the kinetics of sugar detection, conflicting information exists as to the appropriate time which to ingest the sugar probes to optimise the estimation of change in GI permeability. Literature reports a number of studies where the ingestion of the sugar probes occur either pre exercise (Pugh et al., 2017) or mid exercise (vanWijck et al., 2011) the impact of this difference in probe ingestion time upon estimates of permeability remain to be resolved, particularly when considered in conjunction with the above considerations. In urinary assessments pre-absorption factors such as gastric emptying, dilution by secretion and intestinal transit time, and post-absorption factors such as systemic distribution and renal clearance are considered to affect both molecules to a similar extent (Lambert, 2008). As such, the ratio of the two probes will primarily be affected by the state of mucosal permeability (Sequeira et al., 2014). However, permeability estimates are subject to a range of possible complicating factors (Figure 2.5)

Whilst the sugar absorption test has been the clinical gold standard, several issues have been raised as regards the tests correlation with the development of GI related symptoms, inflammation and immune activation. Vojdani et al. (2013) suggests that sugar absorption tests are not indicative of large macromolecules permeation through the GI barrier and fail to correlate with bacterial lipopolysaccharides (LPS) measurements which have been reported extensively in the literature. Several authors have argued that large diameter (high molecular weight > 4000 DA) probes should be used to present a surrogate of macromolecule or food antigen molecules that are implicated in GI dysfunction (Vojdani et al. 2013). Essentially sugar probes markers it is asserted may not represent macromolecular absorption and do not fully represent how the gastrointestinal tract manages luminal antigenic permeability through the GI barrier (Jolanen et al.,1991; Vojdani et al., 2013)

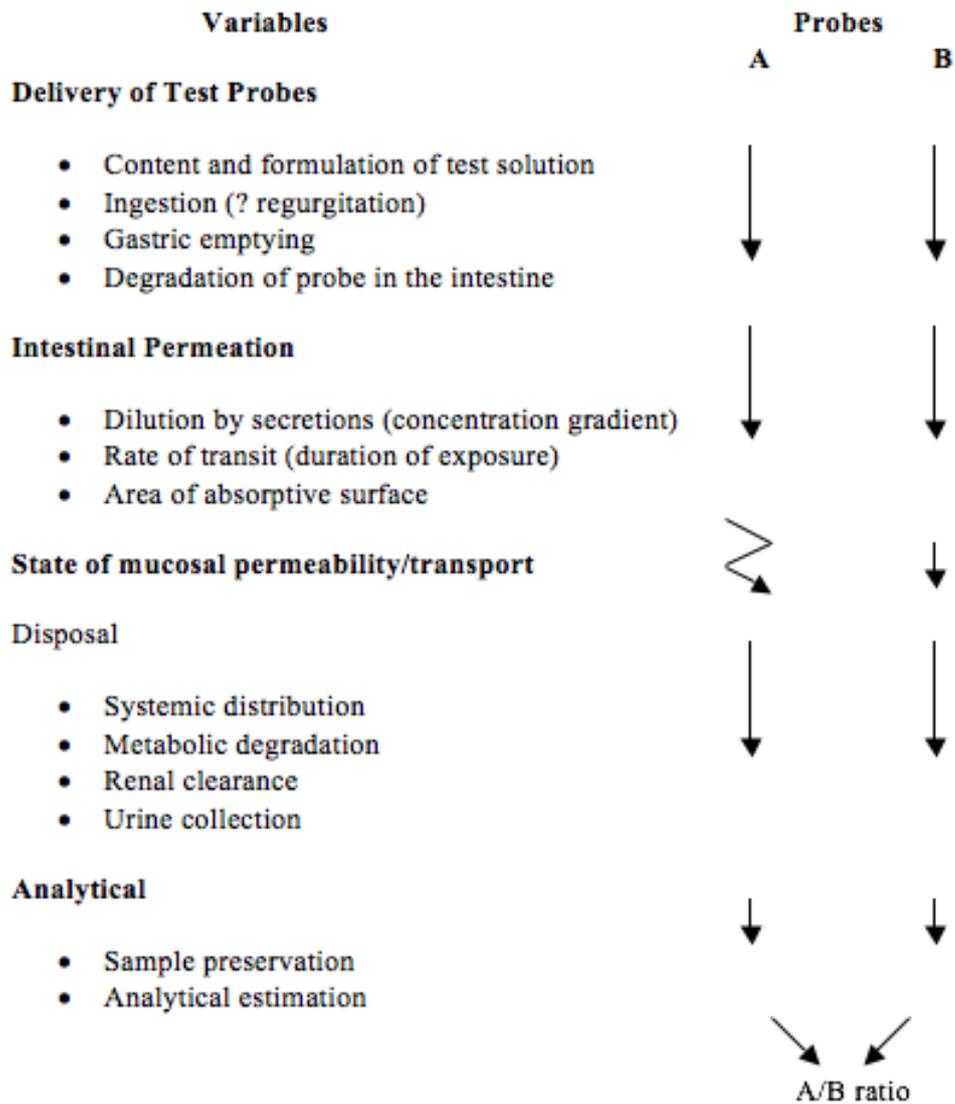


Figure 2.5 Non-Mucosal factors likely to impact upon the measurement of GI permeability, (Travis and Perkins, 1992).

2.6 Biomarkers of Gut Permeability

Alternatively, other biomarkers have been sought to represent a variety of GI dysfunction terms: gastrointestinal ‘dysfunction’, ‘permeability’, ‘damage’ to represent a diverse patho-aetiology (Fengming & Jianbing, 2014). In general, these markers have been developed for and applied in clinical settings with application in the exercise setting based upon their clinical utility profile; interestingly the utility of these parameters in terms of both sensitivity and specificity as well as normal ranges have never been properly established in healthy athletic populations. The range of biomarkers examined that may be a surrogate of GI damage has included Intestinal fatty acid binding protein [I-FABP] a small cytosolic protein

situated in the apical border of the small intestinal villi that is released into the circulation upon cellular damage/hypo-perfusion/hypoxia. It has been the most widely applied biomarker in the exercise setting as a marker of enterocyte tissue damage via putative hypoperfusion and/or hyperthermia (Costa et al., 2017; March et al., 2017; Pugh et al., 2017; Snipe et al., 2017; van Wijck et al., 2012). In general, the efficacy of IFABP as a marker is still debated in the exercise field, as it would seem to be highly liable with reference ranges at rest and during exercise highly variable (Pugh et al., 2017; Van Wijck et al., 2011; Mc Kenna et al., 2017; March et al., 2017). Other markers utilised have included Liver Fatty Acid Binding Protein (van Wijck et al., 2011), D-Lactate (Kondoh, et al., 1992; van der Voort et al., 2014; Wong et al., 2016), calprotectin (Fagerhol et al., 2005; Wang et al., 2013), faecal lipocalin2 (Chassaing et al., 2012), citrulline (Crenn, et al., 2007; van der Velden et al., 2013; van Wijck et al., 2014). However, there exist insufficient data in the exercise field to comment on their potential efficacy as a biomarker.

The prevalence of such biomarkers also raises an important point as mentioned around the use of terminology to describe dysfunction within exercise based gastrointestinal research; leaky gut (Fasano, 2011), GI Permeability (Gutekunst et al., 2013; Lambert, 2008) gut damage (Playford et al., 1999; Snipe et al., 2017) have been utilised somewhat interchangeably however each measures a distinct process and is likely to have different pathway's and time sequences of activation, progression and outcome. Costa et al. (2017) have ascribed the more generic term 'Exercise induced gastrointestinal syndrome' to reflect, probably more correctly the abundance of terms reflecting these collective GI functional issues. Further work to clarify biomarker specificity and sensitivity is required in the exercise field.

The integrity of the GI barrier may be compromised by a variety of factors. Various stressors including, endotoxaemia (O'Dwyer et al., 1988; Brock-Utne et al., 1988), psychogenic stress (Meddings & Swain, 2000), non-steroid anti-inflammatory drugs (NSAID's) and aspirin use (Lambert et al., 2001; Vieth and Montgomery, 2016), exercise stress (Ashton et al., 2003; Pals et al., 1997; Pugh et al., 2017), heat stress (Dokladny et al., 2006; Hall et al., 2001; Lambert et al., 2002), local bacterial or viral infection (Bischoff et al., 2014) and ischemia-reperfusion injury (Bulkley, 1987; Gathiram et al., 1988) can independently, or collectively induce GI barrier dysfunction. The consequences of a loss in GI barrier integrity is an increase in intestinal permeability and a range of clinical symptomologies, inflammation and immunological reactivity that can range from mild to severe (Farhadi et al., 2003; Lambert, 2009; Camilleri et al., 2012). The following section will now consider the effect these putative factors that are associated with GI permeability may express.

2.7 The Gastrointestinal Barrier and Exercise.

Evidence from previous studies suggests that the gastrointestinal tract and specifically the GI barrier may be negatively affected, when exercising (Table 2.2 a,b,c) (Lambert et al., 2002; Lambert, 2009; Pugh et al., 2017). The association between exercise and increased GI permeability has been subject to increasing examination; generally, the exercise stressors utilised have in the majority of studies been continuous, submaximal efforts of running or cycling over a varied range of exercise durations from hours to days. Notionally, the literature indicates that exercise activity increases GI permeability if performed at sufficient intensity and duration i.e. 70% of $\dot{V}O_2$ peak/max a so called 'critical threshold' (Costa et al., 2017; Pals et al., 1997). It has been observed that 60 minutes' steady-rate cycling at 70% W_{max} (van Wijck et al., 2011; van Wijck et al., 2012;), treadmill running at 70% $\dot{V}O_2max$ (Marchbank et al., 2011; Zuhl et al., 2014), 80% $\dot{V}O_2max$ (Davison, et al., 2016; Gisolfi, 1997; March et al., 2017; Pals et al., 1997;), interval running 90%/50% $\dot{V}O_2max$ (Pugh et al., 2017) and marathon/ultramarathon running (Gill et al., 2015; Lambert et al., 1999; Ryan et al., 1998; Smetanka et al., 1999), will increase GI permeability. Pals et al. (1997) demonstrated that increased GI permeability accrued during 60 minutes of treadmill running, could be mediated by exercise intensity in a dose response manner. Higher intensity exercise (80% $\dot{V}O_2$ max), exhibiting a greater permeability than exercise at lower intensities at 40% and 60% $\dot{V}O_2$ max. The majority of similar studies exploring GI permeability have shown similar results; they expand the likely clauses to suggest where exercise in excess of 70% of maximal work or aerobic capacity, and where exercise duration is greater than 50 minutes plus shows increased GI permeability (Davison & Diment, 2009; Jeukendrup et al., 2000; March et al., 2017; Roberts et al., 2016; van Nieuwenhoven et al., 2004; van Wijck et al., 2014). Although, studies several report no significant changes in permeability following treadmill running at 60-70% $\dot{V}O_2max$ (Lambert et al., 2008; Snipe et al., 2017; van Wijck et al., 2014; Yeh et al., 2013). However, across literature there is a dearth of information as regards the assessment of the GI permeability during very high intensity 'interval' exercise. Pugh et al. (2017) more recently has undertaken interval exercise in well trained males and demonstrated increases in GI permeability after a 20 X 400m intervals. Whilst a general interpretation is likely to confirm that stepwise increments in intensity lead to increased permeability they fail to examine an ecologically valid model of stepwise intensity increase or the effect of repeated very short high intensity repeated exercise that is apparent during invasion field games.

2.8 GI Permeability, Hyperthermia, and Hypoperfusion.

Physical activity and exercise result in a significant elevation in metabolic rate which can as a consequence elevate total body heat accumulation. Thermoregulatory mechanisms are therefore essential to dissipate this accumulated heat load and maintain normal physiological function (Crandall & Gonzalez-Alonso, 2010). Under both passive and active conditions body core temperature is maintained within tight boundaries through complex neurological and hormonal negative feedback mechanisms (Gonzalez-Alonso et al., 2008; Pires et al., 2016). Increases in core temperature elicit specific countermeasures which involve the induction of a sweating response to facilitate evaporative heat loss and via peripheral cutaneous vasodilation. However, these responses may induce significant cardiovascular/haemodynamic challenges that impair central venous return and reduce splanchnic blood flow in line with increases in exercise intensity (Rowell, 2004; Van Wijck et al., 2011).

Mechanistic links between the presentation of gastrointestinal symptoms, and disruption to gut mucosa have been partially ascribed to both passive and exertional tissue hyperthermia (Pals et al.1997). Where exercise occurs under different ambient conditions this may mediate secondary to the thermoregulatory challenge imposed reductions in splanchnic blood flow of up to 80%, due to sympathetic mediated vasoconstriction of the splanchnic vascular bed (Crandall & Gonzalez-Alonso, 2010; van Wijck et al., 2011). Briefly, during moderate to strenuous exercise, the release of noradrenaline and its binding to α -adreno-receptors of the sympathetic nervous system, induces a splanchnic vasoconstriction. Such responses result in an increase in the total splanchnic vascular resistance whilst, at the same time, leading to a reduction in vascular resistance in skeletal muscle and skin. The reduction in splanchnic blood flow being inversely proportional to the percentage of maximal oxygen consumption ($\dot{V}O_2$ max) achieved during exercise performance (Ashton et al., 2003; de Oliveira et al., 2014; Gutekunst et al., 2013). As a result, when blood flow is redirected from the splanchnic region to the skeletal muscle and other active tissues GI ischemia and hypoxia may also result (ter Steege et al., 2012b). Elevations in oxidative stress and nitrosative stress in the GI barrier are considered to be causally associated (Lambert et al., 2002; Dokladny et al., 2016). These physiological responses have been associated with several frequently expressed GI symptomology such as nausea, vomiting, abdominal pain, and diarrhea although the strength of association is weak to moderate at best (ter Steege et al., 2012a).

Impairments in splanchnic blood flow under passive, exertional and additional heat stress models seen in literature; may predispose toward build-up of heat in the GI wall and heat mediated disruption to epithelial, mucus and smooth muscle barriers in the (GI) wall (Lambert, 2009; Pires et al., 2016; Selkirk et al., 2008; Zuhl et al., 2014). Mechanistically, hyperthermia-induced morphological disruption of enterocytes and tight junction protein function is noted in rodent models at high gut wall temperatures (≥ 46 °C) (Lambert et al., 2002). *In vitro*, temperatures of 38.3°C have been demonstrated to cause damage to Madin-Darby canine kidney epithelial cells (Moseley *et al.*, 1994). Temperature increases in the physiological range from 37 to 41°C also are reported to cause increased permeability in an *in vitro* intestinal epithelial model through enterocyte cell death, tissue oxidative and nitrosative stress (Bulkley, 1987; Hall et al., 2001; Hayashi et al., 2012; Lambert et al., 2002; Machado et al., 2017; Taylor & Colgan, 2007). However, these responses may be accentuated or attenuated depending upon the athlete's acclimation to and the severity of the environmental conditions in which exercise is undertaken as well as fluid loss (Costa et al., 2017; Guy et al., 2016; Lambert et al., 2001; Pires et al., 2016).

van Wijck et al. (2011) recently discussed that when exercising in the heat there is an extra loss of the total body water and a decrease in plasma volume due to inadequate fluid intake, which can impair cardiac output, further reducing the blood flow to the gut resulting in intestinal hypoxia. Dehydration levels approximating 2 % of body mass may be sufficient to increase GI permeability (Lambert et al., 2008). Through a process of down regulation of Na⁺/K⁺ -ATPase which contributes to normal intestinal fluid balance; fluid balance may be compromised (Lambert et al., 2008). Subsequently, an increased chloride secretion in the enterocyte crypts and decreased sodium absorption in the villus tips [likely due to transient ischemic damage] can lead to a net fluid loss from the small intestine. As noted by others by ensuring the athletes remain relatively hydrated with regular water replacement may mitigate the risk of fluid imbalance effecting intestinal absorption (Costa et al. 2017; de Oliveria et al., 2014; Lambert et al., 2008).

Where core temperature and permeability increase combined with bacterial translocation several authors have ascribed this as a putative mechanism in exertional heat illness and heat stroke, which can in turn result in damage to the organs (Fehrenbach and Schneider, 2006; Selkirk *et al.*, 2008). Marchbank *et al.* (2010) have indicated that running for as little as 20 minutes at 80 % of $\dot{V}O_2$ max elevates core temperature by ~ 2°C and increases gut permeability by ~250 %. Similarly during longer distance exercise intestinal permeability has been increased after the completion of extended duration runs > 2 h (Snipe et al., 2017),

half, full and ultra-marathons marathon (Oktedalen *et al.*, 1992; Gill *et al.*, 2015; Stuempfle *et al.*, 2016).

Pires *et al.* (2016) in a recent systematic review on *in vivo* alterations to GI permeability suggest that impairments in GI barrier dysfunction may be subject to a ‘critical threshold’ whereby core temperatures of up to 38.0°C ‘may likely facilitate’ increased permeability, whereas temperatures of above 39.0°C ‘definitely induce’ GI permeability (Pires *et al.*, 2016). Although others, have examined GI permeability in the heat and found GI permeability to remain unaffected (Snipe, 2017; Yeh *et al.*, 2013). It should be remembered the hyperthermia reported is a function of the core temperature assessment methods which may influence reported values. Rectal thermometry may underestimate the temperature observed in the small intestine GI wall by up to 0.5-2⁰C due to location difference between rectum and small intestine. Pearson *et al.* (2012) have suggest a ‘temporal lagging’ with rectal GI measure of temperature relative to pulmonary artery temperature. The implication that the GI tract may be slower to increase in temperature but also slower to cool down upon exercise cessation. As such the rate and extent of small intestine wall temperature changes may precede the core temperature rise and then likely lag behind in terms of enterocyte heat exposure reduction (Pearson *et al.*, 2012).

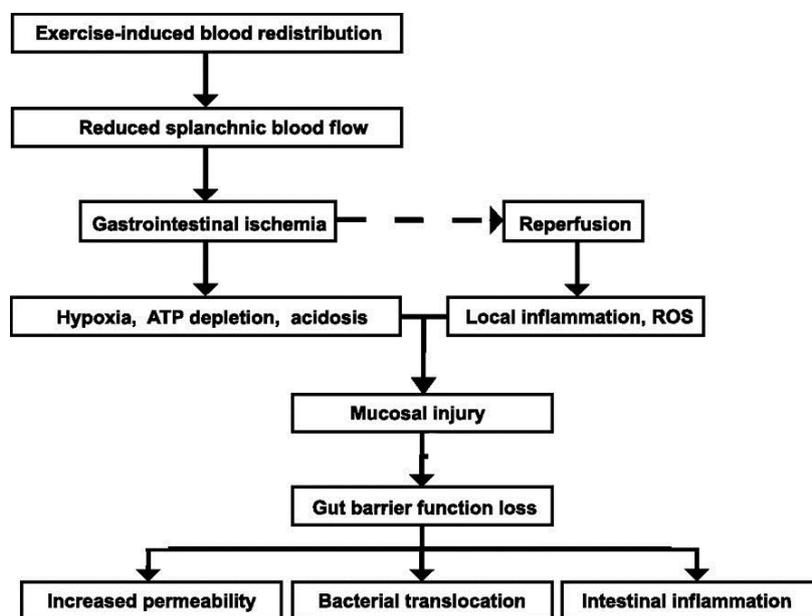


Figure 2.6 Schematic representation (modified) of the potential contributory factors to GI mediated dysfunction and its potential impact. [After van Wijck *et al.* (2012) *American Journal of Physiology-Gastrointestinal and Liver Physiology* **2012**, 303, G155-G168].

2.9 GI Barrier and Exercise Mediated Endotoxaemia.

During exercise splanchnic blood flow may be modulated by various factors including relative exercise intensity ($\dot{V}O_{2\text{ max}}$), duration (Ashton et al., 2003; Bosenberg et al., 1988; Otte et al., 2001; Pals et al., 1997), environmental temperatures (Rowell, 1983; ter Steege et al., 2012) and hydration status (Lambert et al., 2001; Sawka, 1992). As a consequence of this competition between splanchnic, skin and muscle blood flow, intestinal tissue hypoxia events are common leading to a possible paracellular penetration of pathogenic bacteria and toxic luminal antigens including endotoxins due to increased intestinal permeability or as has been described 'leaky gut syndrome' (Ashton et al., 2002; Dokladny et al., 2006; Fasano et al., 2011). Endotoxins are lipopolysaccharides (LPS) derived antigens which can cause a variety of symptoms through activation of cytokine cascades and inflammatory pathways particularly in clinical populations but also in athletes during heat stress (Marchbank et al., 2010; Selkirk et al., 2008). Athletes competing in prolonged exercise events in the heat and in particular marathon runners have reported symptoms such as; fever, nausea, dizziness and in particular, GI problems such as stomach cramps, intestinal cramps, sickness and diarrhoea (Lambert et al., 2008; Selkirk et al., 2008). It has been discussed that many of these symptoms may serve as a warning sign for impending problems such as sepsis and exertional heat stroke (Moncada-Jimenez *et al.*, 2010; Selkirk *et al.*, 2012).

The passage of lipopolysaccharide (LPS) (endotoxaemia) a constituent of gram negative bacterial cell walls from the GI lumen into the GI basal mucosa and circulation following GI barrier dysfunction can act as a major trigger to activate local immune response and secrete soluble factors such as sCD14 via activation of T-lymphocytes, monocytes and tissue macrophages (Jeukendrup et al., 2000; Stuempfle et al., 2016). In exercise studies, LPS has been frequently assayed to examine associations between LPS and GI damage and inflammation (Barberio et al., 2015; Bosenberg et al., 1988; Camus et al., 1997; Jeukendrup et al., 2000; Roberts et al., 2016; Yeh et al., 2013). The mechanism for cytokine activation involves the binding of LPS to serum lipopolysaccharide-binding protein (LBP) to form a LPS-LBP complex. This response increases the production and release of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF- α) and interleukin-6 (IL-6) which further disrupts GI barrier tight junction proteins (Dokladny et al., 2008; Dokladny et al., 2016; Moncada-Jimenez *et al.*, 2010). This release, likely controlled by the expression of nuclear factor kappaB (NF- κ B) which under normal homeostatic conditions is attached and regulated to its inhibitor I κ B (I κ B α) situated in the cytosol (Vargas & Marino, 2016). It would appear initial antigen passage through GI barrier may result in even greater intestinal barrier dysfunction through further phosphorylation of I κ B α , releasing NF- κ B and subsequently promoting further pro-inflammatory cytokine release, although one must access cytokine

correlation across GI literature with caution as data presents high levels of variability (Lambert, 2008; Dokladny et al., 2008; Dokladny et al., 2016). Endotoxaemia has been suggested to play a role in inflammatory responses leading to sepsis, multiple organ failure and can be fatal in certain situations characterized by exercise in hot and humid environments (Camus et al., 1994; Fehrenbach & Schneider, 2006). However across literature there exist a poor correlation between LPS concentrations and GI symptomology (Moncada-Jiménez et al., 2009; Costa et al., 2017). Several factors may contribute to this observation including variances in LPS assay methodologies (Wong et al., 2016). Indeed, inflammation and associated cytokine elevation follow a similar pattern relative to symptom expression that present either no or limited correlations across a range of exercise intensities and durations (Barberio et al., 2015; Gill et al., 2015; Nieman et al., 2006).

2.10 Exercise GI Motility and Gastric Emptying.

As a result of these putative mechanistic pathway alterations in the GI barrier function as a result of possible splanchnic hypo-perfusion, hypoxia and/or hyperthermia interaction mediate an antigenic stimulation, and GI permeability/damage along with possible contributions to GI symptomology (Dokladny et al., 2015; Zuhl et al., 2014). Whilst permeability changes and endotoxin release into circulation are considered to be important factor in the patho-aetiology of exercise mediate GI disturbances. Several other factors have previously been examined in attempts to provide understanding of possible exercise related GI symptomology. Changes in GI motility (i.e. passage of material through the gut) have been documented to be present in the gastrointestinal tract: the esophagus, the stomach, and the intestine. Impaired esophageal peristaltic activity and increased gastro-esophageal reflux during exercise (van Nieuwenhoven et al., 2004; de Oliveria et al., 2014). Impairments in gastric emptying have also been observed although exercise intensity would seem critical with moderate intensity exercise maintaining normal gastric emptying response but very high intensity and /or intermittent activity exercise impairs gastric emptying (Gill et al., 1998).

Table 2.1: Gastrointestinal permeability assessment mono and disaccharide sugar probes used in healthy volunteer and clinical models.

Reference	Group	Probe	In Vivo/In Vitro	Urine Collection Timing, hr	Permeability
Michiel et al. (2000).	Male and Female athletes	Lactulose, L-rhamnose and glucose	In vivo	0-5	Increased
Rao et al. (2011).	Healthy Males	Lactulose and mannitol	In vivo	0-2	Increased
Dunlop et al. (2009)	IBS-D (PI and non-PI), IBS-C	51Cr-EDTA	In vivo	0–3, 3–5, 5–24	Increased
Marshall et al. (1999).	IBS patients	Sucrose, lactulose, mannitol	In vivo	Overnight	Increased
Spiller et al. (2005).	IBS patients	Lactulose, mannitol	In vivo	0–6	Increased
Shulman et al. (2001)	Paedatric IBS and abdominal pain	Sucrose, lactulose, mannitol, sucralose	In vivo	0–3	Increased (sucrose/lactulose)

Table 2.2 Gastrointestinal assessment probes and responses used during and following exercise in healthy volunteers.

Reference	Participant	Probe/method	Exercise	In Vivo/In Vitro	Permeability measurement Urine/blood Collection Timing hr	Gut Permeability
van Nieuwenhoven et al. (2004)	Male and female athletes	Lactulose, L-rhamnose and glucose	Rest, cycling and running on the treadmill	In vivo	Urine 0-5	Increased
Rao et al. (2011)	Healthy males	Lactulose and mannitol.	No exercise	In vivo	Urine 0-2	Increased
Yeh et al. (2013)	Healthy male and Females	No probe involved LPS determination	Running on the treadmill	In vivo	Blood 2-5	LPS Increased
Lambert et al. (2008)	Healthy male and females	Lactulose, L-rhamnose, mannitol	Walk and running	In vivo	Urine	Increased
van Wijck et al. (2011).	Healthy males	Lactulose, sucralose, erythrithol, sucrose and L-rhamnose	Cycling	In vivo	Sugar 5-24 hr	Increased

Table 2.3 Effect of exercise modality, intensity and duration on gastrointestinal permeability across different environmental conditions.

Reference	Sampl	Exercise Mode	Exercise intensity % $\dot{V}O_2$ max	Duration (min)	Permeability protocols	Environmental : T (°C) & RH(%)	GI Permeability
Pals et al. (1997)	6	Active (5 men and 1 woman)	Treadmill	40, 60, and 80%	60	5 h post	Thermoneutral 22; 50 U L/R %R Increased
Lambert et al. (2001)	17	Runners and cyclists (13 men and 4 women)	Treadmill	70%	60	Pre and 4 h post	Thermoneutral 22.4; 48.0 U L/R %R Increased
Lambert et al. (2007)	8	Runners (6 men and 2 women)	Treadmill	70%	60	During and 4 h post	Thermoneutral 23.2; 36 U L/R %R Increased
Lambert et al. (2008)	20	Runners (11 men and 9 women)	Treadmill	70%	60	Pre and 5 h post	Thermoneutral 24.4; 32.7 U L/R %R Increased
Ng et al. (2008)	32	Male runners	21-km road race		~110	Pre and post	Thermoneutral 27; 80 LPS Increased

Table 2.3 cont. Effect of exercise modality, intensity and duration on gastrointestinal permeability across different environmental conditions.

Selkirk et al. (2009)	23	Healthy men (12 trained and 11 sedentary)	Walking	30%	~135	Before and every 0.5 °C increase of body temperature	Hot 40; 30	Plasma LPS Increased
Lim et al. (2009)	18	Male runners	Treadmill	70 %		Pre, post and 1.5 h post	Hot 35; 40	Plasma LPS Increased
Kuennen et al. (2015)	8	Healthy and physically active men	Treadmill	50%	45	Pre and 8 h post	Hot 47.0; 19.7	U L/plasma LPS
Marchbank et al. (2011)	12	Healthy men	Treadmill	80%	20	Pre and 5 h post		U L/R %R
Yeh et al. (2013)	15	Runners (14 men and 1 woman)	Treadmill	70%	60	Pre, 2 and 5 h post	Hot 33; 50 Thermoneutral 22; 62	LPS
Morrison et al. (2014).	15	Male runners (7 trained and 8 untrained)	Cycling and treadmill running	50–80% Reserve heart rate	90	Pre and post run 1, post run 2, and 5 h post-exercise	Hot 30; 50	I-FABP
Shing et al. (2014)	10	Male runners	Treadmill	80% ventilatory threshold	33	Pre, immediately after, and 1 h post	Hot 35; 40	U lactulose/plasma LPS
Zuhl et al. (2014)	8	Runners (5 men and 3 women)	Treadmill	65–70%	60	Pre and 5 h post	Hot 30; 12–20	U L/R %R
Barberio et al.(2015)	8	Healthy men	Treadmill	78%	~25	Pre, immediately after, 1 and 3 h post	Hot 40; 40	Plasma LPS and I-FABP

Table 2.3 cont. Effect of exercise modality, intensity and duration on gastrointestinal permeability across different environmental conditions.

Zuhl et al. (2015)	7	Runners (2 men and 5 women)	Treadmill	70%	60	Pre and 5 h post	Hot 30; 12–20	U L/R %R
Davison et al. (2016)	8	Male (4 runners, 1 cyclist, 1 2 games player,)	Treadmill	80%	20	Pre and 5 h post	-	U L/R %R
Pugh et al. (2017)	10	Recreational active male	Treadmill	70%	60	15 min post exercise	Hot 30;40-45%	Serum L/R
Pugh et al. (2017)	11	male runners	Treadmill	120%			Thermoneutral	Serum L/R
Karhu et al. (2017)	17	active runners	Treadmill	80%	90 min	Post 24 hour	Thermoneutral	Serum L/R
Snipe at al. (2017)	10	Endurance runner	Treadmill	60%	2hr	Pre and post	Hot 35;22	Serum L/R

2.11 GI Symptomology and Exercise Modality (mechanical effects).

Whilst splanchnic perfusion, hypoxia and hyperthermia responses have been advanced as primary aetiological pathways for GI dysfunction. Several other avenues have been explored as to possible causal relationships. Worobetz et al. (1985) has indicated lower frequencies of (GI) symptomology in cycling and swimming activity relative to running. Riddoch & Trinick, (1988) also noted a greater prevalence of GI symptoms whilst running than during cycling or swimming. In addition, Rehrer et al. (1992) and van Nieuwenhoven et al. (2004) have reported triathletes to experience elevated GI symptoms during the run rather than swim or cycling elements of a triathlon. Combined it is postulated that the modality of exercise and the relative mechano-physiological stimuli elicited during each induce changes in GI function, although the mechanism and magnitude of any resultant dysfunction are unquantified (Wright et al., 2011). Mechanical trauma imparts increased vibrations via elevated ground reaction forces transmitted through the abdominopelvic cavity during running than cycling (Rehrer & Meijer, 1991), leading to what has been termed slosh stomach (Bioendich et al., 2016). Others have suggested that this may affect the colon and stool physiology (Simons & Kennedy, 2004). However, there has been little research into this process.

If there was a true, substantial effect of mechanical trauma, it could be presumed that this would be a major cause of GI symptoms in other sports involving changes in abdominal movement from running, as well as jumping, cutting and other jarring movements i.e. team sports. However, there has been little research into sports other than running and cycling. Babic et al. (2001) investigated GI bleeding in rugby players and suggested that mechanical abdominal trauma was not an important factor in GI symptoms as incidence of bleeding was lower than that typically seen in runners, despite the high impact contact nature of the sport. It may be the volume of running undertaken, is more damaging than less frequent traumas that are greater in force i.e. tackles. Verification of this hypothesis is required.

Whilst epidemiological and experimental literature have focused on the perceived negative effects of exercise and adverse symptom reporting it should be remembered that exercise may improve some clinical GI related symptoms particularly in conditions such as Irritable Bowel Syndrome (IBS) or Crohns disease (Johannesson, 2015; Johannesson et al., 2011; Matsuzaki et al., 2016; Pérez, 2009; Stehle et al., 2012). As with other system models such as immunity (Nieman et al., 2011) a dose response model for the induction of GI symptomology may be speculated upon but remains to be properly described (Costa et al. 2017; Pires et al., 2016).

2.12 GI Symptomology and Permeability

Exploration of the associations between subjective GI symptomology and objective markers of GI permeability/damage has been the focus of much of the literature; generally, across most studies where objective and subjective markers have been explored outcome data has been poorly associated. Further consideration is required amongst other forms of exercise activity intensities and durations to determine potential/common association's (Karhu et al., 2017; Pugh et al., 2017). Whilst epidemiological and experimental literature have focused on the perceived negative effects of exercise and adverse symptom reporting it should be remembered that exercise may improve some clinical GI related conditions such as Irritable Bowel Syndrome (IBS) or Crohns disease (Johannesson, 2015; Johannesson et al., 2011; Matsuzaki et al., 2016; Pérez, 2009; Stehle et al., 2012). As with other system models such as immunity (Nieman, et al., 2011) a dose response model for the induction of GI symptomology may be speculated upon but remains to be properly described (Pires et al., 2016).

2.13 NSAIDS and GI damage and permeability.

Whilst exercise may mediate changes in GI function and contribute to subjective symptom expression its combination with pharmaceutical agents and its clinical impact on the gut has come to prominence as an increasing problem (McAnulty et al., 2007; Nieman, et al., 2006; Sanabria & Zabala, 2017;Tscholl & Dvorak, 2012). Nonsteroidal anti-inflammatory drugs (NASIDs) are widely available over the counter agents used in the acute and chronic treatment and management of soft-tissue injuries and for analgesic purposes (Tscholl et al., 2016). It is generally assumed, incorrectly, that the use of NSAIDs can help performance due to their facilitation of more frequent, intense training sessions by acting to mask adverse musculoskeletal issues; therefore, they are regularly used by athletes in a variety of sports (Alaranta et al., 2008; Gorski et al., 2011; Tscholl & Dvorak, 2012). The high prevalence rates for NSAID consumption particularly from a prophylactic perspective is often accompanied by limited awareness of the side effects of use particularly on a chronic basis (Didier et al., 2017; Gorski et al., 2011; Warden, 2009). Several reports on the use of NSAID medication across team sports has indicated an unexpectedly high level of both prescribed and un-prescribed consumption of NSAIDs (Holgado et al., 2017; Tscholl & Dvorak, 2012; Tscholl et al., 2015). Due to NSAIDs analgesic, anti-inflammatory and antipyretic effects, they have evolved into one of the most commonly used class of pharmaceutical agent by athletes to ameliorate a range of musculoskeletal pathologies including post exercise muscle soreness (Da Silva et al., 2015; Holgado et al., 2017; Vaso et al., 2015). Clinically, NSAID

induced GI mucosal damage in the form of mucosal erosion and ulceration is a well described adverse effect of their usage (Marlicz et al., 2014). Significantly, NSAIDs such as ibuprofen have previously been found to increase gastrointestinal GI permeability at rest (Blackler et al., 2014; Sostres et al., 2017) and following exercise particularly after prolonged, sub-maximal endurance events such as marathon and triathlons (Jeukendrup et al., 2000; Küster et al., 2013; McNulty et al., 2007; Nieman et al., 2006; Smetanka et al., 1999; Whatmough et al., 2017). NSAID [Ibuprofen] induced complications are thought to be caused as a result of the inhibition of cyclooxygenase (COX) isotypes 1 and 2 and in particular (COX-2), via a reduction in localised nitric oxide (NO) production and inhibition of prostaglandin release (Vieth & Montgomery, 2017). These responses to NSAID ingestion can cause an inflammatory response of the GI barrier and may impair perfusion of the upper GI tract (Holgado et al., 2017; Lambert et al., 2007; Lanas et al., 2003). Such modifications altering mucosal cytoskeleton integrity and causing GI damage, permeability and necrosis (Vieth & Montgomery, 2017).

Since the use of NSAIDs in a variety of sports and individual events is widespread, the effect they have on the GI barrier function alone or when combined with exercise (predominately endurance exercise) have been subject to limited review. Given NSAIDs widespread use amongst invasion field sports such data would provide insight into potential adverse effects on GI function (Tscholl & Dvorak, 2012; Vaso et al., 2015). Lambert *et al.* (2001) report increased gastroduodenal and intestinal permeability after ingesting 1,300 mg of aspirin prior to 60 minutes of running at 70% $\dot{V}O_2$ max. Lambert et al. (2007) further reported different responses between aspirin and ibuprofen, with aspirin increasing intestinal permeability relative to ibuprofen again following steady state running. Aspirin effects the COX-1 pathway, which is involved with synthesis of the mucosa and ibuprofen predominately effects the COX-2 pathway, which is involved with inflammation (Iwamoto et al., 2013). van Wijck et al. (2012) have reported increased GI permeability and damage after one hour of moderate intensity exercise following acute NSAID ingestion. Such data inform the view that NSAIDS are known to accentuate GI injury particularly under physical stress (Audet et al., 2016) and have also been shown to mediate lethality between NSAIDS, hyperthermia and/or exercise in rodents; although to date this has not been reported in humans (Takahashi et al., 2001; Audet et al., 2017).

2.14 Summary of literature.

It is apparent from the proceeding literature that the coincidence of exercise, environmental challenge and variations in both the duration and intensity of exercise leading to perturbations in GI perfusion may predispose the gastrointestinal system to adverse changes notably in its ability to partition the content of GI lumen from systemic circulation. Epidemiological data clearly report increased symptomology of gastrointestinal disturbance (nausea, vomiting, diarrhoea cramps and bloating) in both male and female runners and athletes, with symptomology perhaps increasing as a function of exercise modality, distance run, and gender (Peters *et al.*, 1999; van Nieuwenhoven *et al.*, 2004; ter Steege *et al.*, 2008). Mechanistic links between the presentation of the gastro-intestinal symptoms, the exercise challenge, and disruption to gut mucosa have been ascribed to reductions in gastrointestinal blood flow due to vasoconstriction of splanchnic vasculature (ter Steege *et al.*, 2012). This redistribution of cardiac output away from the splanchnic vasculature to active skeletal muscle in order to maintain exercise activity is proportional to the increase in exercise intensity. As such splanchnic blood flow may be reduced by up to 80% of resting blood flow, leading to gastro-intestinal ischemia, transient hypoxia and oxidative and nitrosative stress for as long as exercise stress is maintained. A further corollary of the increase in exercise intensity is a progressive hyperthermia (Lim and Mackinnon, 2006; Gonzalez-Alonso *et al.*, 2008; Selkirk *et al.*, 2008). Increased thermal strain from both the exercise itself and exercise undertaken in different environmental temperatures (Lim and Mackinnon, 2006; Gonzalez-Alonso *et al.*, 2008; Selkirk *et al.*, 2008) and in different exercise models (Yano *et al.*, 2002; Lambert, 2004; Lambert, 2008) may also be contributory to GI damage. The alteration GI permeability and damage in hyperthermia conditions being characterised as subject to a threshold effect (Pires *et al.*, 2016). Several recent models have summarised this conceptual framework (Figure 2.6). Determination of the effects of the interaction between intensity and modality of exercise on GI function remains to be fully elucidated.

Chapter 3 - General Methodology

3.0 General Method

The present chapter describes the measurement techniques used within this thesis for the collection of physiological, thermoregulatory and metabolic data and common biochemical analysis undertaken. The procedures, equipment, test equipment and test presented in this thesis are employed in at least two of four studies are described in this chapter, whereas those utilised in only a single study can be found in the methods section of that study.

3.1 Ethics

All studies were granted full ethical approval from the Ethics Committee of Liverpool John Moores University in advance of the studies being undertaken. All participants who volunteered did so by their own accord, and were finally informed of the nature, purpose and possible risks before they provided written informed consent that was initial in the presence of a third-party witness. Exercise and biochemical analysis were carried out in the physiology and biochemical laboratories respectively at the Research Institute for Sport and Exercise Sciences Liverpool John Moore's University. Moreover, all measurements were taken at the same time of day in order to avoid circadian variation in internal body temperature (Reilly & Brooks, 1990). Analysis of Lactulose and L-Rhamnose was performed at Royal Cornwall Hospital Trust Cornwall UK, one of only two accredited UK labs. All other assay procedures were performed at Liverpool John Moore's University. Assistance is acknowledged where required.

3.2 Participants

All participants were non-smokers with no history of neurological disease or musculoskeletal abnormality. Participants were asked to abstain from alcohol, drugs and spicy foods in the preceding 48 hours before testing. A minimum of three days were scheduled between tests. This was based on the complete turn over rate for small intestine enterocytes i.e. whole small intestine enterocytes are replaced every 72 hours.

Table 3.1 – Participants characteristics.

	Study 1	Study 2	Study 3	Study 4
Age (years)	24 ± 2.4	24 ± 3	19.6 ± 0.7	29 ± 10
Height (m)	1.80 ± 0.09	1.78 ± 0.1	1.78 ± 0.06	1.78 ± 0.06
Body mass (kg)	74.4 ± 11.9	79.6 ± 4	75.1 ± 5.9	78.4 ± 10.1
$\dot{V}O_{2\text{ peak}}$ (mL·kg ⁻¹ ·min ⁻¹)	57.1 ± 8.1	53.6 ± 7	-	56.4 ± 5.0

3.3 Body Mass and Height

Participants body mass was assessed to the nearest 0.1 kg using a Seca weighing scales (Seca, model 702, Germany), and height, to the nearest 0.1 m, using the Seca stadiometer (Seca, Model 217, Germany) during the initial visit to the laboratories that coincided with their assessment of physiological fitness.

3.4 CARDIO-RESPIRATORY MEASUREMENTS

3.4.1 Heart Rate

Participants were fitted with a short-range radio telemetry system for the measurement of heart rate (Polar S610i, Kempele, Finland) in all exercise and passive rest experiments. In all studies, heart rate ($\text{b}\cdot\text{min}^{-1}$) was continually measured at 5-second intervals; data as presented in each chapter represents an average over designated time periods as outlined in that chapter's specific methodology. The chest strap was worn directly below the chest at V5 level with the transmitter positioned centrally on the xipho-sternum.

3.4.2 Assessment of expired respiratory gases during exercise

Participants were required to wear a Hans Rudolph oro-nasal facemask (7450 Hans Rudolph, Cranlea UK Birmingham for measurement of expired fractions of oxygen ($\dot{V}O_2$ $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and carbon dioxide during breath-to-breath measurement and averaged over each 10-second period via an on-line open circuit spirometry system (Oxycon Jagger, Netherlands) or the Metalyzer 3B (Cortex, Germany) across experiments (Figure 3.1) Within each experiment the same system was utilised across all time points and participants. The systems both utilise a low resistance two-way valve that has an integrated infra-red flow transducer and expired gas collection tube. The expired gases are sampled into a negatively pressured tube as gases enter the flow transducer. These are dried as they progress down the tube and then analysed via changes in conductance of an electrical signal across a fuel cell and infra-red CO_2 analyser. The Oxygen and Carbon dioxide sensors were calibrated with both ambient air and an α - gravimetric gas (Oxycon Jagger, Netherlands) which contained 16 % O_2 and 4 % CO_2 with the balance nitrogen. Calibration of the volume transducer was performed with a three-litre syringe (Model 5330, Hans Rudolf, MO, USA), being pumped through the transducer at varying flow rates to match system designated requirements. Respiratory data were expressed as Standard Temperature and Pressure Dry.

3.5 Treadmill

A motorised treadmill (H/P Cosmos Pulsar, Germany) was used in all studies for maximal exercise assessments during running and experimental exercise protocol performance.

3.6 Maximal/Peak Aerobic Power ($\dot{V}O_{2\text{ peak/max}}$)

Participants $\dot{V}O_2$ peak was determined using a progressive incremental protocol on a motorized treadmill (HP/Cosmos Pulsar, Germany). This evaluation characterised physiological capacity and facilitated adjustment of the workload for each participant in the subsequent work protocols employed across the thesis protocols to appropriate % of $\dot{V}O_2$ peak. The protocol commenced at 8 km·h⁻¹ at 0 % inclination with 2 km·h⁻¹ increments at 2 minute intervals to a maximal velocity of 16 km·h⁻¹ with 2.5 % inclinations occurring every 2 minutes thereafter until volitional exhaustion. The criteria of the British Association of Sport and Exercise Sciences (BASES) were used to classify $\dot{V}O_2$ response attained (Winter, Jones, Davidson, Bromley, & Mercer, 2007). The attainment of $\dot{V}O_{2\text{max}}$ was accepted as being achieved based on the following and point criteria: 1) heart rate within 10 b·min⁻¹ of age predicted maximum, 2) respiratory exchange ratio >1.15, and 3) lactate blood >8mmol·L⁻¹. 4) volitional exhaustion. Participants were also verbally encouraged during the final stages of the test to maintain exercise for as long as possible to ensure that they reached exhaustion.



Figure 3.1 Expired gas fraction analysis during exercise (Borg, 1982).

3.7 Rating of perceived exertion (RPE)

RPE was assessed utilising the 15-point 6-20 ratio scale outlined by Borg (1982). Participants were familiarised with using the scale during the initial maximal incremental exercise test to exhaustion. Each end of the scale was ‘anchored’ during the maximal exercise protocol so as to introduce the perception of effort associated with progression through the scale from 6 [rest] through 20 [maximal effort], (Birk & Birk, 1987). In doing so this provides a construct against which participants can gauge subsequent efforts during exercise trials.

Rating	Description
6	No Exertion At All
7	Extremely Light
8	
9	Very Light
10	
11	
12	
13	Somewhat Hard
14	
15	Hard
16	
17	Very Hard
18	
19	Extremely Hard
20	Maximal Exertion

Figure 3.2 Borg scale for reporting subjective RPE during exercise (Borg, 1982).

3.8. Thermal Comfort

At the same time points for assessment of Subjective Shivering, participants also reported subjective Thermal Comfort (Young, Sawka, Epstein, Decristofano, & Pandolf, 1987)(Figure 3.4).

THERMAL COMFORT SCALE

1	Very Cold
2	Cold
3	Cool
4	Slightly Cool
5	Neutral
6	Slightly Warm
7	Warm
8	Hot
9	Very Hot

Figure 3.3: Thermal comfort scale used for subjective rating of thermal strain.

3.9 Thermoregulatory Variables

3.9.1 Rectal temperature

Core temperature was assessed via a flexible rectal thermistor (Mini-thermistor; Grant instruments Ltd, Shepreth, UK) self-inserted 10 cm beyond the external anal sphincter. Rectal temperature was recorded at regular intervals throughout the experimental protocol day using an electronic data logger system Squirrel 1000 data logger (Grant Instruments Ltd, Shepreth, UK). The rectal probe was inserted, at least 30 min prior to any measures.

3.10 Gastrointestinal Permeability Assessment.

In order to determine gastrointestinal permeability each participant will undergo a GI permeability assay. This will involve consuming a water solution containing the following mono and disaccharide sugar probes. The probes consist of 5 g Sucrose, 5 g Lactulose, 1 g Rhamnose and 0.5 g D-Xylose, in solution. Fifteen min after ingestion of the sugar probe, the participant commences the exercise protocol. Sugar probe permeability will be determined in this thesis through two methods 1).via a continuous pooled urine collection for 5 h (300 minutes) into a polypropylene container that will be refrigerated and contain agents (thymol) for inhibition of bacterial growth. 2) Through serum collection to determine sugar probe concentration's in the circulation after 2 h collection window. Briefly, for urine collection, to assess sugar probe presence will be performed at each bladder voiding. If a participants are unable to void at 5 h (300 min) then the bladder void immediately following this time frame will be taken (van Nieuwenhoven, et al., 1999b). The sugar probes present in the urine will be determined by High performance liquid chromatography. Blood samples taken pre dosing and 2 h post drink ingestion will be collected following the model of van Wijck et al. (2011). Ratios of the sugars will be determined and used to indicate changes in GI permeability across different regions of the GI tract.

3.10.1 Gastrointestinal Permeability Profiling Beverage.

A standard beverage was maintained for every allocation throughout the study. Participants were asked to ingest a drink containing, the sugar test solution; 5 g lactulose (L), 1 g L-rhamnose (R), and 5 g sucrose (S) in 115 ml of water, whereby 5 h urine collection period commenced. They were instructed to consume the beverage within one minute; upon ingestion the clock was started. Participants remained in a fasted state, but were allowed water at a rate of 1 mL·kg every 15 min. This water selection was utilised to minimise what has been termed “slosh stomach” (Biondich & Joslin, 2016).

3.11 Procurement, storage and analysis of blood samples.

Differing volumes of whole blood were required for the different trials performed in this thesis. As such two methods to procure these blood samples were applied; via an indwelling cannula (BD Nexiva Closed IV Catheter 22G Blue, Becton Dickinson, Oxford, UK) or butterfly needle (BD safety set 21 G $\frac{3}{4}$, Becton Dickinson, Oxford, UK) dependent upon participant preference although all were encouraged to have the former procurement method were feasible. In relation to venepuncture the needle was placed into a superficial vein in the anti-cubital fossa of the forearm using standard venepuncture techniques (Vacutainer Systems, Becton Dickinson, Oxford, UK) or the cephalic vein on the lateral aspect of the forearm for cannulation. Patency of the cannula was maintained with a 5 mL 0.9 % sodium chloride flush (Posifush, Becton Dickinson, Oxford, UK) after each blood draw. Briefly, for venepuncture a tourniquet was applied to the mid bicep to induce an increase in intravascular pressure and venous return; the median antecubital vein was cleaned with several medi-swab isopropyl alcohol wipe BP 70 % (Seaton Health Care Group, Oldham, UK). The needle was inserted, once a flashback was observed, it was advanced, secured and the collection tubes were attached to correspond to the order of draw recommendations i.e. gold, green, lavender (Vacutainer Systems, Becton Dickinson, Oxford, UK). Upon completion of the sampling the needle was removed and pressure applied to the puncture site to minimise any possible subcutaneous haematoma. All blood samples were collected into vacutainers™ of differing volumes (6.0 mL – 10 mL) (Becton Dickinson, Northampton UK). The samples were collected into four vacutainers; a serum separation tube (SST), Ethylenediaminetetraacetic acid (EDTA) and lithium heparin (LH) which was used as a chelating agents for the blood. Briefly the serum separation tube was allowed to sit at room temperature for approx. 30 min until clotted then placed on ice until centrifugation. The samples were then centrifuged at 1500 x g for 15 minutes at 4°C. The plasma/serum was then carefully removed from the vacutainer using a pipette (Fisherbrand, Finnpipette, U.S.A.) and aliquoted into triplicate replicates for future analysis. Following this, plasma and serum were then stored at -80°C (Thermo Forma – 970 ULT Freezer, Ohio U.S.A).

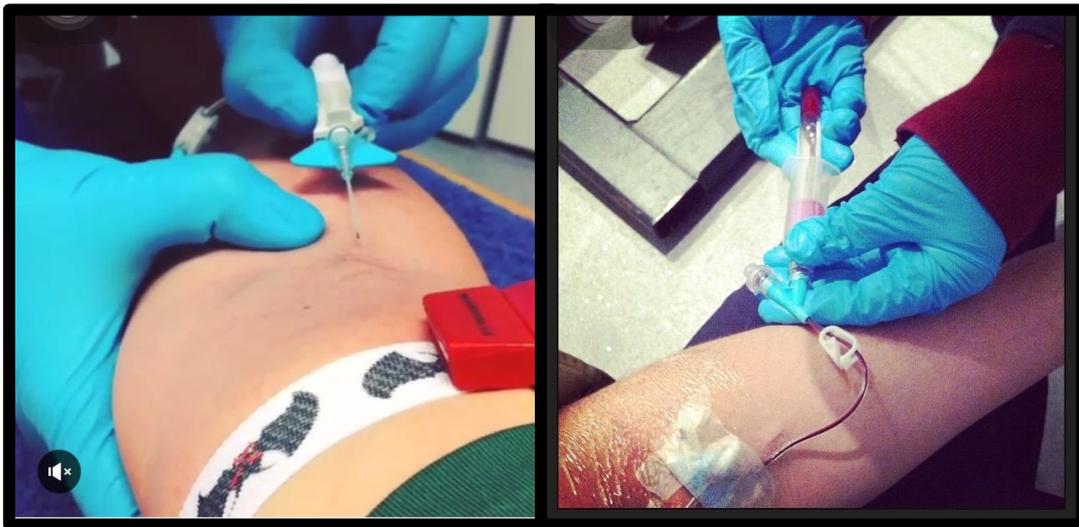


Figure 3.4 Procurement of blood via insertion and management of Nexiva cannula.

3.11.1 Haemoglobin whole blood photometry and haematocrit determination.

The quantitative determination haemoglobin was determined via a calibrated HemoCue™ β -Haemoglobin analysers (HemoCue™, Derbyshire, UK). Photometers were calibrated prior to use to variance of $0.3 \text{ g}\cdot\text{dL}^{-1}$ with photometry calibration cuvette. The CV were the equation were $\sim 6 \%$ for hb. The β -Haemoglobin technique is based upon drawing up a small sample of blood $10 \mu\text{l}$ via capillary action into the measurement chamber of the microcuvette where the internal reagents (40 % w/w sodium deoxycholate, 20 % w/w sodium nitrite, 18 % sodium azide) mix with the sample. The microcuvette is then placed in the HemoCue™ photometer where the light transmittance through the microcuvette is determined and the level of haemoglobin quantitated. The test principle is based on the conversion of methaemoglobin to azidemethaemoglobin. Briefly, the reagent 40 % w/w sodium deoxycholate cause lysis of the erythrocyte membranes, the 20 % w/w sodium nitrite converts the haemoglobin iron from ferrous to ferric state to form methaemoglobin which finally forms azidemethaemoglobin.

3.11.2 Measurement of Haematocrit and Plasma Volume Change.

Prior to centrifugation, two micro-haematocrit tubes (LIP, Shipley, England) were filled 2/3 capacity with blood. The micro-tubes were sealed at one end using critical-seal putty (Oxford Labware, Sherwood, Medical St Louis, MO, USA) and centrifuged (Hettich Zentrifugen, Tuttlingen, Germany) at $10000 \text{ rev}\cdot\text{min}^{-1}$ for 5 min to resolve samples into a plasma and cellular component for the determination of haematocrit (HCT) and plasma ratio in each tube. Samples were then removed from the centrifuge and read on a Hawksley reader

(Hawksley and Son, England). Plasma volume alterations were calculated using the equations of Dill and Costill, (1974)

3.12 Gastro-Intestinal Symptomology Questionnaire (Pre and Post protocol):

The gastrointestinal questionnaire was usually presented to the participants pre-exercise, post exercise, post 1 hour after exercise This consists of visual analogue scale (VAS) to assess GI symptoms such following the model of Moncada-Jimenez et al.,(2009). Participants completed a 16-question visual analogue scale questionnaire (100-mm lines) with the participant indicating via a mark on a line their perception of how they feel, and quantitated in terms of percent full scale (i.e., 0 % = none, 100 % = severe). Issues around gastrointestinal upset with reference to upper and lower gastrointestinal tract to be assessed included; side stitch, nausea, bloating, urge to burp, urge to vomit, urge to defecate, diarrhoea, stomach cramps, stomach upset; intestinal cramps, dizziness, shivering and heart burn. GI VAS data will be collected at time points corresponding to chapter specific protocols. An abdomino-pelvic segmented model was included to determine from, participants any areas where they felt specific pain or discomfort during protocols.

3.12.1 Gastro-Intestinal General Symptomology Questionnaire (within protocols)

Further to the pre and post questionnaire gastrointestinal symptoms were recorded during each experimental protocol using a GI discomfort scale (Pfeiffer et al., 2009). Participants rated their symptoms on a 10-point scale, ranging from 0 ('no problem at all') to 9 ('the worst it has ever been'), with a score > 4 being regarded as serious.

3.13 HPLC Assessment of intestinal permeability

Intestinal permeability for the recovery of Lactulose and L-Rhamnose was assessed by analysing pooled 5 h urine samples using a previously published protocol (Fleming et al., 1996), with the modification of using L-rhamnose instead of mannitol as the monosaccharide probe. The various sugars were separated using high-pressure liquid chromatography (HPLC) and quantitated by use of a pulsed electrochemical detector using a gold working electrode and silver/silver chloride reference electrode. The detection potential was -0.01 V (0-0.5 s), the oxidation potential was +0.75 V (0.51-0.64 s), the reduction potential was -0.75 V (0.65-0.75 s), and the integration period was 0.05 to 0.5 s. Retention times were 2.7 min for L-rhamnose and 6.1 min for lactulose. The coefficient of variation for the samples in the trial in this thesis using this method has been found to be between 1.8 – 8.5 %.

3.14. Assessment of Serum Cortisol.

Serum cortisol across studies was determined via automated Roche COBAS electro-chemiluminescence immunoassay procedure following the manufacturer's instruction. The CV was <10 %. The lower and upper limits of measurements were 0.5 and 1750 nmol/L, respectively.

3.15 Statistical Analysis

SPSS software (version 23; SPSS inc., Chicago, IL) was used for data entry and analysis for all studies. Excel was utilised to calculate mean and SD across the chapters. In each chapter a description of the analysis process is provided.

3.15.1 Sample size.

In this thesis the sample size across the studies were relatively small only chapter 5 has a sample size close to the recommendations of 12 per study (Julious, 2005). Throughout the thesis, the temptation to cite sample size as a restriction on the studies has been resisted. Retrospective sample size power estimation in order to assess how many participants would be required to turn a non-significant result in a significant result should be avoided. Such an approach is statically incorrect, data from the chapters can be used to estimate effect sizes for planning future work but not to explain lack of statistical significance in current data (Beck, 2013).

3.16 Pilot study 1.

Effect of steady state exercise relative to rest on gastrointestinal damage: exploration of faecal calprotectin utility in short term exercise.

3.17 Introduction.

The gastrointestinal barrier has a role in protecting the internal environment from harmful substances entering the blood stream (Camilleri et al., 2012; Fasano & Shea-Donohue, 2005). A breach in this barrier may be the primary event in the pathogenesis of intestinal inflammatory disorders (Farhadi et al., 2003; Farhadi et al., 2007). However, a main concern is that a variety of physiological, psychological and immunological challenges such as prolonged strenuous exercise affects the physical and chemical integrity of the intestinal barrier (Lambert et al., 2008). Epidemiological data report increased expression of gastrointestinal (GI) disturbance symptomology in athletes after exercise of varying durations and intensity across gender and fitness status (Haaf et al., 2014; Keeffe et al., 1984; Peters et al., 1999; ter Steege et al., 2008). These symptoms have been associated with a loss of GI epithelial barrier integrity secondary to hyperthermia, splanchnic hypo-perfusion, GI hypoxia (Calder et al., 2013; Dokladny et al., 2006; Pires et al., 2016; van Wijck et al., 2011; Zuhl et al., 2014b). Determination of the magnitude and extent of changes to the GI intestinal barrier as a result of exercise have utilised a range of biomarkers borrowed from clinical practice to quantitate the extent of potential damage/inflammation to the GI barrier (Snipe, et al., 2017; van Wijck et al., 2011). A broad range of sugar absorption tests designed to determine breaches in membrane integrity, and a range of other bio-markers such as cytosolic proteins have been utilised (Calder et al., 2013; Crenn et al., 2010; Lostia et al., 2008; van der Voort et al., 2014). Recently, calprotectin, a 36-Kda cytosolic zinc binding protein marker of GI inflammation has been postulated as a biomarker of exercise related perturbations in GI function (van Wijck et al., 2011). Calprotectin is found in the cytoplasm of activated macrophages and neutrophil representing ~30-60 % of the cytosolic proteins present and has potent morphological disruption and antibacterial effects upon release (Pimentel et al., 2015; Soubières & Poullis, 2016). Calprotectin shows high levels of correlation to morphological, histological, immunological and cytokine derived markers of inflammatory bowel diseases (Sherwood & Walsham, 2016). Its presence in faecal samples is indicative of GI barrier breach and inflammatory cascade activation (Soubières & Poullis, 2016). It has been extensively used in clinical settings to diagnosis and classify a range of GI inflammatory related conditions such as inflammatory bowel disease, Crohn's disease, irritable bowel syndrome; as a clinical test it presents a high level of diagnostic sensitivity and specificity of 95 % and 91 % (Chang et al., 2014; Wang et al., 2013) It presents with a

normal diagnostic range of <50 ug/g, with concentration's between 50-180 µg/g associated with morphological disruptions to the gastric and GI barrier, 100-200 µg/g related to ulceration disorders, and concentrations of >220 µg/g metastatic disorders (Lehmann, et al., 2015; Wang et al., 2013). It is feasible that faecal calprotectin concentrations of >50 µg/g may provide an indicator of GI inflammation as a result of exercise. Calprotectin responses to exercise have previously been reported, however as these were derived from serum estimates they may fail to adequately reflect GI concentrations (Fagerhol et al., 2005; Janssen Duijghuijsen et al., 2017a; Mortensen et al., 2008).

This pilot study aims to explore the effect of steady state exercise upon GI permeability, with faecal calprotectin and GI discomfort being an indicator of inflammation of the gut. The hypothesis tested was that following a bout of steady state exercise, there will be an increase in measurements of faecal calprotectin (µg/g) and GI discomfort (AU) when compared to resting activity.

3.18 Test protocol

3.18.1 Preliminary testing:

Aerobic capacity ($\dot{V}O_2$ peak):

Participants were required to attend the sports science lab of the TRB to undergo a graded maximal exercise treadmill exercise running test. Participants warmed up for 7 minutes at intensity self-selected intensity. After this period, the exercise protocol commenced at 8.0 km·h⁻¹ after every 2-minute interval the speed increases by 2.0 km·h⁻¹ until 16 km·h⁻¹. Subsequent to this the gradient increased by 2.5 % until maximal voluntary exhaustion. Expired gas fractions were measured to assess the volume of oxygen uptake and CO₂ and excretion. The resultant $\dot{V}O_2$ peak estimates were used to regress $\dot{V}O_2$ against velocity to determine running velocities/ $\dot{V}O_2$ relationships equivalent to 70% of $\dot{V}O_2$ peak for use in future steady state exercise protocols. RPE 6-20 scale was determined to anchor effort perceptions as outlined by Birk and Birk, (1987).

3.19 Experimental Design:

3.19.1 Participants. *Four male participants (age 20.5 ± 0.5 years; mass 72.6 ± 11.2 kg; height 1.78 ± 0.11 m; $\dot{V}O_{2\text{ peak}}$ 4.03 ± 0.49 L·min⁻¹) undertook a counter balanced repeated measures design of 50 minutes of rest (Control) or steady state running upon a motorised treadmill (~70% $\dot{V}O_{2\text{ max}}$). Participants gave informed consent in line with the institutional*

ethical procedures of Liverpool John Moore's University. Participants were required to abstain from exercise for 24 hours and alcohol and spicy food (Section 3.2). All confirmed their compliance with these criteria upon arrival at the laboratory.

3.19.2 Experimental Conditions: Rest (Control) and Exercise:

An EasySampler[®] Stool Sample Collection kit (Alpha Laboratories, UK) was given to participants before coming into the lab to collect their last faecal output from the previous day for the determination of calprotectin. The faeces sample from the day before testing was collected and placed in faecal collection tube via a sample spatula by the participant's double bagged and sealed for transportation to the lab following NHS guidelines on stool collection and storage. Faecal calprotectin samples remain viable at room temperature for at least 7 days (Sherwood & Walsham, 2016). Participants were then asked to insert a rectal probe 10 centimetres (cm) past the external anal sphincter and attach T31 coded™ transmitter (Polar™, Kempele, Finland) around their chest at V5 just under the pectoral muscle and wear a FT2 watch (Polar™) linked to the T31 coded™ transmitter. In the rest condition the participants were then allowed to pass the allocated experimental time by whatever way they chose as long as they stayed seated for the full 50 minutes. Conversely in the exercise condition participants commenced running at a speed equivalent to 70% of their individual $\dot{V}O_2$ peak for 50 min. During both protocols, heart rate HR ($b \cdot \text{min}^{-1}$) was recorded every minute and every 3 min' core body temperature ($^{\circ}\text{C}$), rate of perceived exertion (RPE), thermal comfort and GI comfort (Pfeiffer et al., 2009) were measured. Once the 50 min had expired participants took another EasySampler[®] Stool Sample Collection kit to collect the next faecal sample i.e. post exercise and recorded how long after exercise it was passed. This sample was then returned labelled and stored at -80°C . A minimum of 5 days between trials was

3.19.3 Faecal calprotectin analysis.

The analysis of the faecal calprotectin involves three key steps; extraction of the stool sample, sample processing and lateral flow assay procedure and readout (Figure 3.5). The extraction of the stool sample involved defrosting the sample, once fully defrosted the faecal sample was taken out of the test tube and placed on a sterile surface, the dosing tip was then placed 2cm into the faecal sample until all collection groves were completely full then placed back into the dosing tube with extraction solution and firmly closed. The sample was then homogenized by vortexing for 30 s at 2500 rpm by a VWR Signature Digital Vortex Mixer. This process was repeated until all the faecal sample had been completely removed from the dosing tip. The solution was left for 10 min to allow the precipitation to settle at the bottom of the tube. The supernatant was then diluted using a ratio of 1:16 with the extraction

buffer (i.e. 20 µl of sample to 300 µl of extraction buffer) this was again vortexed at 2500rpm for 30 s. After this all samples were centrifuged at 3000 x g for 5 minutes then left to settle for 10 min again. The calibration of Quantum Blue® Reader was set with an internal reference cassette. 60 µl of extracted supernatant was then pipetted onto the sample loading port of the test cassette and incubated at room temperature for 12 min. The test cassette was then loaded into the reader and scanned. Data was expressed as ug/g.

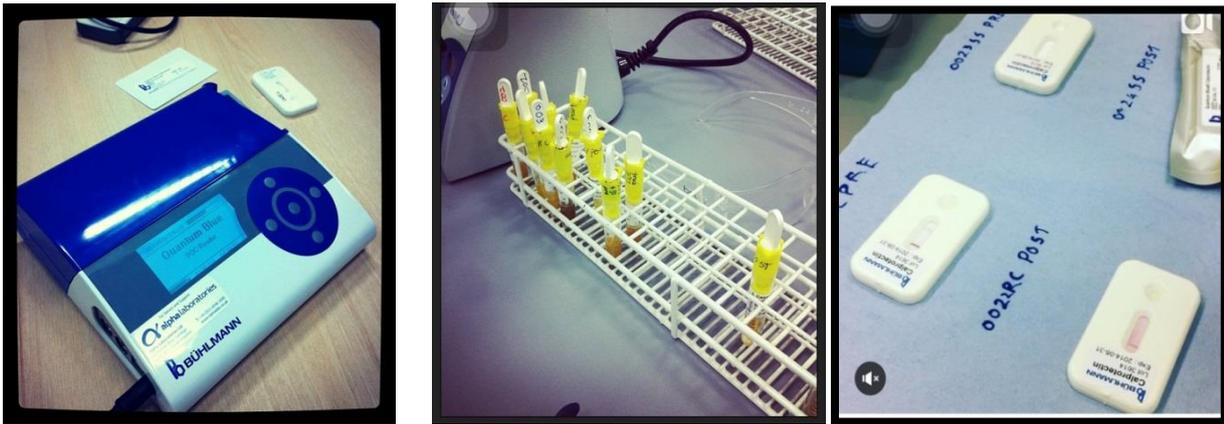


Figure 3.5 Extraction, processing and lateral flow analysis of faecal calprotectin (right to left).

3.20 Statistical analysis

Calprotectin concentrations were not statistically analysed due to small sample size. Data interpretation will therefore be descriptive. Microsoft Excel 2010 (Microsoft) was used to calculate averages of each variable across the time frame as well as standard deviation.

3.21 Results

3.21.1 Gastrointestinal Damage

3.21.1.1 Faecal Calprotectin

Faecal calprotectin in the pre-exercise rest condition was not higher than the mean of faecal calprotectin post-exercise rest condition. Similarly, little difference between the pre-exercise sample and the post-exercise sample from the steady state exercise condition were noted (Figure 3.6). There was no difference between the pre-exercise samples from both the rest condition and steady state exercise faecal calprotectin was much lower in the post samples of the rest condition than observed after steady state exercise (Figure 3.6).

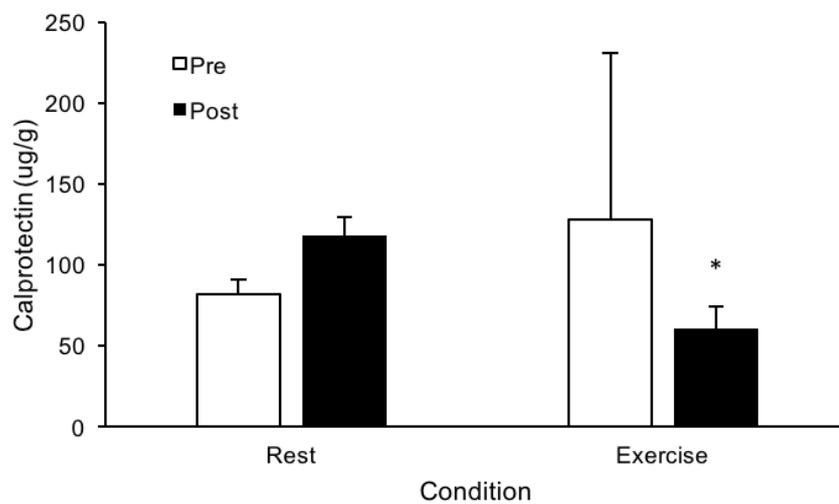


Figure 3.6 Faecal calprotectin responses during exercise and rest.

3.21.2 Physiological variables

3.21.2.1 Heart Rate

The mean heart rate during steady state exercise was elevated above that seen during the rest condition (Figure 3.8).

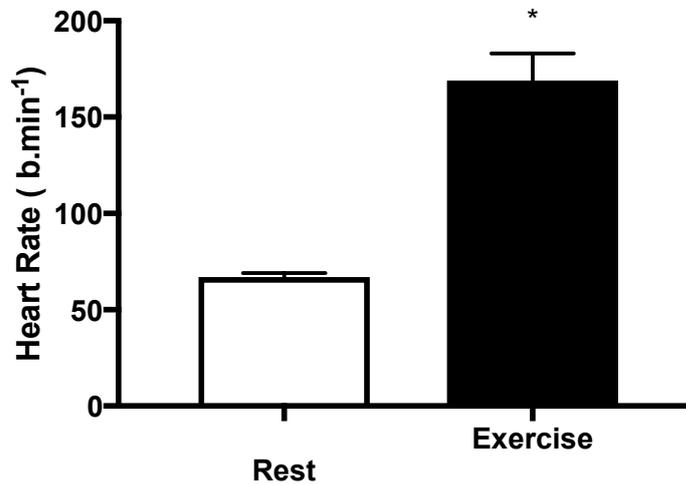


Figure 3.7 Average heart rate responses during both protocol completions.

3.21.2.2 Core Body Temperature:

Core body temperature varied as a function of protocol undertaken. The mean core body temperature during steady state exercise was significantly higher than during rest (Figure 3.9).

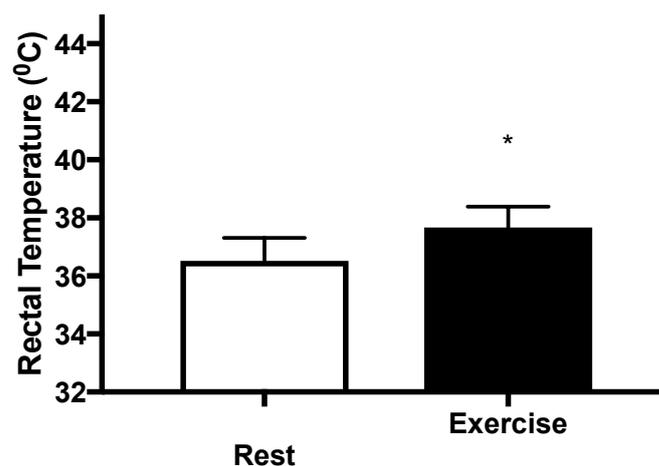


Figure 3.8 Average rectal temperature responses during both protocols completion.

3.21.2.3 Rate of Perceived Exertion

The mean rate of perceived exhaustion during rest) was lower than during steady state exercise (Figure 3.10).

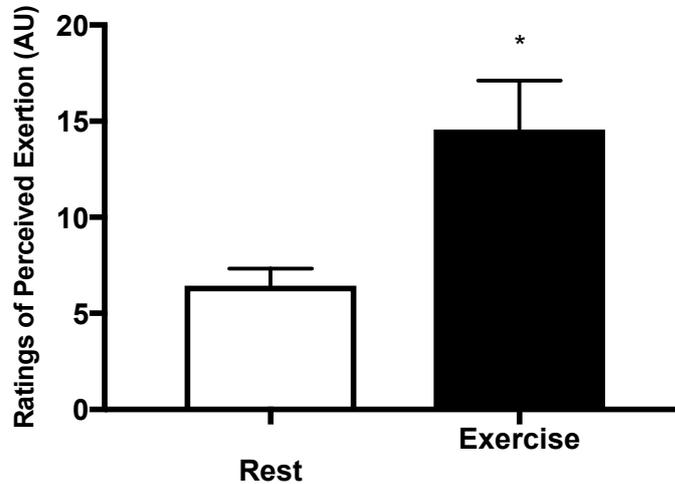


Figure 3.9 Average RPE responses during both protocols completion.

3.21.2.4 Thermal Comfort Scale:

The average response to the thermal comfort scale was higher during steady state exercise than at rest. All participants reporting generally higher scores heat perception during the steady state exercise.

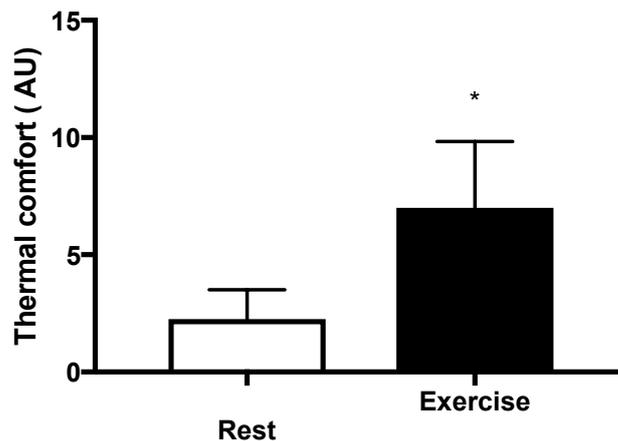


Figure 3.10 Thermal Comfort subjective ratings responses during both protocols completion.

3.21.2.5 Gastrointestinal Comfort Scale.

The average GI comfort scale response was not significantly lower during rest than during exercise at 70% of $\dot{V}O_2$ peak. Only one participant displayed signs of GI comfort above minor problems (>4) in both rest and steady state exercise.

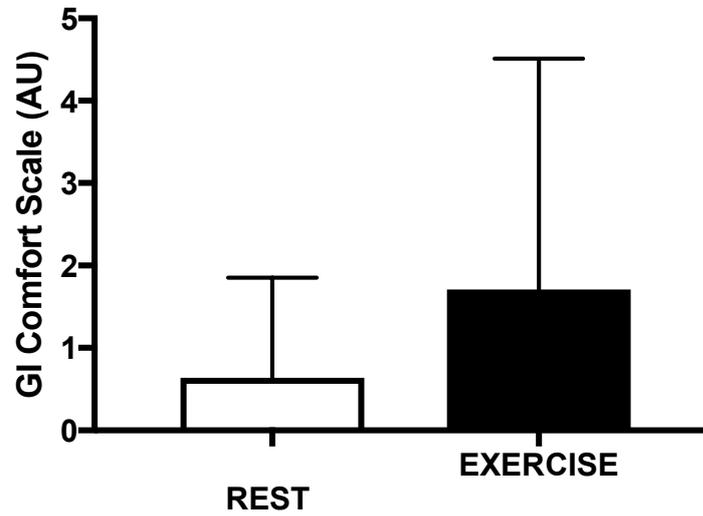


Figure 3.11 Average Thermal Comfort ratings responses during both protocols completion.

3.22 Discussion

The aim of this pilot study was to examine the potential efficacy of Faecal calprotectin as a marker of GI damage/inflammation. Upon an inflammatory response/damage in the intestinal mucosa, degranulation of neutrophils, monocytes and activated macrophages releases calprotectin and its concentration increases in the intestinal lumen as such this makes it a specific marker for gastrointestinal inflammation (Poullis et al., 2004; Wang et al., 2013). Therefore, calprotectin's potential as a non-invasive biomarker of intestinal inflammation has been advocated in clinical scenarios (Fengming & Jianbing, 2014). In the present study, we extended this concept to determine if faecal calprotectin may show efficacy due to exercise mediated inflammation in the GI tract. It was hypothesised that the concentrations of faecal calprotectin would be elevated post exercise relative to the non-exercise day due to increased GI permeability leading to endotoxin leakage and a subsequent neutrophil activation and recruitment to the gut (Soubières & Poullis, 2016).

However, this scenario of elevated faecal calprotectin was not reflected in the data. High levels of faecal calprotectin from the pre steady state exercise sample were indicative of an activation of inflammatory cascades at rest relative to and greater than that observed following exercise (Figure 3.8). This inherent variability is inconsistent with the idea that faecal calprotectin presents a sensitivity to detect exercise mediated increases in GI inflammation (van Wijck et al., 2011). The faecal calprotectin concentrations expressed are higher than the traditional clinical cut off for the initiation of further investigative procedures (ref). Since this work, several studies have utilised the faecal calprotectin approach in larger samples during running based studies, the absolute values and magnitude of changes in their faecal calprotectin concentrations were much lower than noted herein and may reflect the analysis methodology applied (Karhu et al., 2017; Snipe et al., 2017). Van Wijck *et al.* (2011) has also shown that faecal calprotectin concentrations rise slightly after 60 minutes of cycling at high intensity but not significantly above resting values and in line with observation of others (Snipe et al., 2017). Others have noted more significant elevations in calprotectin after exercise (Fagerhol et al., 2005) however this was determined in the blood compartment rather than the GI tract; it has been noted that exercising skeletal muscle presents a significant source of circulating calprotectin as such its usefulness in this compartment as a GI biomarker is therefore unsubstantiated (Mortensen et al., 2008). Compartment specific assessment of calprotectin is therefore critical if it is to reflect the local gut inflammatory responses.

There are several factors both intrinsic and extrinsic to the assay procedure which may have contributed to the elevated calprotectin observations noted i.e. sample collection, management, extraction and preparation. Firstly, the measurement of faecal calprotectin, are based on immunochemical techniques utilising either polyclonal or monoclonal antibodies targeted at various epitopes on the calprotectin molecule (Sherwood & Walsham, 2016). These can be divided into those that produce a quantitative (ELISA/lateral flow) result and those that produce a positive or negative result, i.e. qualitative result high/low. In the present study we utilised the BÜHLMANN. • Quantum Blue® calprotectin solid phase assay, which is a rapid bench top lateral flow assay with antibody embedded into a cassette. The addition of a supernatant extracted from faecal samples allows determination of calprotectin through a colorimetric reaction and reading which varies from that applied during ELISA. Second, sample management, extraction and preparation are critical factors. Stool samples are collected, temporarily stored and transported by participants, whilst several studies have indicated the stability of faecal calprotectin at room temperature variation from room temperature may alter bacterial degradation within the sample (Lasson et al., 2015; Whitehead et al., 2015). In terms of sample preparation and extraction faecal calprotectin is suggested to be uniformly distributed throughout a faecal sample thus minimising the impact of sampling site from the primary stool sample impacting upon the final estimates of faecal calprotectin (Sherwood & Walsham, 2016). In relation to faecal calprotectin extraction the use of the 'easy sampler extraction system' is designed to minimise inter-sample variability in relation to the faecal mass presented into the extraction buffer medium through a grooved system that collects the requisite amount required; although this view hasn't been supported (Whitehead et al., 2015).

A more fundamental consideration with faecal calprotectin determination is the duration of time allowed to pass between exercise/rest protocol performances until the collection of the faecal sample. The temporal disassociation between protocol execution and stool collection means it cannot be ascertained whether the release of calprotectin in the gut is due to normal GI mediated background inflammatory response or as a result of exercise related GI inflammation. Participants are free living and unless diet is restricted and standardised post protocol this may impact local gut inflammatory response (Mendall et al., 2016). Whilst non-exercise day samples presented may provide a representation of the background inflammation levels, the 12-18-hour gap between protocol execution and sample acquisition does not provide confidence in the provenance of the faecal calprotectin concentrations acquired. Indeed time of day variations are likely within clinical population from morning to evening (Lasson et al., 2015). If several baseline measurements were taken then the faecal calprotectin background could be established to compare the results obtained after the

exercise period, particularly as the results are somewhat counter intuitive to what was hypothesised and expected. Snipe et al. (2017) has also raised questions over the sensitivity of faecal calprotectin as a marker on similar grounds, i.e. resolving the temporal relationship between a likely but transient elevation in exercise induced inflammation and the ability to collect a faecal sample that match this transient inflammation.

Beyond the methodological factors, explanation of the data may rest with non-adherence to the protocol on rest/control days. Furthermore, whilst participants were requested to abstain from NSAID's and exercise prior to the assessment days, adherence to these guidelines is predicated upon participants' verbal confirmation. Clearly from the variation in calprotectin levels between rest and exercise, other variables were affecting the faecal calprotectin concentrations. It may also be that the exercise didn't stimulate sufficient GI distress because the intensity was not high enough and/or the duration not long enough; gut discomfort subjective ratings were low and did not exceed '4' a level which Pfeiffer *et al.* (2009) considers noteworthy in terms of subjective symptoms. However, some studies have noted this intensity and duration of exercise doesn't always increase GI permeability (Lambert et al., 2008; Snipe et al., 2017; Yeh et al., 2013) it does increase markers of tissue damage and potential inflammation (March et al., 2017; McKenna, 2017; Pugh et al., 2017; Snipe et al., 2017) Many other studies however have related increases in GI permeability with exercise of this intensity (Pals et al., 1997; Pugh et al., 2017; van Wijck, et al., 2011). These types of events cause reduction in splanchnic blood, hyperthermia and dehydration which causes mucosal barrier damage (Costa et al., 2017). Therefore, based on the faecal calprotectin concentrations observed it could be argued that no significant exercise mediated damage to the mucosal structure occurred. However, because faecal calprotectin was the only measure of gut damage/inflammation utilised the application of dual sugar tests and or other markers such as Intestinal Fatty Acid Binding Protein and/or others such as cytokine profiles, lactoferrin or antitrypsin-1 would be needed to confirm this view.

In conclusion we observe that steady state exercise for 50 min had no clear effect on faecal calprotectin levels. We observed a wide variability in the faecal calprotectin values obtained, with values exceeding some clinical guidelines thresholds. The lack of established 'normal' calprotectin ranges for young healthy athletic male population limits the conclusions that may be drawn from present data as clinical guidance indicates raised values can be associated with normal GI morphology and inflammation levels. The role of faecal calprotectin as a marker of exercise mediated gut inflammation/damage is thus unproven particularly with the assay methodology applied. Further research is required to validate the use of this

biomarker. In terms of application of faecal calprotectin in this thesis the application of this test will not move forward in the following studies.

Chapter 4

Gastrointestinal and physiological responses to soccer specific exercise performed under differing environmental conditions.

4.0 Gastrointestinal and physiological responses to soccer specific exercise performed under differing environmental conditions.

The aim of this chapter was to examine the effect soccer specific intermittent exercise has upon GI permeability and symptoms and how these may be modified by differing thermal environmental conditions. This study was presented orally at the *Conference on Movement, Health and Exercise (MoHE 2017)*, Kuala Lumpur, Malaysia, September 2017.

4.1 Introduction

Soccer is an intermittent, invasive field based sport characterized by repeated bouts of high intensity exercise super imposed upon a background low intensity exercise or static recovery (Bangsbo et al., 2006; Mohr et al., 2003). Typically, work rate analysis data indicates that during match play, players cover on average 10-14 km dependent upon position (Di Salvo et al., 2009), of this activity most is executed at low to moderate intensity (Bradley & Noakes, 2013). However, high intensity activity is critical to performance and accounts for approximately 8 % of the distance covered, with on average >1400 m acceleration and deceleration activities and >500 plus change of direction executed which vary as a function of player role (Bradley et al., 2010; Carling et al., 2012; Di Mascio & Bradley, 2013; Di Salvo, et al., 2012). More recently, the adoption of a metabolic cost paradigm to training and match play have ascribed higher levels of energetic and physiological load than that attributed to work rate analysis alone (Gaudino et al., 2014; Osgnach et al., 2010). Typically, players achieve average heart rates of ~85 % age predicted maximum which may correspond to an average of ~70 % maximal oxygen consumption ($\dot{V}O_2 \text{ max}$) (Bangsbo et al., 2006; Krstrup et al., 2006). Taken together both approaches are indicative of a performance environment that imposes high levels of both physical and metabolic load upon players (Di Salvo et al., 2012; Gregson et al., 2010; Suarez-Arrones et al., 2015).

In particular, the prolonged aerobic and intermittent nature of soccer activity imposes significant thermal loads which elevates core body temperature during soccer-match play, changes which are strongly associated with impaired physical, skills and cognitive performance across a range of performance metrics independent of performance environment (Kurdak et al., 2010; Mohr et al., 2012; Nybo et al., 2014; Ozgüven et al., 2010). These responses may be further compounded when match play is undertaken across different environmental conditions particularly where ambient temperatures are elevated (Aldous et al., 2016; Chmura et al., 2017; Maughan et al., 2010). Exercise in 'Hot' conditions i.e. >32°C is associated with an elevation in body temperature reducing the gradient for heat

exchange between the skin and environment (Nielsen et al., 1993). The imposition of humidity (>45 %) may further exacerbate such increases impairing evaporative heat loss and thermoregulatory response (Dvorak & Racinais, 2010; Grantham et al., 2010). Consequently, the combined exercise activity and heat stress imposed, mediates a number of well described compensatory physiological responses in molecular, metabolic and cardiovascular systems (González-Alonso et al., 2008; Nybo et al., 2014; Racinais & Sawka, 2015). In particular, the haemodynamic challenge of meeting exercising skeletal muscle demands and meeting thermoregulatory demands of exercise activity in hot conditions (>32°C) places significant stress on the gastrointestinal system in relation to thermal load management and maintenance of splanchnic perfusion (Hayashi et al., 2012; Perko et al., 1998). Appreciation of such challenges are vital in light of the frequent single and multiple exposure players have to match play particularly in hot and /or humid ambient condition. Qatar (2022) will see world cup match's played and training undertaken in conditions of high thermal load (Maughan et al., 2010; Sofotasiou et al., 2015). Hence, the effects of heat exposure during match play are important factors in performance and recovery management from games in light of the well documented effects hyperthermia may have on the induction of fatigue and impact upon the gastrointestinal system (Grantham et al., 2010; Kurdak et al., 2010).

Gastrointestinal disturbances as a result of exercise activity has been extensively reported across a range of athletic events and populations (de Oliveira et al., 2014; Riddoch & Trinick, 1988; ter Steege et al., 2008). It has been suggested that 4 % to 90 % of endurance sport participants experience some adverse GI symptoms related to exercise including nausea, vomiting, abdominal cramps and the urge to have a bowel movement (Haaf et al., 2014; Pfeiffer et al., 2009; Pfeiffer et al., 2012; Wilson, 2017). Whilst it is clearly evidenced that sustained endurance exercise of varying durations and intensity mediate increased permeability and damage in the GI tract (Jeukendrup et al., 2000; Pals et al., 1997; Roberts et al., 2016b; van Nieuwenhoven et al., 1999; van Wijck et al., 2011), the effect of the combined endurance and intermittent activity of soccer related intermittent exercise on GI permeability remains unknown. It also remains to be determined what impact the performance of such intermittent exercise under different environmental condition that challenge the maintenance of splanchnic blood and thermoregulation have on GI permeability and the expression of GI symptomology. The aim of this study was therefore twofold; **1)** to assess changes in GI permeability after combined continuous and intermittent i.e. soccer specific intermittent exercise (SSIE) for 90 min' relative to no exercise and **2.)** to determine the impact of environmental conditions i.e. Hot (32°C) vs Cold (12°C) on GI permeability and symptomology.

4.2 Method

Initially 12 recreational soccer players volunteered and were recruited to participate in the study, all providing signed informed consent in accordance to the Liverpool John Moore's university ethics committee procedures, however due to illness and inability to commit to testing schedules only six male university soccer players were included in the final analysis (age 24 ± 2.4 years; body mass 74.4 ± 11.9 kg; peak oxygen uptake ($\dot{V}O_2$ peak; 57.1 ± 8.1 mL·kg⁻¹·min⁻¹). Players were recruited during the pre-and early season period and were actively participating in soccer matches and team training. Sample size estimates were determined a priori based upon data of Pals et al. (1997). Assuming an exercise to rest GI permeability ratio difference of 0.05 arbitrary units and an anticipated SD of 0.02. the initial sample of 12 was determined. Assuming a type I error of .05, a type II error rate (i.e. power of 80%) a total of 12 participants were estimated as required for this study (V.18, Minitab Inc, PA, USA)..

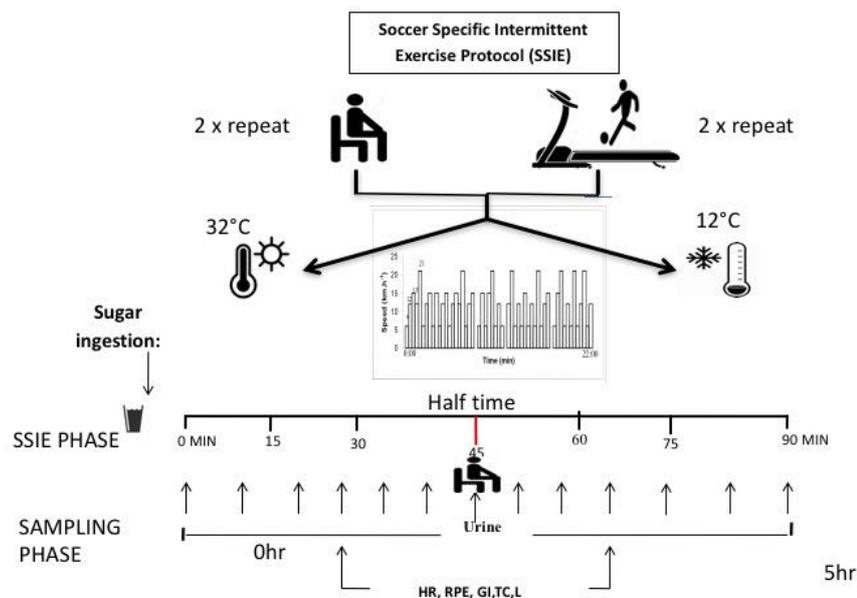


Figure 4.0 Schematic diagram of experimental design of the gastrointestinal and physiological responses to soccer specific intermittent exercise performed under differing environmental conditions.

4.3 Experimental design

This study was a, counterbalanced repeated measures design. Five testing sessions were organised; one preliminary assessment session followed by two rest and activity sessions, each interspersed by 3-7 days (Figure 4.0). All participants were tested in a post-absorptive state from an 8 h overnight fast, between 08:00-11:00 h. Participants were instructed to refrain from strenuous exercise 48 h prior to data collection sessions. During the 24 h period

prior to testing, each participant recorded a nutrition diary to reduce nutritional variation and were asked to avoid consumption of non-steroidal anti-inflammatory drugs and ergogenic aids, such as caffeine and alcohol during 24 h prior data collection. Participants were excluded if they used non-steroidal anti-inflammatory drugs. Environmental temperatures were set at 12°C and 32°C, relative humidity of 45 % for cold and hot environmental conditions, respectively in a environmental chamber (TISS,UK). These temperatures were selected as they represented the typical playing conditions experienced during transit from the start to the end of the typical European season. The soccer-specific intermittent exercise protocol was performed on a motorized treadmill (h/p/cosmos pulsar, Germany) and consisted of different exercise intensities that are observed during a 90-minsoccer match (e.g. walking, jogging, cruising, and sprinting) (Drust, et al., 2000). The rest protocol required no physical or psychological stressing activities, whilst seated upright for an identical duration and environmental conditions utilised for the soccer-specific intermittent protocol.

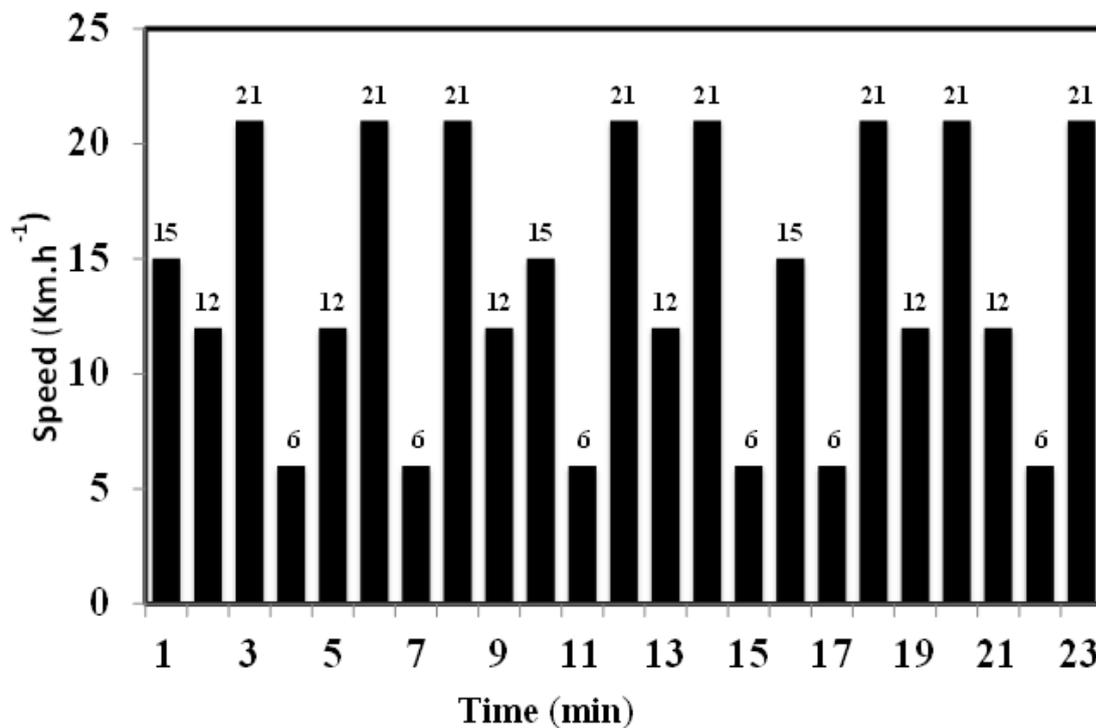


Figure 4.1 Schematic representation of the typical activity segment following the Drust et al. (2000) soccer specific intermittent exercise protocol.

4.4 Preliminary testing

Participants $\dot{V}O_2$ peak was determined using a progressive incremental protocol on a motorized treadmill (h/p/cosmos pulsar, Germany); temperature of 22°C and 45 % relative humidity. The protocol followed that previously outlined in (**Chapter 3; section 3.6**). The

criteria of the British Association of Sport and Exercise Sciences (BASES) were used to clarify if $\dot{V}O_2$ peak/max was attained (Winter et al., 2007). Expired respiratory gases were analysed by breath-by-breath, automated gas-analysis system (Metalyser, 3B Cortex, AZ, USA). (Chapter 3; section 3.6).

4.5 Experimental protocol

Upon arrival to the laboratory, each participant provided a urine sample, whereby volume and osmolality was recorded (Pal-Osmo, Vitech Scientific Ltd., Japan). Nude mass (SECA, 704 Birmingham, UK), resting heart rate (Polar Electro Oy, F-90440 Kempele, Finland), rectal temperature (VALSUITE, ellab A/S, Copenhagen, Denmark) and fingertip capillary blood lactate (Lactate Pro LT-1710, Arkray, Japan) was also recorded. Resting perceptions of effort (RPE) (Borg, 1982) and Thermal Comfort (Young et al., 1987) was also recorded. The participant then ingested the sugar test solution; 5 g lactulose (L), 1 g L-rhamnose (R), D-Xylose (0.5 g) and 5 g sucrose (S) in 115 ml of water, whereby 5 h urine collection period commenced. A gastrointestinal symptomology visual analogue scale (GI-VAS) (Moncada-Jiménez et al., 2009) to assess upper and lower GI symptoms such as heartburn, nausea, abdominal cramps, urge to defecate, was completed 15 min post ingestion of the test sugar solution. Participants were asked to place a mark on a line pertaining to their perception, and these were quantified in terms of percent full scale (i.e. 0 % = none, 100 % = severe). During exercise, heart rate and rectal temperature were continuously monitored and recorded at 1 min intervals. Participants RPE and thermal comfort were also continuously monitored and recorded at 2 min intervals. Fingertip capillary blood lactate was recorded prior to beginning of each half, and upon completion of each 22.5 min cycle of the soccer-specific intermittent exercise protocol (Figure 4.1)(Drust et al., 2000). Urine samples were collected and stored, with volume and osmolality recorded at 0 and 90-min time intervals. GI-VAS to document incidence of GI symptoms during each respective 45 min periods of the soccer-specific intermittent exercise protocol were completed. Participants ingested $\sim 1 \text{ mL}\cdot\text{kg}^{-1}$ of water (temperature 10°C) at 15 min intervals. Participants were removed from the environmental chamber during the 15-min half-time interval, whereby they rested in a seated position in environmental temperature of 22°C and relative humidity of 45 %. Participants ingested $\sim 1 \text{ mL}\cdot\text{kg}^{-1}$ of water immediately prior to re-entering the environmental chamber to commence the second period of the soccer specific exercise protocol.

Post-exercise nude mass was recorded to determine fluid loss (Pre- Post [+fluid ingested]). Participants then ingested water to replace mass loss and to encourage urination. Urine samples were collected and stored, with volume and osmolality recorded at over the

remaining time period up to 5 h after sugar ingestion. Total urine volume and osmolality (5 h urine collection period) was recorded (mL). If participants needed to urinate during exercise, post sugar probe ingestion and outside of allotted times, these were recorded and urine added to total volume. All urine samples collected throughout the 5 h collection period was stored and frozen at -80°C for analysis, by HPLC assay. Participants were allowed to consume non-sucrose containing foods 3 h post sugar test solution ingestion, during each rest and activity testing sessions. A final GI-VAS was completed 5-h post-test solution ingestion.

4.6 Analytical procedures

Assessment of intestinal permeability

Intestinal permeability for the recovery of Lactulose and L-Rhamnose was assessed by analysing pooled 5 hour urine samples using a previously published protocol (Fleming et al., 1996), with the modification of using L-rhamnose instead of mannitol as the monosaccharide probe. (Chapter 3, section 3.10).

4.7 Statistical analysis

Descriptive statistics were produced for all data sets to check for normal distribution as indicated by Kolmogorov-Smirnov (accepted if $P > 0.05$). Data was examined utilising a two-way within subject design general linear model Mode: [Exercise vs Rest] and Environment [Hot vs Cold] to determine the effects of discrete parameters of GI Permeability and GI Symptomology. Physiological and perceptual responses to soccer-specific intermittent exercise over time for the parameters heart rate, RPE, thermal comfort, rectal temperature, were determined via the addition of a third main effect [time] (Three way) ANOVA. Where a significant main effect was determined, pairwise comparisons were analysed according to Bonferroni post hoc in order to locate specific differences. If Mauchley's test of sphericity indicated a minimum level of violation, as assessed by a Greenhouse Geisser epsilon (ϵ) of ≥ 0.75 , data were corrected using the Huynh-Feldt ϵ . If Mauchley's test of sphericity was violated, data were corrected using Greenhouse Geisser ϵ . Paired *t*-test analysis was use where appropriate. Statistical significance was set at $p < 0.05$. Statistical analysis was performed using SPSS statistical software (SPSS 23.0, SPSS, Inc., Chicago, IL, USA). Visual representations of experimental data were produced using Microsoft Excel software package. Data are presented throughout as Mean \pm SD.

4.8 Results

4.8.1 Physiological Responses Rest. During exercise heart rate, rectal temperature, lactate, and body mass losses were all significantly increased in relation to their corresponding rest and environmental condition. Ratings of perceived exertion and thermal sensation were also significantly increased in relation to rest and their corresponding environmental condition ($P < 0.05$) (Table 4.1).

Table 4.1 Physiological response to SSIE and rest in Cold (12°C) and Hot (32°C) environmental conditions.

Variable	Rest		Exercise	
	Cold	Hot	Cold	Hot
Average Heart rate (b·min ⁻¹)	59 ± 5	70 ± 7†	139 ± 15*	163 ± 7*^
Peak Heart rate (b·min ⁻¹)	56 ± 5	66 ± 7†	155 ± 18*	185 ± 4*^
Peak rectal temperature (T _c °C)	36.7 ± 1.0	37.0 ± 0.3	38.3 ± 0.5*	39.1 ± 0.5*^
Lactate (mmol·L ⁻¹)	0.9 ± 0.1	0.9 ± 0.1	2.0 ± 0.1*	4.2 ± 0.7*^
Pre-post protocol mass loss (kg)	0.2 ± 0.3	0.4 ± 0.3	0.9 ± 0.3*	1.6 ± 0.5*^
RPE (AU)	6 ± 0	6 ± 0	12 ± 2*	13 ± 2*^
Thermal comfort scale (AU)	2 ± 0	7 ± 0†	5 ± 0*	7 ± 1*^

*Significant from rest ($P < 0.05$), † significance from cold rest ($P < 0.05$), ^ significant from cold exercise, ($P < 0.05$), RPE- Rating of perceived exertion. (AU) = Arbitrary Units.

4.8.2 Physiological Responses: Soccer Specific Exercise.

4.8.2.1 Heart Rate.

Heart rate was significantly elevated relative to rest and during 90 min' SSIE exercise in 32°C than 12°C ($F_{1,25} = 2.993$, $P = 0.0001$). (Figure 4.1).

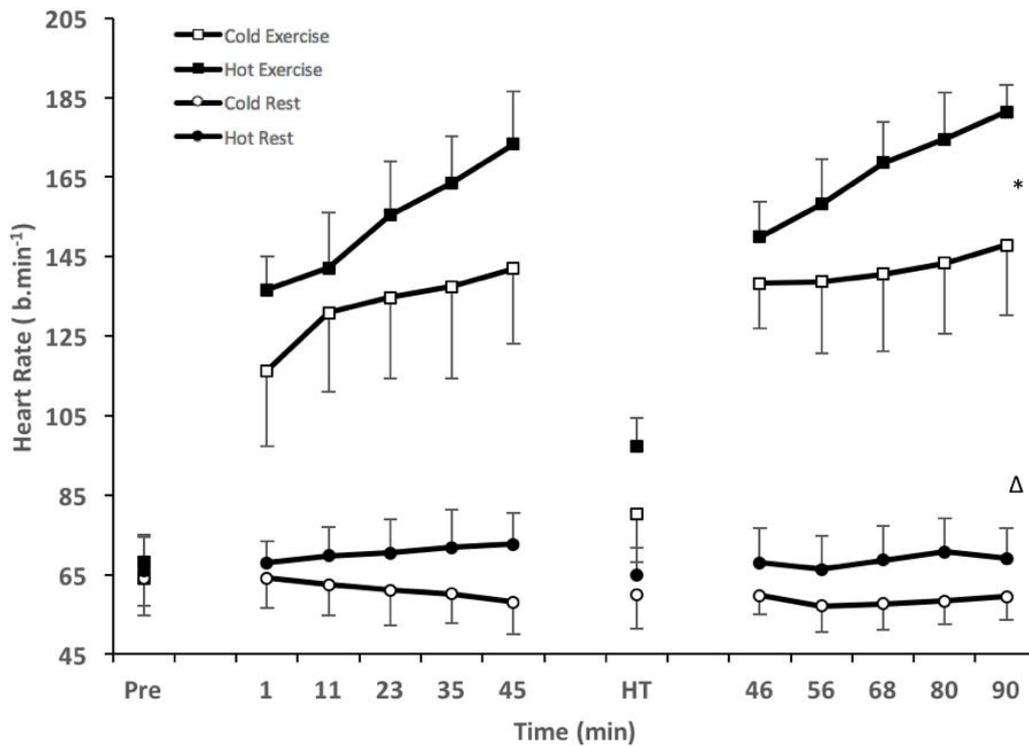


Figure 4.1 Heart rate responses at rest and during soccer-specific intermittent exercise in Cold (12°C) and Hot (32°C) temperatures. * Significant difference between exercise vs rest and environmental conditions Δ ($P < 0.005$).

4.8.2.2 Rectal Temperature. There was a significant main effect of condition, environmental temperature on rectal temperature responses during exercise ($F_{1, 5} = 21.257, P = 0.006$) (Figure 4.2). Rectal temperatures responses were significantly elevated during exercise in 32°C relative to 12°C. There was a significant main effect of time upon temperature elevation during soccer-specific exercise with greater increases observed during second period than the first period ($F_{11, 55} = 42.901, P < 0.001$). There was a significant interaction ($F_{11, 55} = 9.411, P < 0.001$) with the increase in rectal temperature higher during hot (1.2°C) than during cold (0.9°C) conditions.

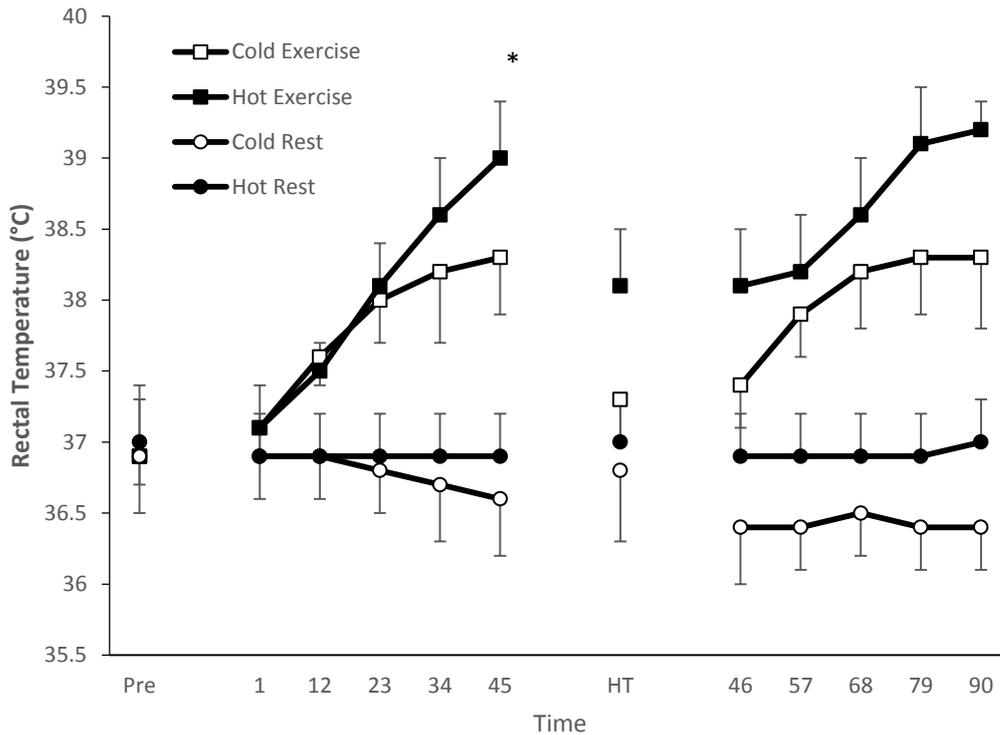


Figure 4.2 Rectal temperature response at rest and during soccer-specific intermittent exercise in Cold (12°C) and Hot (32°C) temperatures. * Significant difference within and between exercise conditions and rest conditions ($P < 0.001$).

4.8.2.3 Lactate. There was a significant main effect of environmental temperature upon capillary lactate accumulation during exercise ($F_{1,5} = 15.577$, $P = 0.011$) (Figure 4.3). There was a significant main effect of time of soccer-specific exercise ($F_{1,25} = 7.887$, $P < 0.001$). There was a significant interaction, such that lactate increased to a greater extent under hot relative to cold exercise condition's ($F_{1,25} = 2.993$, $P = 0.03$).

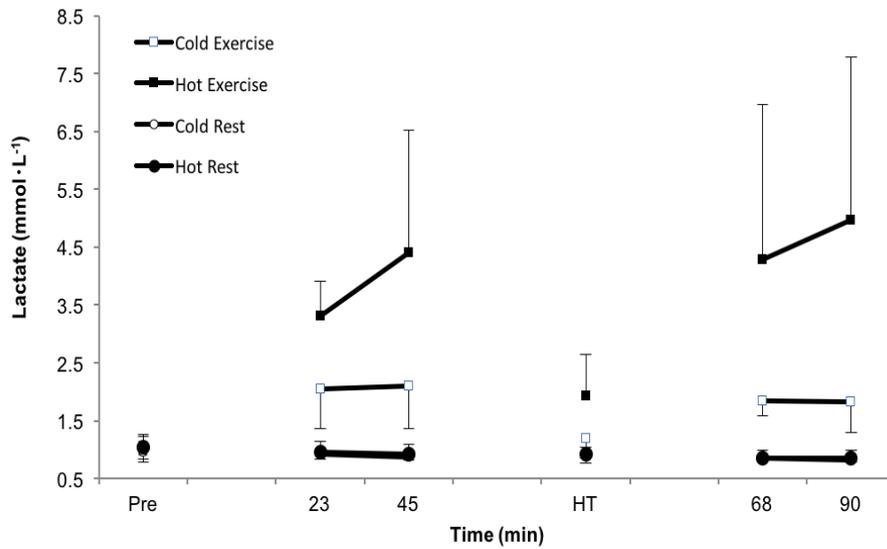


Figure 4.3 Lactate response at rest and the soccer-specific intermittent exercise in Cold (12°C) and Hot (32°C) temperatures * Significant difference between exercise conditions.

4.8.2.4 Pre- Post Body Mass Loss. Body mass loss (kg) was significantly greater during hot than cold exercise (1.7 ± 0.5 kg VS 0.9 ± 0.3 kg) ($t_5 = -3.945$, $P = 0.011$), with greater losses observed during hot than cold corresponding to a $2.2 \pm 0.7\%$ and $1.2 \pm 0.4\%$ body mass loss, respectively (Figure 4.4).

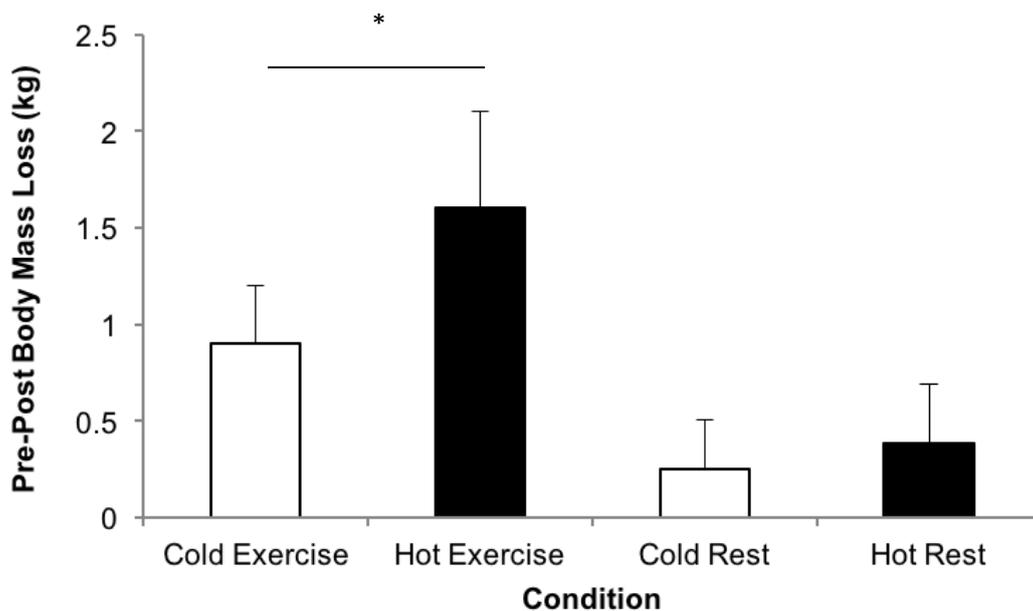


Figure 4.4 Body mass loss (sweat loss) following rest and the soccer-specific intermittent exercise in Cold (12°C) and Hot (32°C) temperatures. * Significant difference between environmental conditions ($P < 0.05$).

4.8.3 Psycho-physiological components

4.8.3.1 Ratings of Perceived Exertion. There was a significant main effect of environmental temperature upon ratings of perceived exertion during exercise with RPE responses were significantly elevated during exercise in 32°C than 12°C (Figure 4.5) ($F_{1,5} = 17.652, P = 0.008$). There was a significant main effect of time upon soccer-specific exercise ($F_{11,55} = 29.166, P < 0.001$), with greater increases observed during second period than the first period ($P < 0.001$). There was a significant interaction ($F_{11,55} = 7.355, P < 0.05$) with RPE elevated to a greater extent during hot than during cold exercise .

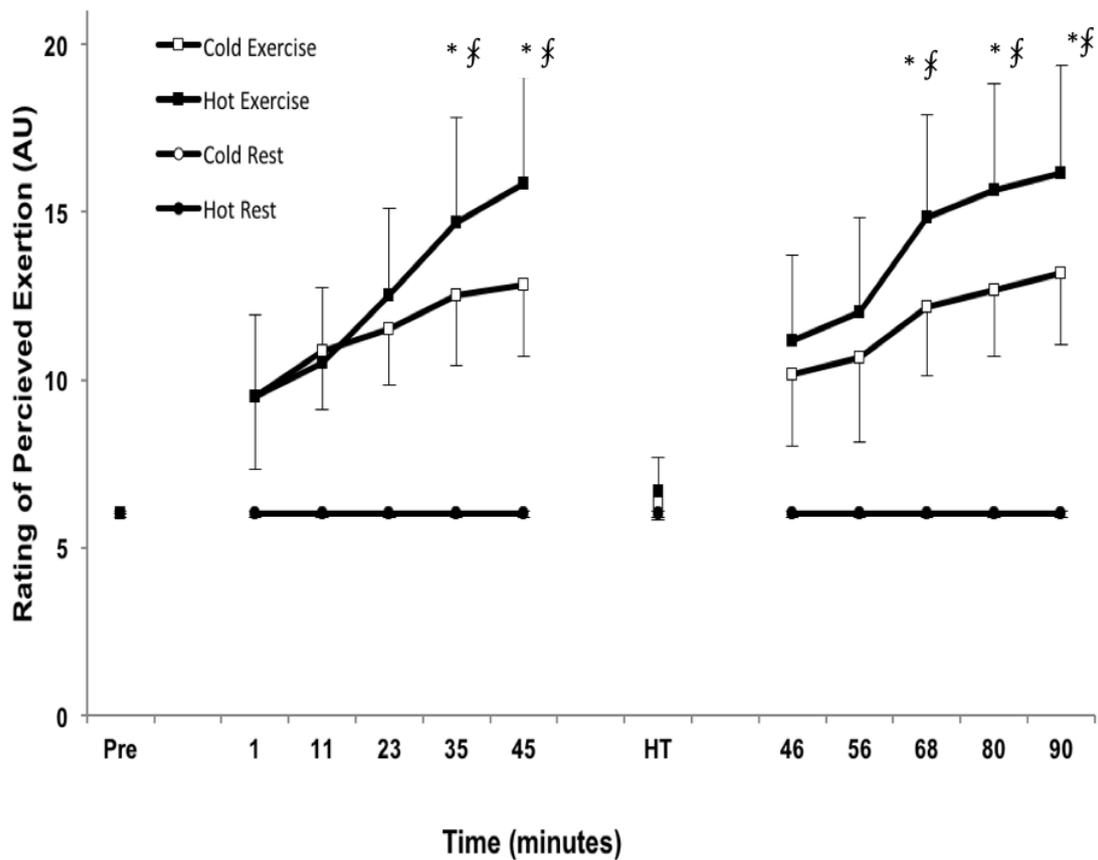


Figure 4.5 Rating of perceived exertion during rest and soccer-specific intermittent exercise in the Cold (12°C) and Hot (32°C) temperatures . * ‡ Significant difference between exercise conditions ($P < 0.05$). Note standard error bars are obscured due to $SD=0$ on rest hot and cold conditions which overlie each other. AU = Arbitrary units

4.8.3.3 Thermal Comfort. There was a significant main effect of environmental temperature upon ratings of perceived thermal comfort during exercise ($F_{1,5} = 41.967, P < 0.001$). Mean thermal comfort responses were significantly elevated during exercise in 32°C relative to

12°C (Figure 4.6). There was a significant interaction such that thermal comfort increased more in the hot relative to the cold conditions ($F_{11,55} = 3.957, P < 0.05$).

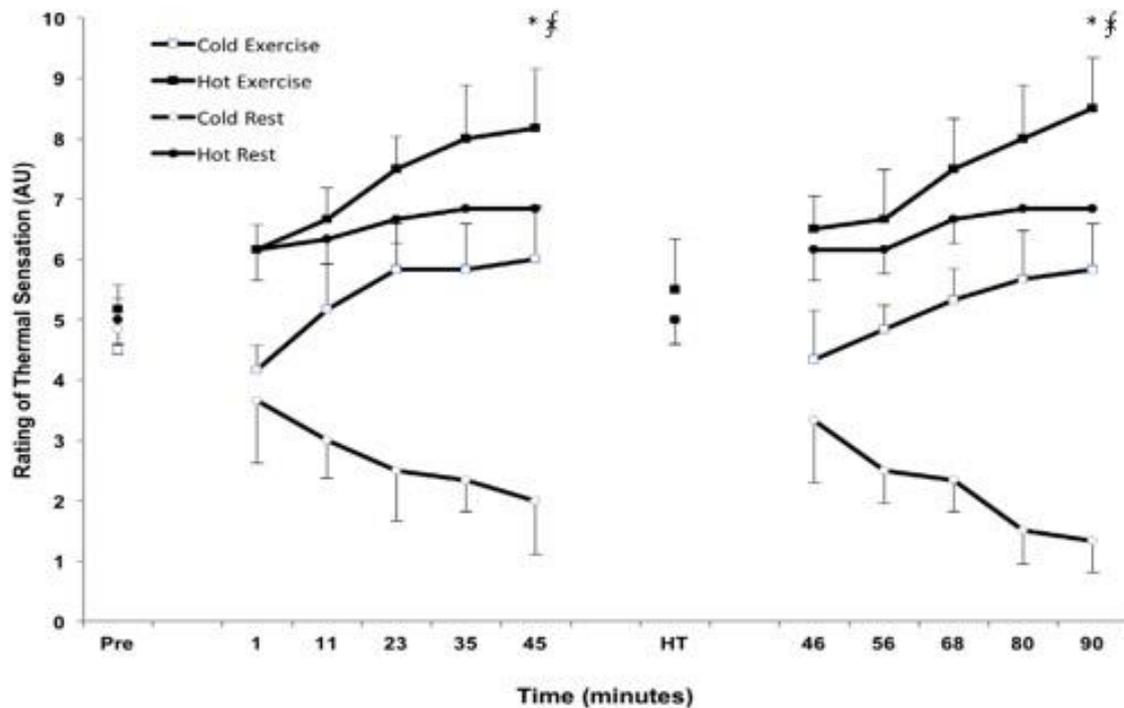


Figure 4.6 Ratings of thermal comfort (sensation) during soccer-specific intermittent exercise in Cold (12°C) and Hot (32°C) temperatures. *‡ Significant difference between exercise conditions and temperatures ($P < 0.05$). AU = Arbitrary units.

4.8.4 GI Permeability. Evaluation of gastrointestinal permeability via Lactulose/L-rhamnose ratio indicated there was a no main effect of soccer-specific intermittent exercise upon intestinal permeability as assessed by lactulose/L-rhamnose ratio, compared to rest ($F_{1,5} = 3.30, P = 0.129$). There was however a significant main effect of environmental temperature ($F_{1,5} = 8.35, P = 0.034$). No significant interaction ($F_{1,5} = 1.56, P > 0.05$) effect was apparent. Urinary excretion ratio of Lactulose/L-rhamnose increased during exercise in both hot (86 %) and cold (46 %) relative to their corresponding rest conditions (Figure 4.7).

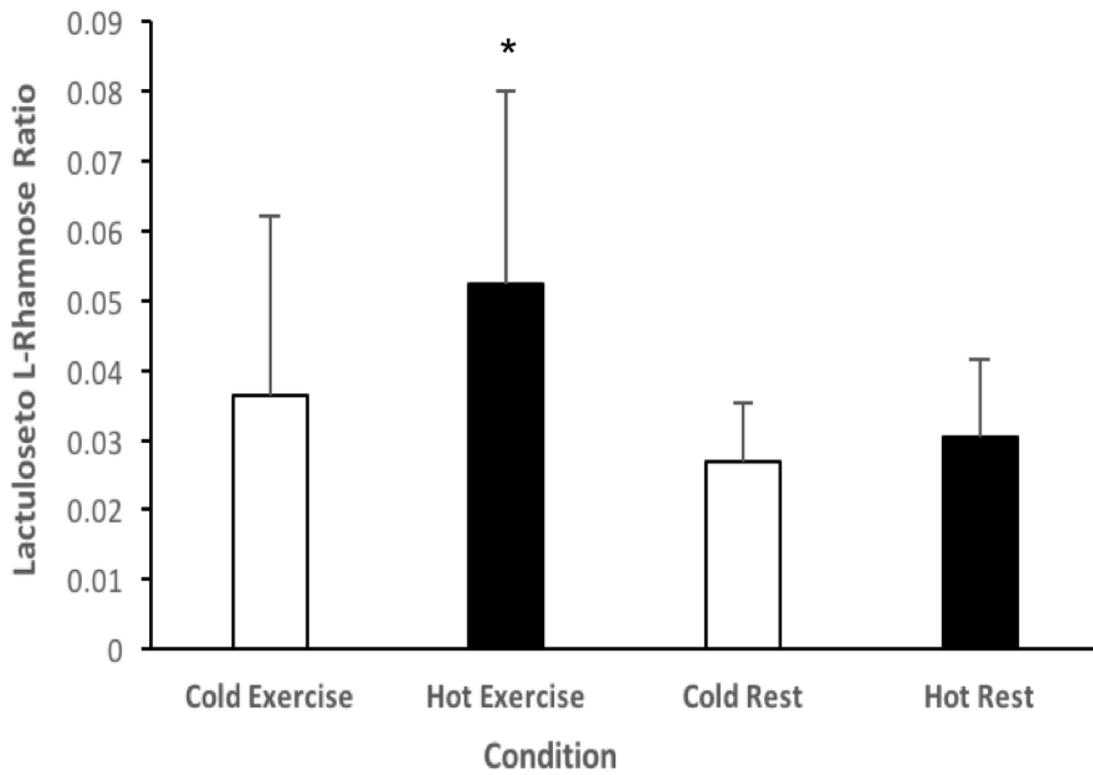


Figure 4.7 Urinary excretion (5 h post ingestion) Lactulose/L-Rhamnose ratio following rest and soccer-specific intermittent exercise in cold (12°C) and hot (32°C) temperatures. * Significant main effect of environment ($P < 0.05$).

4.8.4.1 .Gastrointestinal Symptoms. Gastrointestinal symptoms expressed during exercise and rest under both hot and cold environmental conditions are represented in Table 4.2.

Table 4.2 Pooled symptomology severity (mm) for all time points (pre, half-time, full-time, 3 h post) during exercise testing sessions in cold (12°C) and hot (32°C) temperatures

Gastrointestinal Symptoms	Cold Exercise	Hot Exercise	Cold Rest	Hot Rest
Side stitch	0.1 ± 0.3	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
Nausea	0.0 ± 0.0	0.2 ± 0.8	0.0 ± 0.0	0.0 ± 0.0
Bloating	0.0 ± 0.0	0.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
Urge to burp	0.0 ± 0.2	0.5 ± 1.1	0.0 ± 0.0	0.0 ± 0.0
Urge to Vomit	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Urge to Defecate	1.6 ± 2.3	1.0 ± 1.8	0.0 ± 0.0	0.0 ± 0.0
Diarrhoea	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Need to fluctuate	1.2 ± 2.0	0.5 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
Flatuation	1.5 ± 2.4	0.4 ± 0.9	0.0 ± 0.0	0.0 ± 0.0
Stomach Cramps	0.1 ± 0.4	0.5 ± 1.3	0.0 ± 0.0	0.0 ± 0.0
Stomach Upsets	0.0 ± 0.0	0.6 ± 1.2	0.0 ± 0.0	0.0 ± 0.0
Intestinal Cramps	0.0 ± 0.0	0.3 ± 1.1	0.0 ± 0.0	0.0 ± 0.0
Dizziness	0.0 ± 0.0	0.9 ± 1.6	0.0 ± 0.0	0.0 ± 0.0
Shivering	0.0 ± 0.0	0.8 ± 1.8	0.0 ± 0.0	0.0 ± 0.0
Heart Burn	0.0 ± 0.0	0.2 ± 0.6	0.0 ± 0.0	0.0 ± 0.0

4.9 Discussion

The primary findings of this study indicate that relative to rest the soccer specific intermittent exercise model (SSIE) applied in this study does not significantly elevate GI permeability or increase the expression of subjective symptoms of GI dysfunction. However, data does indicate a significant additive effect of environmental temperature upon GI permeability but not GI symptoms in the Hot (32°C) relative to Cold (12°C) conditions across both exercise and rest conditions. The findings suggest that prolonged exposure to heat creates a greater gastrointestinal and systemic burden than in cold conditions. This is the first study to investigate the effect of SSIE upon GI permeability and the expression of subjective symptoms of GI dysfunction as well as examining permeability responses when undertaken under different environmental conditions. The hypothesis tested was based upon the general consensus that exercise activity would increase GI permeability if performed at sufficient intensity and duration (Pals et al., 1997). Superimposed upon that initial consideration was the idea that by ‘clamping’ the SSIE activity under two divergent environmental temperatures it would be possible to evaluate the effects of SSIE exertional induced permeability/symptomology and the additive/synergistic effect of SSIE undertaken in a low and high external thermal load environment.

The novel data herein indicate that SSIE does increase GI permeability by 86 % and 46 % relative to rest in Hot vs Cold conditions; these changes were however, non-significant. These non-significant changes in permeability following 90 min of SSIE exercise relative to rest are consistent with some (Lambert et al., 2008; Snipe et al., 2017; van Wijck et al., 2014; Yeh et al., 2013) following treadmill running at 60-70% $\dot{V}O_2$ max; but not all literature. Increases in GI permeability are more frequently reported and have been described across differing exercise intensities and durations. It has been observed that 60 minutes’ steady-rate cycling at 70 % maximal power output (van Wijck et al., 2012; van Wijck et al., 2011), treadmill running at 70% $\dot{V}O_2$ max (Marchbank et al., 2011; Zuhl et al., 2014), 80 % $\dot{V}O_2$ max (Davison et al., 2016; March et al., 2017; Pals et al., 1997), interval running (Pugh et al., 2017) and marathon/ultramarathon running (Gill et al., 2015; Ryan et al., 1998; Smetanka et al., 1999), all elevate GI permeability. The discrepancy between the current data and aforementioned studies may be due to a variety of situational factors that impact upon small intestine GI barrier function. Pires et al. (2016) has recently summarised a widely held idea that exercise intensity alone may be only a part of an inter-related network of factors that bring about changes in GI permeability status and for permeability to manifest requires other factors to be co-expressed; a view echoed by others to include a complex mix of exercise

modality and duration, hyperthermia, splanchnic perfusion changes, initial training and acclimation status, hydration status, and antioxidant status (Costa, et al., 2017; Dokladny et al., 2008; 2016; Pires et al., 2016; van Wijck et al., 2012). In addition, possible methodological issues relating to GI permeability detection methods may also contribute to observed differences (Pugh et al., 2017). Varied approaches relating to gut permeability probe selection, timing of probe administration, detection protocols of the sugar-based permeability tests combined with the use of different analytical techniques during these studies make it difficult to compare quantitative permeability indices directly across studies (Pugh et al., 2017; van Wijck et al., 2012; van Wijck et al., 2013).

Exercise intensity and duration are thought to be critical factors in driving and regulating GI permeability responses through modulating hyperthermia and splanchnic perfusion responses (Costa et al., 2017; Lambert et al., 2008; Pals et al., 1997; Pugh et al., 2017). As such, the physical strain imposed in the current simulation is critical to the consideration of the GI permeability responses noted. It should accurately reflect that seen in soccer match play, and mimic the physiological strain it imposes on the GI system. The physiological strain imposed during soccer under a range of environmental conditions, means players may typically achieve average heart rates of ~85 % - 95 % of age predicted maximum which can correspond to an average of ~70 % -90 % ($\dot{V}O_2$ max) dependent upon the environmental conditions under which the activity is implemented, the match-play conditions and playing position (Gregson et al., 2010; Kurdak et al., 2010; Mohr et al., 2012). Soccer match play however expresses a high level of variability that impacts upon performance and physiological metrics returned during any given match scenario (Aldous et al., 2016; Di Salvo et al., 2009) Understanding the impact of soccer performance across physiological system, has necessitated the development of a range of soccer simulation protocols to restrict this variability and facilitate the replication of soccer specific stress with more precise experimental control (Aldous et al., 2016; Roelands et al., 2015).

In the present study a motorised treadmill protocol was utilised (Drust et al., 2000; Sari-Sarraf et al., 2011; Sari-Sarraf et al., 2008) in order to impose a soccer match play match specific physiological strain upon the participants. Physiological data (Table 4.1) would indicate that the soccer specific protocol in the cold and the hot conditions elicited ~70% and 84% of maximal heart rate equating to estimated ~<60 % and ~<74 % $\dot{V}O_2$ peak. Such observations reflects the present population were relatively well conditioned with $\dot{V}O_2$ peak measures of ~57 mL·kg⁻¹·min⁻¹ and the estimated level of fractional utilisation of maximal exercise capacity elicited during both protocols lower than is noted in other similar activities

(Harper et al., 2016; Mohr et al., 2012). Additionally, the blood lactate responses indicate a significantly lower exercise intensity in the cold than the hot conditions and values below that noted in literature for both temperate and hot conditions (Dvorak & Racinais, 2010; Mohr et al., 2012). Taken together with the perceptual response data, it may be concluded that the exercise stress imposed as a result of the simulation protocol was insufficient particularly in the cold condition to mirror a 'realistic' soccer specific match-play load or that imposed during other simulation protocols (Aldous et al., 2016; Grantham et al., 2010; Harper and Hunter, 2016; Mohr et al., 2012). It is only with the superimposed 'heat stress' that physiological responses become similar to 'match play' observed under thermo-neutral conditions (26°C) (Gregson et al., 2010; Kurdak et al., 2010; Mohr et al., 2012). The lower exercise intensities of the SSIE protocol may explain the failure to achieve a significant increase in GI permeability when SSIE was considered alone. Several recent investigations indicate that 70 % of $\dot{V}O_2$ max for 50- 60 min may be a critical threshold for increases in permeability to be observed (Pires et al., 2016) with several studies at similar intensities also reporting no GI permeability alterations (Lambert et al., 2008; Snipe, 2017; Yeh et al., 2013). Present data should be considered in light of these observations.

During SSIE the onset of physical activity imposes a haemodynamic challenge in order to meet the increasing metabolic demands of skeletal muscle and the thermoregulatory demands of SSIE activity under varying environmental stress (Ozgunen et al., 2010). This challenge places significant stress on the gastrointestinal system in relation to managing splanchnic perfusion, thermal load and oxidative stress (Hayashi et al., 2012; Knight et al., 2017; Lambert, et al., 2002; Perko et al., 1998). The onset of SSIE is likely to bring about a redistribution of cardiac output with reductions in splanchnic blood flow of up to 80 % noted (Crandall & Gonzalez-Alonso, 2010; Knight et al., 2017; Rowell, 2004; van Wijck et al., 2012). Consequently, splanchnic hypo-perfusion both passively and during activity have been aetiologically associated with the expression of GI symptomology and changes in small intestine GI permeability (Otte et al., 2001a; ter Steege et al., 2012). Contributory to this association will be factors such as exercise intensity, pattern of load application, exercise duration and environmental temperature/humidity (Gutekunst et al., 2013; ter Steege & Kolkman, 2012). van Wijck et al. (2011) suggests as little as 20 minutes exercise at 70 % of $\dot{V}O_2$ max will impair GI splanchnic perfusion. Whilst Crandall et al. (2010) suggest both exertional and passive heat stress will accentuate splanchnic hypo-perfusion in ranges from 10-80 % reduction in blood flow. Pugh et al. (2017) following high intensity interval exercise have suggested a similar mechanism may be responsible for increased GI permeability as well as tissue injury although perfusion data is absent.

In the context of experimental protocols undertaken herein a significant hyperthermia during both the rest and SSIE protocols, was apparent with the highest temperatures achieved toward the end of the 1st and 2nd periods of play in the SSIE during the hot relative to cold conditions (Figure 4.2). This pattern of increase, and core temperatures achieved reflects that observed in similar studies during both passive hyperthermia and sustained soccer related activity in the heat and during other simulation protocols despite the lower relative exercise intensities achieved with the present protocol (Aldous et al., 2016; Chmura et al., 2017; Kurdak et al., 2010; Ozgüven et al., 2010). Passive and exercise-induced hyperthermia in the heat are both associated with elevated physiologic strain when compared to similar task execution in temperate or cold conditions (Crandall & Gonzalez-Alonso, 2010; González-Alonso et al., 2008; Snipe et al., 2017). Present finding concurs with this observation.

The elevations in small intestine GI permeability under passive, exertional and additional heat stress models presented here; are likely associated with epithelial barrier dysfunction (Lambert et al., 2002). It is thought that, hyperthermia-induced morphological disruption of enterocytes and tight junction protein function is noted in rodent models at high gut wall temperatures (>46 °c) (Lambert et al., 2002). *In vitro*, temperatures of 38.3°C have been demonstrated to cause damage to canine kidney epithelial cells (Moseley *et al.*, 1994). Additionally, elevations in core temperature in the physiological range (37°C to 41°C) cause increased permeability in an *in vitro* intestinal epithelial model (Bulkley, 1987; Hall et al., 2001; Lambert et al., 2002; Taylor & Colgan, 2007; Ward et al., 2014). Such interactions are potentially brought about via disruption of the tight junction family of protein structures including, Zonulin-1 (ZO-1), occludin, and claudin proteins within the GI barrier (Derikx et al., 2010; Grootjans et al., 2016; Matthijsen et al., 2009). These modifications likely impacting the structural integrity of adjoining epithelial cells, as well as the stabilization of the internal myosin light chain structures (Derikx et al., 2010; Grootjans et al., 2016; Zuhl et al., 2014). Disruption which accentuates GI barrier impairment (Barberio et al., 2015; Lambert, 2004; Vargas & Marino, 2016) .

Taken in tandem, hyperthermia and perfusion-reperfusion changes are considered important as a source of gut morphological damage in clinical and non-clinical scenarios and may contribute to observed GI permeability changes observed herein (King et al., 2015; Lambert et al., 2002; Zuhl et al., 2014). *In vivo* changes in small intestine GI permeability may be subject to a 'critical threshold' whereby core temperatures of up to 38.0°C 'may facilitate' increased permeability, whereas temperatures of above 39.0°C 'definitely induce' permeability (Pires et al., 2016). Present data would suggest that these thresholds where

exceeded with peak temperature in excess of these thresholds (Figure 4.2). Although others, have examined GI permeability in the heat using a slightly different exertional intensity/duration and thermal model heat (35°C) vs temperate (22°C) and found GI permeability to remain unaffected (Snipe et al., 2017; Yeh et al., 2013).

It should be remembered the level of hyperthermia reported is a function not only off the exercise and environmental stress but off the core temperature assessment methods utilized to assess it. In this study core temperature was determined with rectal thermometry; this approach may underestimate the temperature observed in the small intestine GI wall by up to 0.5-2°C due to location difference between rectum and small intestine. Pearson et al., (2012) suggest a 'temporal lagging' occurs between rectal GI measure of temperature relative to pulmonary artery temperature. The implication for the present work being the GI tract may be slower to increase in temperature but also slower to cool down in the post exercise period. As such the rate and extent of small intestine wall temperature changes may precede the core temperature rise and then likely lag behind in terms of enterocyte heat exposure reduction. Such extended thermal strain/exposure beyond the initial exercise phase may bring about further disruption to GI barrier integrity beyond the 90 minute window reported in the current model (Dokladny et al., 2006). This may provide an explanation for the increased between treatment GI permeability changes in high ambient heat environment.

A further confounding factor during the SSIE, is that core temperature elevation and splanchnic hypo-perfusion are likely to be accentuated with fluid loss. It is well established that total body water loss through sensible and insensible pathways reduces plasma volume, and reduces exercise performance (>2 % body mass loss), particularly in hot environments. The 2.2 % observed herein despite fluid ingestion is sufficient to impair performance (Edwards et al., 2007; Maughan et al., 2010; Nuccio et al., 2017). Such progressive losses in total body water may contribute to splanchnic blood flow reductions and impairment in the dissipation of thermal load from the GI wall.

Subjective reports of gastrointestinal disturbances are frequently reported, particularly amongst endurance sports and athletes (de Oliveira et al., 2014; Hoffman et al., 2016; Stuempfle & Hoffman, 2015; Wilson, 2017). In the present study, subjective expression of gastrointestinal symptomology was disassociated from the objective permeability marker (Table 4.2). The low symptomology scores expressed during both passive and SSIE, across both Hot and Cold conditions for both upper and lower GI symptoms is the first for a team sport activity profile, how this data reflects real world scenarios remains to be determined. Parallels may be drawn with Pugh et al. (2017) who described a similar dissociation

following high intensity interval exercise. These observations of no or mild symptoms are in line with that reported more recently following acute exercise across a range of exercise intensities, modalities, and environmental conditions particularly in controlled laboratory studies (Karhu et al., 2017; Lambert et al., 2008; Marchbank et al., 2011b; Morrison et al., 2014; Pugh et al., 2017; van Wijck et al., 2011; Zuhl et al., 2014). At present the narrative is generally consistent; a clear divergence in symptomology expression to that expressed in field studies (Pfeiffer et al., 2012; Rehrer et al., 1990; Riddoch & Trinick, 1988; ter Steege et al., 2008; ter Steege et al., 2012). This apparent discrepancy between field and laboratory symptom expression may be due to a number of factors. The variances may be reflective of the young trained population utilized, the administration of fluids thorough-out, carbohydrate ingestion, as well as the previously discussed protocol /exercise intensity effects. Exercise modalities used in laboratory studies have often been shorter in duration and lower in relative intensity than those typically seen in competitive endurance races with more heterogeneous populations.

In relation to the practical importance of these changes in GI permeability, small intestine permeability can have major implications for the digestion and absorption of carbohydrates and dietary protein in the acute recovery phase following exercise (Janssen Duijghuijsen et al., 2016; Janssen Duijghuijsen et al., 2017; van Wijck et al., 2014). Whilst we have observed increases in L/R ratios as a surrogate of increased GI permeability which are similar to the endurance-type exercise, this may indicate that soccer-specific intermittent exercise may compromise the small intestines integrity and impact upon its primary functional role to digest and absorb nutrients, impairing vital recovery processes.

4.10 Conclusions.

This study indicates GI permeability is elevated when un-acclimated well-trained soccer players are exposed to acute periods of both passive and exercise stress under hot conditions. Findings are supportive of the hypothesis linking additional thermoregulatory strain induced by environmental heat exposure to the observed elevated GI permeability. This change being brought about via possible exacerbation of splanchnic hypo-perfusion, intestinal ischemia, and hyperthermic strain upon the intestinal epithelium (Pires et al., 2016; ter Steege et al., 2008; Zuhl et al., 2014). However, confirmation of these mechanistic aspects requires further experimental verification as no assessment of GI perfusion has been undertaken during SSIE performance to corroborate these considerations. We also reported a clear divergence between objective and subjective markers of permeability and symptomology which poses questions as to the usefulness of these subjective scales under such settings of soccer specific intermittent exercise.

Chapter 5

Effect of high intensity intermittent (HIIT) vs steady state exercise (SS) in a cold (12° C) versus hot (32° C) environment on GI permeability and GI symptomology.

5.0 Effect of high intensity intermittent (HIIT) vs steady state (SS) exercise in a cold (12°C) versus hot (32°C) environment on GI permeability and GI symptomology.

This chapter develops the concept outlined from Chapter 4, in that it isolates and examines the relative impact of exercise pattern i.e. HIIT vs steady state continuous exercise upon GI permeability and symptoms and how these may be impacted under different environmental. It is hypothesised that both will increase GI permeability relative to rest and that permeability will increase to a greater extent under the Hot relative to Cold conditions.

5.1 Introduction

Gastrointestinal disturbances resulting from exercise activity have been extensively reported across a range of athletic events and populations (de Oliveira et al., 2014; Riddoch & Trinick, 1988; ter Steege et al., 2008). Wide variances in the numbers of individuals that report GI symptoms exist with 4 % to 90 % of endurance sport participants experiencing what they perceive to be some form of adverse GI symptoms (Haaf et al., 2014; Pfeiffer et al., 2009; 2012; Wilson, 2017). Literature supports the view that sustained endurance exercise of varying durations and intensity mediate increased GI permeability and damage (Jeukendrup et al., 2000; Pals et al., 1997; Roberts et al 2016; van Nieuwenhoven et al., 1999; van Wijck et al., 2011). The mechanisms of these subjective GI disturbances remain to be fully clarified, although possible mechanisms highlight increases in paracellular and transcellular intestinal permeability to luminal antigenic molecules (Lambert, 2009); secondary to the initiation of splanchnic hypo-perfusion (ter Steege et al., 2008; van Wijck et al., 2011), splanchnic hyperthermia (Dokladny et al., 2006; Lambert et al., 2002), tissue hypoxia and disruption to enterocyte tight junction proteins (Dokladny et al., 2016; Zuhl et al., 2014) with subsequent endotoxaemia development (Barberio et al., 2015; Brock-Utne et al., 1988). It is apparent from these studies that as exercise intensity increase there is a corresponding reduction in splanchnic perfusion (Otte et al., 2001; Rowell, 2004). In addition this exercise-hypo-perfusion response mirrors the inverse relationship apparent between exercise intensity and intestinal permeability (Pals et al., 1997). Consequently, it may be hypothesized that changes in blood flow and permeability respond as a function of how exercise intensity and duration are programmed. As such, it may be that the type of exercise training undertaken will predispose toward increased GI permeability and symptom expression.

High intensity interval training (HIIT) has become increasingly used as a mechanism for driving cardiovascular and skeletal muscle adaptation in a time efficient manner when considered relative to traditional sustained intensity endurance training (Buchheit & Laursen, 2013). However, whilst HIIT may provide an optimal training stimulus for improving aerobic

fitness, knowledge of how these different types of training HIIT and sustained endurance training influence the GI system is unknown as comparative evaluations haven't been completed. To date only one study has examined GI permeability response during Intermittent exercise noting that GI permeability and damage were increased. However no steady-state exercise protocol was implemented to evaluate if the activity profile (Pattern) impacted the level of GI permeability or symptoms expressed (Pugh et al., 2017). The question as to whether one form of exercise programming i.e. HIIT vs Steady state is more damaging to the GI system than another remains unresolved. A hypothesis may be advanced that would imply that HIIT exercise would be more damaging to the GI tract than steady state continuous exercise. That argument would proceed as follows; interval training which follows a 'work-recover-work-recover....' model will set up an oscillatory pattern of splanchnic blood that moves between periods of hypo-perfusion (exercise) followed by reperfusion (recovery). Consequently, this perfusion-reperfusion pattern is likely to induce local tissue hypoxia (exercise), and mediate possible increases in perfusion-reperfusion oxidative stress (recovery) all of which may impact upon GI barrier function. Whether the GI permeability and symptoms elicited following this model are higher relative to those observed following sustained steady state exercise is unknown (Otte et al., 2001; van Wijck et al., 2011). A separate analysis of HIIT vs sustained endurance exercise vs passive rest is therefore necessary to determine more precise effects of these exercise programming approaches on gut permeability and function.

Given that these exercise programmes are undertaken across a range of environmental conditions it also remains to be determined what impact performance of such intermittent and steady state exercise under different environmental conditions that challenge the maintenance of splanchnic blood and thermoregulation have on GI permeability and the expression of GI symptomology. The aim of this study was to therefore; 1) to assess changes in GI permeability and symptom expression after steady state continuous and intermittent exercise relative to rest and 2.) to determine the affect of environmental conditions i.e. Hot (32°C) vs Cold (12°C) on GI permeability upon the continuous and intermittent exercise conditions. It is hypothesised that **1.** Exercise relative to rest will increase GI permeability and symptom expression **2.** HIIT exercise would increase permeability and symptom expression to a greater extent than that observed during steady state exercise **3.** GI permeability and symptomology would increase across all protocols when undertaken in the Hot relative to the Cold conditions.

5.2 Methods

Participants. Initially 23 recreational athletes volunteered and were recruited to participate in this study all providing signed informed consent in accordance to the Liverpool John Moore's university ethics committee procedures, however due to illness and inability to commit to testing schedules, failure to adhere to guidelines only ten male recreational athletes (age 25 ± 3 years; body mass 74.4 ± 6.7 kg; Peak oxygen uptake ($\dot{V}O_2$ peak) 56.5 ± 3.8 mL·kg⁻¹·min⁻¹) were included in this final analysis. None of the participants had any previous history of GI related diseases or other gastric problems and were not regularly consuming non-steroidal anti-inflammatory drugs (NSAIDs). Participants were asked to abstain from exercise and alcohol at least 24 hours prior to experimental assessment and refrain from using NSAID during the study. Participant's confirmed verbally compliance with these requirements prior to experimental data collection. All experimental procedures and potential risks/discomforts were explained in detail and written informed consent was obtained prior to testing. The study was approved by the Liverpool John Moores University Ethics Committee. Sample size estimates were determined a priori based upon the data of Pals et al. (1997). Assuming an exercise to rest GI permeability ratio difference of 0.05 arbitrary units and an anticipated SD of 0.02. the initial sample of 12 was determined. Assuming a type I error of .05, a type II error rate (i.e. power of 80%) a total of 12 participants were estimated as required for this study (V.18, Minitab Inc, PA, USA).

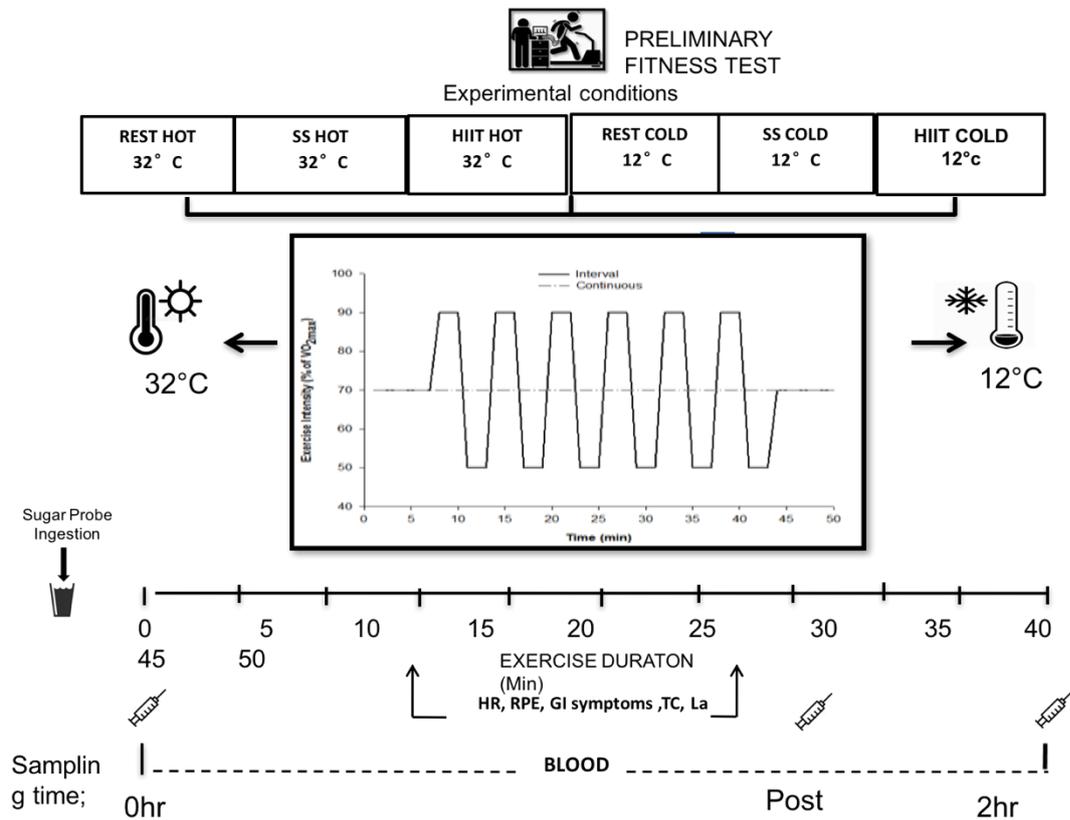


Figure 5.0. Schematic representation of the gastrointestinal and physiological responses to soccer specific exercise performed under differing environmental conditions.

5.2.1 Experimental design.

This study employed a counterbalanced repeated measures design. Seven data collection sessions were organised; one preliminary assessment session (section 5.2.1) proceeded by two passive rest (R) protocols R_{HOT}/R_{COLD} ; two steady state (SS) treadmill runs SS_{HOT}/SS_{COLD} and two high intensity intervals (HIIT) $HIIT_{HOT}/HIIT_{COLD}$ treadmill runs, each interspersed by 5-7 days (section 5.2.4). These protocols were performed under two different environmental conditions either a Cold (12°C) or Hot (32°C) condition. Passive rest and exercise protocol duration was set at 50 min. All participants were tested in a post-absorptive state from an 8 h overnight fast, between 08:00-11:00 h. Participants were instructed to refrain from strenuous exercise 48 h prior to data collection sessions. During the 24 h period prior to testing, each participant recorded a nutrition diary to reduce nutritional variation and were asked to avoid consumption of non-steroidal anti-inflammatory drugs and ergogenic aids, such as caffeine and alcohol during 24 h prior data collection. Environmental temperatures were set at 12°C and 32°C, relative humidity of 45 % for the cold and hot environmental conditions, respectively. The protocol-specific steady state or intermittent exercise protocol was performed on a motorized treadmill (H/P/cosmos pulsar, Nussdorf-Traunstein, Germany).

The rest protocol required no physical or psychological stressing activities, whilst seated upright for an identical duration and environmental conditions utilised for the alternate exercise protocol(s). All participants provided verbal confirmation as to compliance with the pre experimental instructions.

5.2.2 Preliminary testing:

Participants $\dot{V}O_2$ max/peak was determined using a progressive incremental protocol on a motorized treadmill (H/P/cosmos pulsar, Nussdorf-Traunstein, Germany) as outlined in (Chapter 3 Section 3.6); lab temperature of 22°C and 60 % relative humidity. The criteria of the British Association of Sport and Exercise Sciences (BASES) were used to classify attainment of $\dot{V}O_2$ max/peak (Winter et al., 2007), (Chapter 3, Section 3.6). Expired respiratory gas fractions were analysed via a Hans Rudolph oro-nasal mask continuously sampling using an online gas analysis system (Oxycon Pro, Jaeger, Netherlands) (Chapter 3, section 3.6) to allow for the subsequent determination (linear regression) of the workload required for the experimental steady state 70% $\dot{V}O_2$ peak or HIIT trials (90 % - 50 % $\dot{V}O_2$ peak).

5.2.3 Exercise Intensity Verification Trial.

Subsequent to the maximal treadmill running test participants rested for 30 min and then undertook a treadmill verification run to determine a $\dot{V}O_2$ /velocity relationship associated with the relative exercise intensity desired in the experimental protocols i.e. the high intensity (90 %), steady state (70 %), and recovery (50 %) of $\dot{V}O_2$ peak. Briefly participants, commenced running at the speed calculated from the regression analysis (section 5.2.3) to equate to either 50 % 70 % or 90 % of $\dot{V}O_2$ max; oxygen uptake (Oxycon Pro, Jagger, Netherlands) was measured as participants ran for 5 min' blocks at the speed corresponding to the required fraction of $\dot{V}O_2$ peak. Treadmill speed was adjusted either up or down to equate speed to $\dot{V}O_2$. Where values remained with $\pm 3 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ this was accepted as the protocol running velocity. This was repeated across all three intensities (50 %, 70 %, 90 %) with a 5-min break between each starting at 50 % intensity and working upward.

5.2.4 Experimental Protocol

On arrival at the laboratory, participants were seated for 15 min and had a venous cannula inserted into an ante-cubital vein for serial blood sampling for the assay of GI permeability following the time frame; pre protocol, immediately post protocol completion and 2 h post sugar probe ingestion (Chapter 3, section 3.10). Nude body mass was recorded (Seca 704, Birmingham, UK). Sample procurement and management and storage followed that outlined in Chapter 3 (section 3.13). Core body temperature was assessed via a rectal thermistor

(Mini-thermistor; Grant instruments LTD, Shepreth, UK) and monitored throughout exercise using an electronic data logger (Grant Squirrel 1000 series, Grant Instruments, Cambridge, UK) (Chapter 3, section 3.9.1). A Polar FT1 heart rate monitor transmitter band was positioned across the chest level with the xipho-sternum (Polar Electro Oy, Kempele, Finland) and heart rate was recorded at 1 min intervals. Pre and post-exercise haematocrit was determined using a fingertip blood sample collected into micro-capillary tubes. Tubes were spun at 10,000 rpm for 5 min (Heraeus Pico 17, Thermo Scientific, UK) and haematocrit was measured using a micro-haematocrit reader (Hawksley, Sussex, UK). Fingertip capillary blood for haematocrit and haemoglobin (Hb 201+, HaemoCue AB, Angelholm, Sweden) were determined using standard auto analysers (Chapter 3, Section 3.15). After instrumentation was complete, participants consumed the GI sugar permeability probe solution (chapter 3 section 3.10), 15 min for gastric emptying then elapsed whereby the 2 h collection period commenced (Chapter 3, Section 3.11). During exercise, rate of perceived exertion (RPE; 6-20) and GI comfort scale (0-10) were recorded every 3 min (Chapter 3, Section 3.7). Subjects consumed water at a rate of at least $\sim 1 \text{ mL}\cdot\text{kg}^{-1}$ every 15 minutes to alleviate fluid loss. Total fluid intake was recorded and post exercise nude body mass was obtained to determine estimated sweat rate. Upon completion of exercise a further blood sample was immediately obtained at 2 hours post sugar probe ingestion.

5.2.5 Experimental HIIT and Steady State Exercise Protocols.

In order to effectively address the issue of activity profile impact on GI permeability (i.e. HIIT and SS) we utilised a protocol system following the model of Bartlett et al., (2011) that were matched for total oxygen consumption and energy expenditure after being matched for average intensity, duration and distance ran. Briefly, each protocol consisted of running on the same motorised treadmill (H/P/cosmos pulsar, Nussdorf-Traunstein, Germany). The HIIT protocol began with a 7-min warm up at a running velocity corresponding to $\sim 70\% \dot{V}O_{2\text{max}}$ followed by 6 X 180 s- repetitions at a velocity corresponding to $\sim 90\% \dot{V}O_{2\text{max}}$. The HIIT intervals were separated by further 180 s active recovery periods at a velocity corresponding to $50\% \dot{V}O_{2\text{max}}$. Upon completion of the interval and recovery phase, participants then undertook a 7-minute cool down at a running velocity corresponding to $70\% \dot{V}O_{2\text{max}}$. The exercise protocol gave a cumulative time of 18-min of interval exercise and 18-min of active recovery, providing for a total interval exercise time of ~ 36 min. Including the warm-up and cool-down times, the total duration of the exercise protocol was 50-min ensuring the protocols were matched for duration. The steady state (SS) protocol consisted of 50-min of continuous running at a velocity corresponding to $\sim 70\% \dot{V}O_{2\text{max}}$. The average intensity during the HIT and the SS protocol, when quantified according to average

running velocity, equated to $\sim 70\% \dot{V}O_{2\max}$. The exercise protocols and their relationship to each other are presented in Figure 5.1.

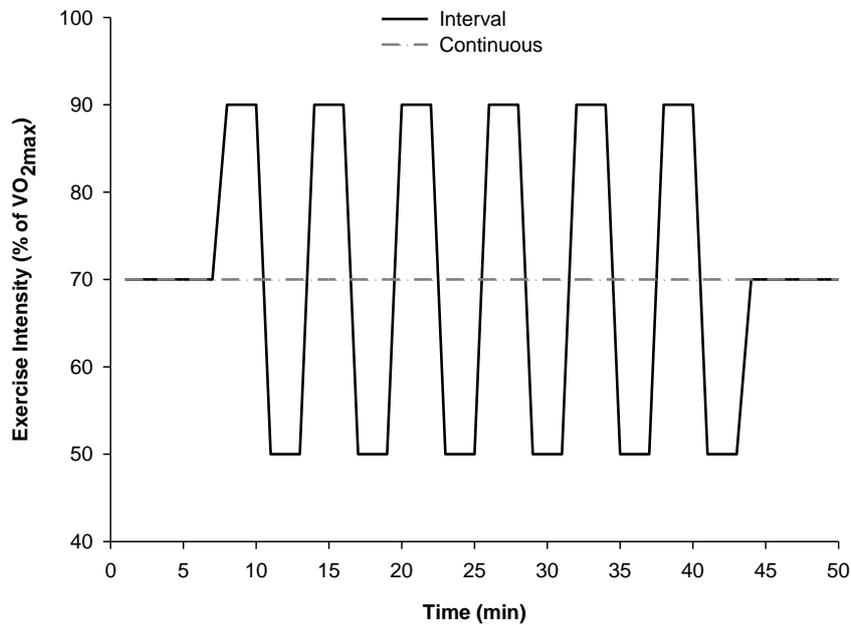


Figure 5.1: Schematic representations of the exercise steady state and intermittent exercise protocols. Redrawn after the model of Bartlett *et al.*, (2011).

5.3 Analytical procedures.

5.3.1 Assessment of intestinal permeability

Intestinal permeability for the recovery of Lactulose and L-Rhamnose was assessed by analysing serum samples using a previously published protocol (Fleming *et al.*, 1996), (Chapter 3, section 3.10).

5.3.2 Assessment of Serum Cortisol

Serum cortisol concentrations determined via automated Roche COBAS electrochemiluminescence immunoassay procedure following the manufacturer's instruction. The CV was <10%. The lower and upper limits of measurements were 0.5 and 1750 nmol·L⁻¹, respectively.

5.4 Statistical analysis.

Descriptive statistics were produced for all data sets to check for normal distribution as indicated by Kolmogorov-Smirnov (accepted if $P > 0.05$). Data was examined utilising a two-way within subject design general linear model Mode: [Exercise vs Rest] and Environment [Hot vs Cold] to determine the effects of discrete parameters of GI Permeability and GI Symptomology. Physiological and perceptual responses to rest, steady state and high intensity intermittent exercise over time for the parameters heart rate, RPE, thermal comfort, rectal temperature, were determined via the addition of a third main effect [time] (Three way) ANOVA. Where a significant main effect was determined, pairwise comparisons were analysed according to Bonferroni post hoc in order to locate specific differences. If Mauchley's test of sphericity indicated a minimum level of violation, as assessed by a Greenhouse Geisser epsilon (ϵ) of ≥ 0.75 , data were corrected using the Huynh-Feldt ϵ . If Mauchley's test of sphericity was violated, data were corrected using Greenhouse Geisser ϵ . Paired t -test analysis was used where appropriate. Statistical significance was set at $p < 0.05$. Statistical analysis was performed using SPSS statistical software (SPSS 23.0, SPSS, Inc., Chicago, IL, USA). Visual representations of experimental data were produced using Microsoft Excel software package. Data are presented throughout as Mean \pm SD.

5.5 Results

Exercise significantly elevated the parameters of heart rate, rectal temperature above the corresponding resting protocols when considered across both environmental condition ($P<0.05$). Ratings of perceived exertion, and thermal comfort were also significantly increased in relation to rest and their corresponding environmental condition ($P<0.05$) (Table 5.1). All parameters showed a significant pre-to post exercise increase in the exercise condition in the hot and cold conditions ($P<0.05$) with no significant elevations apparent in the resting protocols across both environmental conditions ($P>0.05$).

Table 5.1 Physiological and perceptual responses to rest, steady state and HIIT exercise in Cold (12°C) and Hot (32°C) conditions.

Variable	Rest		Steady State(SS)		HIIT	
	Cold (12°C)	Hot (32°C)	Cold (12°C)	Hot (32°C)	Cold (12°C)	Hot (32°C)
HR (b·min ⁻¹) (Average)	61 ± 10	63 ± 7	161 ± 12†	167 ± 12†	162 ± 13†	166 ± 12†
HR (b·min ⁻¹) (Peak)	61 ± 10	63 ± 7	174 ± 14†	177 ± 17†	173 ± 15†	180 ± 11†
Rectal Temp(°C) (Average)	37.1 ± 0.4	36.9 ± 1.0	37.7 ± 0.3	38.1 ± 0.2*	37.6 ± 0.3	38.0 ± 0.3
Rectal Temp(°C) (Peak)	37.1 ± 0.2	36.9 ± 0.4	38.0 ± 0.5	38.9 ± 0.1*†	38.4 ± 0.1	38.9 ± 0.1*†
Pre- post protocol mass loss (kg)	0.1 ± 0.2	0.1 ± 0.2	0.4 ± 0.3	1.0 ± 0.4	0.5 ± 0.5	0.9 ± 0.7
Thermal Comfort Scale (AU)	2.4 ± 0.8	3.3 ± 1.5	4.7 ± 1.0	7.0 ± 1.6*†	5.4 ± 1.5	7.7 ± 0.8*†
RPE (AU)	6 ± 0	6 ± 0	11.9 ± 1.1†	13.6 ± 1.6†	12.4 ± 2.0†	13.8 ± 2.0†
GI Discomfort Scale (AU)	0.4 ± 0.6	0.5 ± 0.6	2.0 ± 1.2	1.6 ± 1.0	1.5 ± 0.8	1.6 ± 0.9

† Significantly different to both rest conditions

* Significantly different to equivalent condition in the cold. (AU) Arbitrary Units

5.5.1 Physiological Responses:

5.5.1.1 Heart Rate. Average heart rate was elevated relative to rest and during 50 min' of HIIT and SS exercise in both hot and cold conditions ($P < 0.05$). Heart rate was significantly higher during exercise relative to rest ($P < 0.05$). No significant interactions were apparent between Hot and Cold conditions ($P > 0.05$) (Figure 5.1). There was a significant effect of exercise duration apparent ($P > 0.05$). However, we noted higher heart rate peak heart rates in the heat relative to the cold for HIIT exercise only.

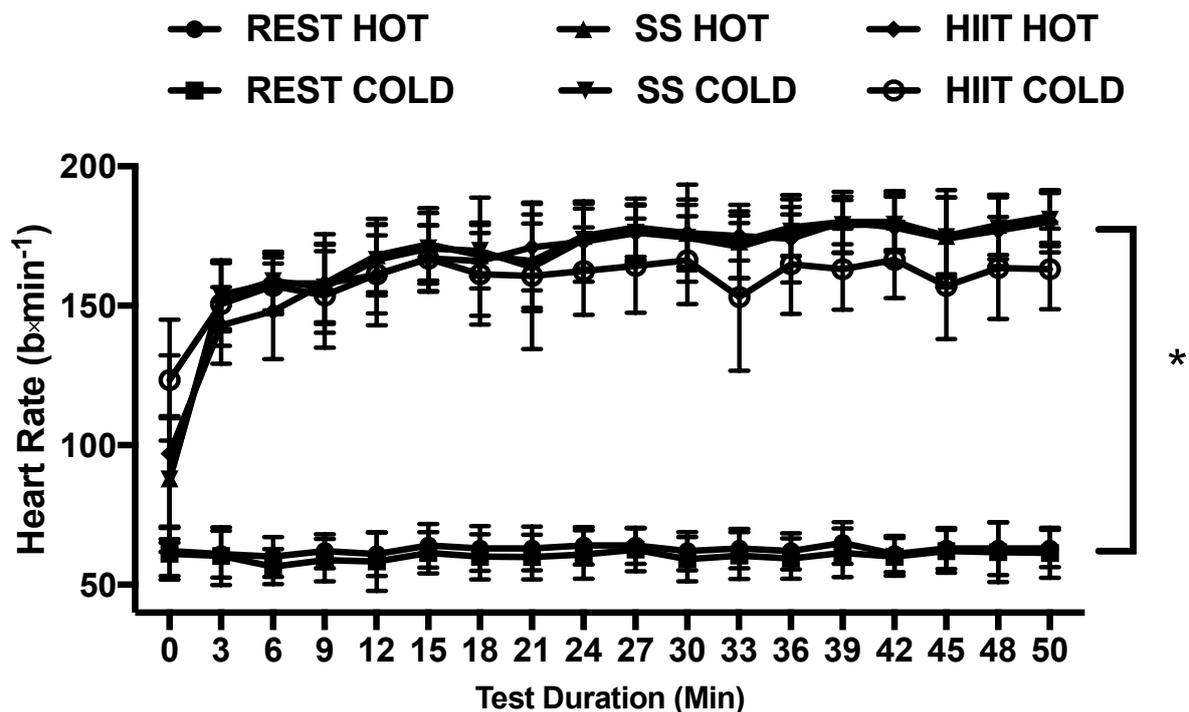


Figure 5.1: Heart rate response ($b \cdot \text{min}^{-1}$) to HIIT, steady state exercise and rest protocols in Cold (12°C) and Hot (32°C) conditions. * significant difference rest vs exercise across both cold (12°C) and hot (32°C) conditions.

5.5.1.2 Rectal Temperature. There was no significant main effect of exercise and environmental condition upon rectal temperature responses, however a main effect of time during all exercise conditions but not rest was apparent ($P < 0.001$). (Figure 5.2). Average rectal temperatures responses were higher but did not attain not significance during exercise in 32°C relative to 12°C ($P < 0.05$). There was a significant main effect of time upon temperature elevation during SS and HIIT exercise with temperature rising as exercise duration increased ($P < 0.001$).

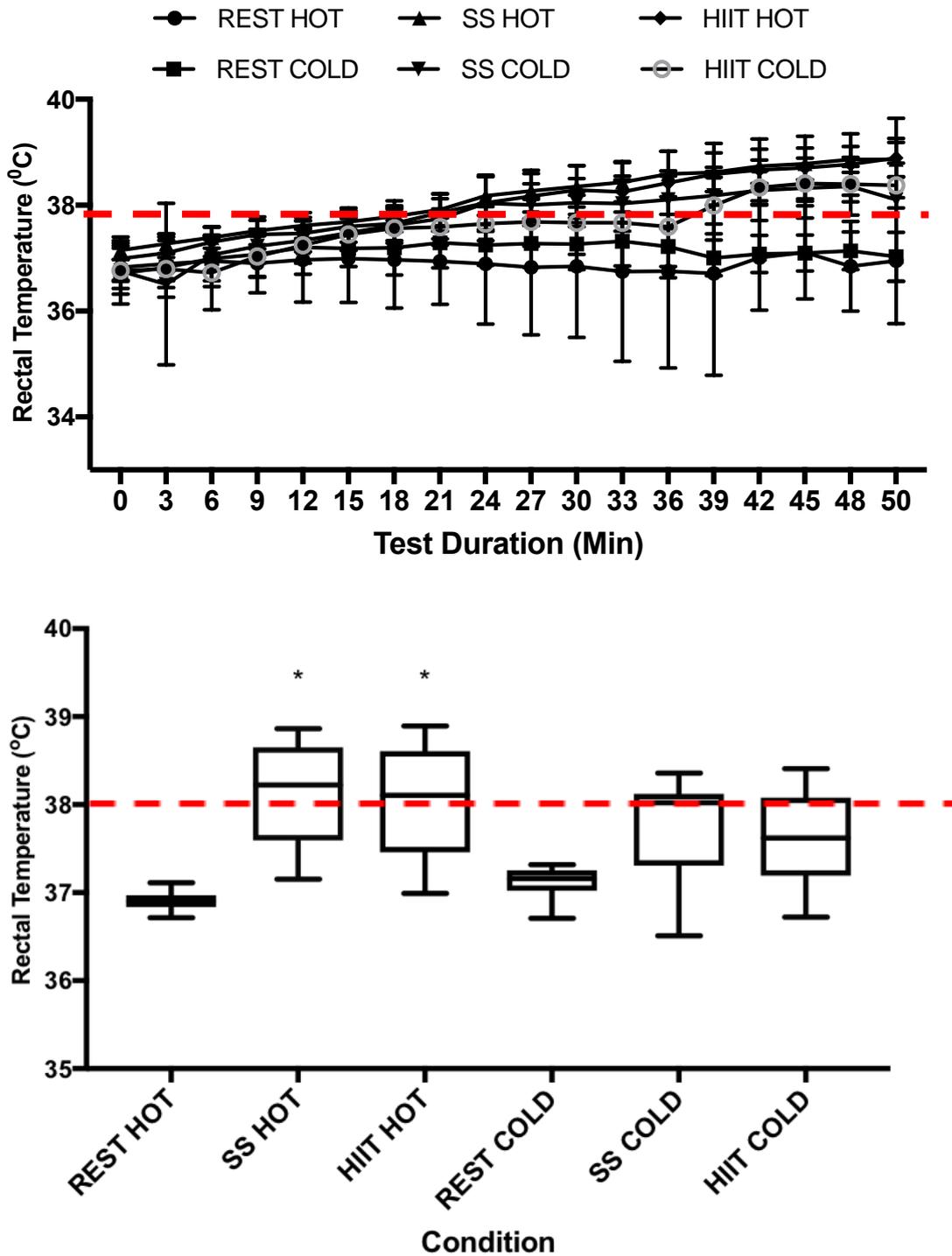


Figure 5.2 A. and B Core temperature response to HIIT and steady state exercise and rest protocols in Cold (12°C) and Hot (32°C) conditions over time and by condition expressed as mean and 95% CI (B). Broken line indicates the postulated Pries et al. (2017) threshold for temperature derived permeability induction hypothesis.

5.5.2 Psycho-Physiological Responses.

Ratings of Perceived Exertion. There was a significant main effect of exercise upon ratings of perceived exertion relative to rest (Figure 5.3). There were no significant interactions between exercise activity (SS and HIIT) and environmental temperature.

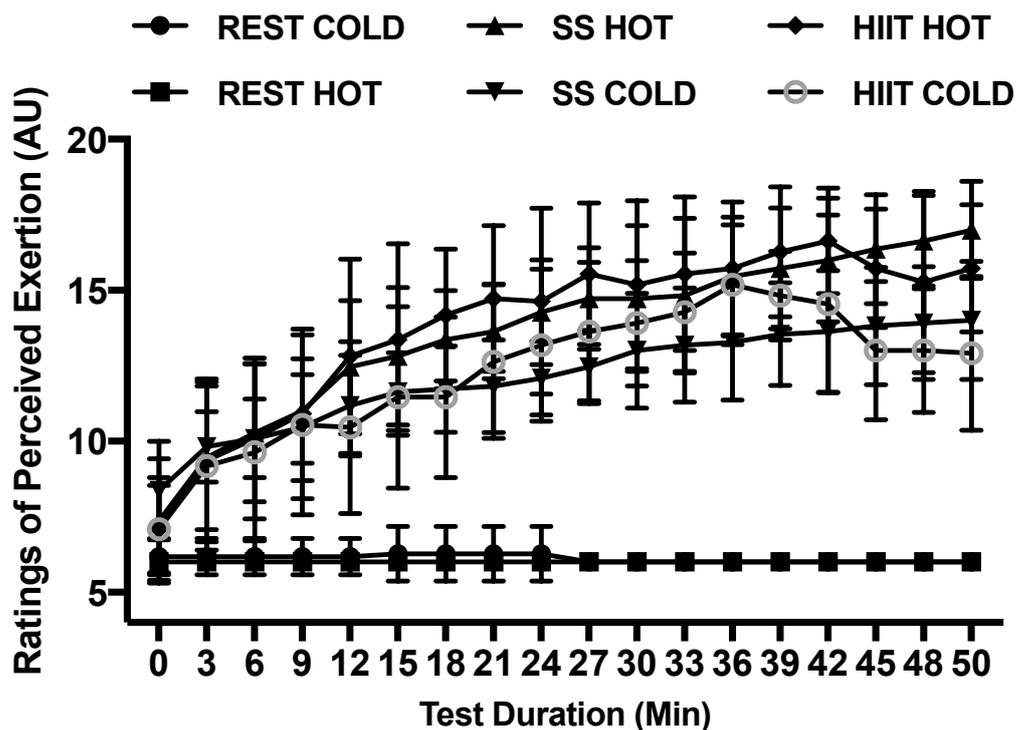


Figure 5.3 Rating of perceived exertion response to HIIT, SS exercise and rest protocols in Cold (12°C) and Hot (32°C) conditions.

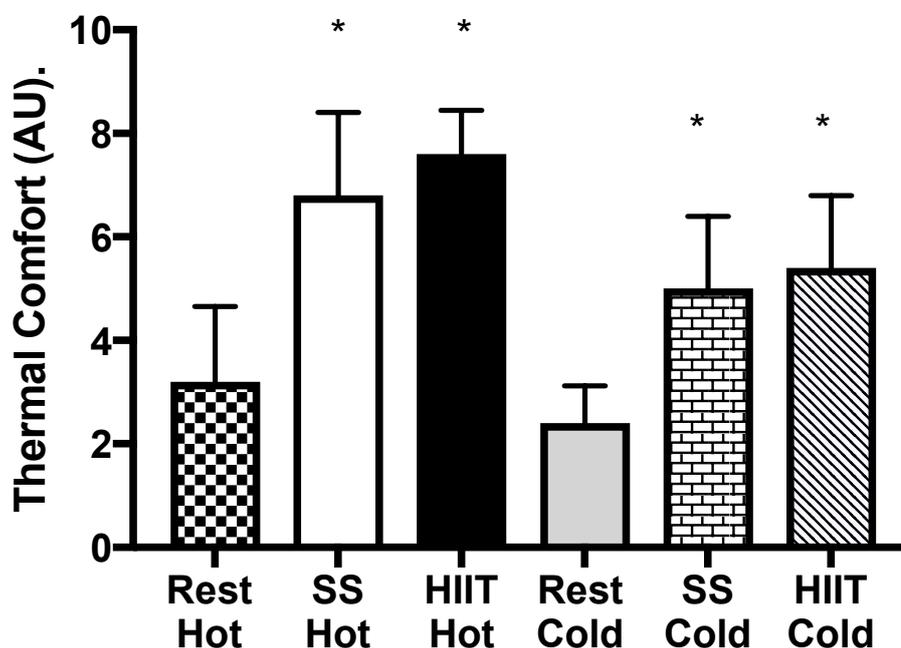


Figure 5.4: Thermal comfort responses to HIIT, steady state exercise and rest condition in Cold (12°C) and Hot (32°C) conditions.* Significant main effect of exercise alone relative to rest conditions ($P < 0.05$).

5.5. Thermal comfort scale. There was a significant main effect of exercise relative to rest upon for ratings of perceived thermal comfort during exercise ($P < 0.01$). Mean thermal

comfort responses were significantly elevated during exercise in 32°C than 12°C (Figure 5.4). There was a significant main effect of SS and HIIT duration of ($P \leq 0.001$).

5.5.3 Gastrointestinal Responses.

5.5.3.1 GI Symptoms. Gastrointestinal symptoms expressed during exercise and rest under both hot and cold environmental conditions are represented in Figure 5.5. There was a main effect for exercise relative to rest ($P < 0.05$), however there was no main effect for environment apparent ($P < 0.05$).

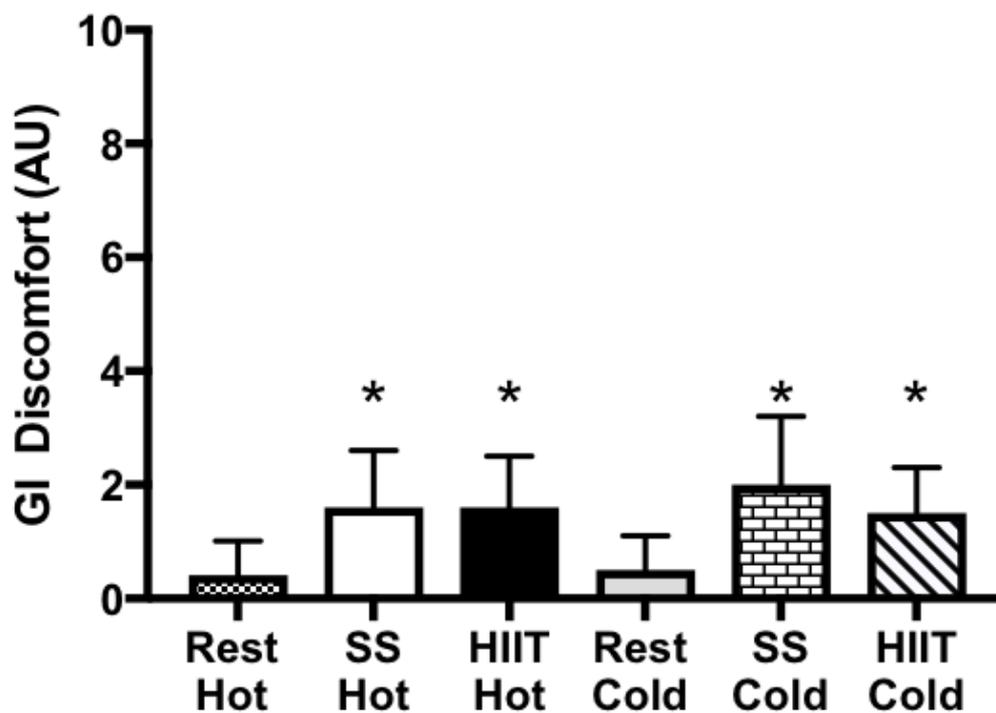


Figure 5.5: GI discomfort scale response to HIIT, steady state exercise and rest protocols in cold (12°C) and hot (32°C) conditions. * Significant main effect of exercise alone relative to rest conditions ($P < 0.05$).

5.5.3.2 GI Permeability. Evaluation of GI permeability via Lactulose/L-rhamnose ratio indicated there was a main effect of exercise upon intestinal permeability, compared to rest ($F_{2, 12} = 5.28, P > 0.05$) with HIIT and steady state exercise elevating permeability above the resting condition. There was no significant main effect of environmental temperature upon exercise related GI permeability ($F_{1, 6} = 1.78, P > 0.05$.) No interaction ($F_{2, 12} = 2.68, P > 0.05$) effects were apparent with both steady state and HIIT responding in a similar manner across the hot and cold conditions (Figure 5.6).

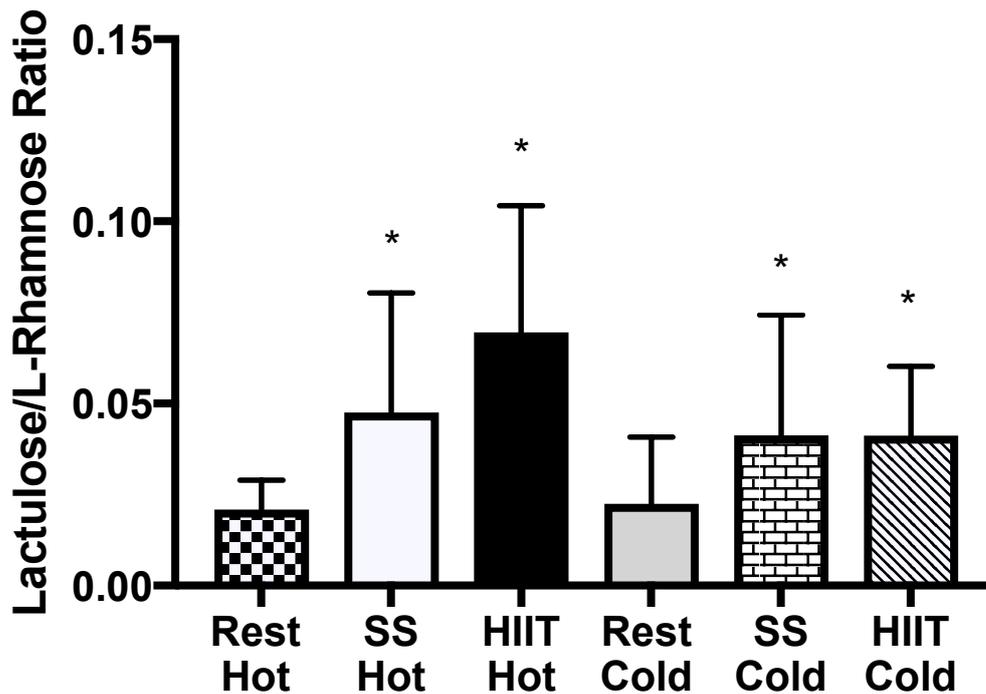


Figure 5.6: Serum recovery (2 hrs post ingestion) of Lactulose/L-Rhamnose ratio following rest, steady state and HIIT exercise in Cold (12°C) and Hot (32°C) conditions. * Significant main effect of exercise relative to rest conditions ($P < 0.05$).

5.5.4 Serum Cortisol responses

Serum cortisol showed a main effect for activity and time alone ($P < 0.05$) with no main effect for environment apparent ($P > 0.05$) (Table 5.2).

Table 5.2 Serum cortisol responses to the rest, steady state and HIIT protocols in cold (12°C) and Hot (32°C) conditions.

Variable	Rest		Steady State Exercise		HIIT Exercise	
	Cold	Hot	Cold	Hot	Cold	Hot
Pre-Protocol (nmol·L ⁻¹)	414 ± 239	414 ± 247	380 ± 167	397 ± 177	435 ± 186	422 ± 195
Post-Protocol (nmol·L ⁻¹)	375 ± 190	308 ± 153	486 ± 196	671 ± 214 [†]	606 ± 245 [†]	691 ± 162 [†]
2 Hrs Post (nmol·L ⁻¹)	315 ± 140	290 ± 133 [†]	382 ± 156	554 ± 179	399 ± 214	505 ± 285

[†] Significantly different to both rest conditions

5.6 Discussion

The aim of this study was to examine the effects of continuous 'steady state' and HIIT exercise upon GI permeability and GI symptomology and to determine the role environmental stress may play upon the expression of these markers (Camilleri et al., 2012; Fasano & Shea-Donohue, 2005; Karhu et al., 2017; Pals et al., 1997; Pugh et al., 2017). The primary findings indicate that relative to rest both the steady state and HIIT exercise when performed at the relative intensity of 70 % of $\dot{V}O_2$ max significantly elevated GI permeability but did not increase subjective ratings of GI dysfunction. Data also indicates an additive effect of environmental temperature upon GI permeability in the Hot (32°C) relative to Cold (12°C) conditions with HIIT exercise GI permeability elevated by 68 % relative to all other cold conditions and 48 % to the Hot protocols.

This is the first study to undertake a comparative evaluation of the effects of steady state vs HIIT exercise upon GI permeability and subjective symptoms of GI dysfunction. The hypothesis advanced in this work was based upon the idea that exercise would increase GI permeability, particularly if performed at sufficient intensity and duration (Pals et al., 1997). In addition, we sought to undertake these activities under divergent environmental temperatures to understand possible additive effects of a low and high external thermal load may have upon GI permeability. Pires *et al.* (2016) has recently summarised that exercise intensity alone whilst important may only be a part of an inter-related network of factors that bring about changes in GI permeability and for GI permeability to occur requires other factors to be co-expressed; a view echoed by others to include a complex interaction of exercise modality and duration, hyperthermia, splanchnic perfusion, training and acclimation status of participants, and hydration status (Costa et al., 2017b; Dokladny et al., 2008; 2016; Lambert et al., 2008; Pires et al., 2016; van Wijck et al., 2012). In this study, we sought to partition out and examine several of these factors by looking at the interaction between the way the exercise intensity is applied [patterned] i.e. steady state vs HIIT and its impact upon GI permeability under different environmental condition hot and cold. The novel data in this chapter indicates that steady state and HIIT exercise increases GI permeability relative to rest in both Hot vs Cold conditions. These observed changes are in line with previously expressed views that increases in GI permeability are a likely outcome of exercise activity when intensity and duration exceeds a 'critical exercise intensity threshold'. Notionally this threshold has been ascribed to activity equating to or exceeding 70 % of $\dot{V}O_2$ max (Pals, et al., 1997; Marchbank et al., 2011; Zuhl et al., 2014; Davison et al., 2016; Pugh et al., 2017). The present data extends current literature in that we show under hot conditions when the average exercise intensity is held constant but the pattern of exercise activity varies above and below this average i.e. interval running it leads to GI permeability been elevated. It also

shows an additive effect of thermal load in hot relative to cold conditions upon GI permeability. However, the elevations in GI permeability noted herein are inconsistent with others that note no changes in GI permeability following treadmill running at 60-70 % $\dot{V}O_{2max}$ (Lambert et al., 2008; van Wijck et al., 2014; Yeh et al., 2013).

The experimental approach undertaken in this study modelled the exercise intensities of the HIIT and steady state protocols to achieve an average intensity profile of ~70 % of $\dot{V}O_{2max}/peak$ each, independent of the activity pattern. In this study, we utilised the validated protocol of Bartlett et al., (2011) to match the interval and continuous exercise patterns for this average exercise intensity [quantified as running velocity corresponding ~70 % $\dot{V}O_{2max}$], and exercise duration, (Bartlett et al., 2012; Bartlett et al., 2013). Physiological, cortisol and perceptual data (Table 5.1 and Table 5.6) would indicate that the steady state and HIIT protocols elicited similar average loads across conditions. The cortisol response are in line with that reported for exercise in similar environments (Bergeron, 2014).

Further, the mechanisms of these changes in GI permeability will result directly from the manipulation of the intensity and duration of the 'exercise stimulus'. Upon commencement of both steady state and the HIIT exercise a redistribution of cardiac output from the splanchnic organs with reductions in splanchnic blood flow of up 80% is likely (Crandall & Gonzalez-Alonso, 2010; Knight et al., 2017; van Wijck et al., 2012). van Wijck *et al.* (2011) suggests as little as 20 minutes exercise at 70 % of $\dot{V}O_2$ max will impair GI perfusion. Consequently, splanchnic hypo-perfusion has been associated with the expression of GI symptomology and changes in GI permeability (Otte et al., 2001; Otte et al., 2005; ter Steege et al., 2012).

In this comparison of steady state and HIIT exercise, it is likely that whilst hypo-perfusion may occur the pattern(s) it follows will likely differ. HIIT is likely to establish an oscillatory pattern of splanchnic blood flow with perfusion and re-perfusion occurring as exercise intensity increases and decreases. Conversely, with steady state exercise a reduction in perfusion will occur that will remain approximately constant until the exercise stimulus is removed (Crandall & Gonzalez-Alonso, 2010; van Wijck et al., 2011). This reduction in blood flow places significant stress on the gastrointestinal system in relation to managing splanchnic perfusion and dissipating thermal load (Hayashi et al., 2012; Knight et al., 2017; Lambert et al., 2002; Perko et al., 1998; Ward et al., 2014).

In the context of hyperthermia contributing towards an increased GI permeability (Figure 5.6) observed under the steady state and HIIT exercise models and the observed step wise increase from rest through steady state to HIIT both exercise protocols presented significant

corresponding hyperthermic responses, with the highest temperatures achieved toward the end of the 50 min of steady state and HIIT exercise activity in the heat relative to cold (Figure 5.3 A and B). This pattern of increase and core temperatures achieved reflects that observed separately in steady state and interval exercise (March et al., 2017; Pugh et al., 2017). Data indicates that heat stress is associated with an additive impact, which extends GI permeability beyond that noted for exercise in the cold (Figure 5.6). This is consistent with the view that exercise in the heat is associated with elevated physiologic and thermal strain when compared to similar task execution in temperate or cold conditions (Crandall & Gonzalez-Alonso, 2010; González-Alonso et al., 2008; Snipe et al., 2017). Lloyd et al. (2016) has recently expressed the idea that when two stressors are co-expressed i.e. exercise intensity/pattern x environment an additive and/or synergistic level of stress is applied with the overall response governed by what they call the 'worst strain takes precedence model'. In light of this model present data suggest that the increased GI permeability observed in HIIT exercise hot condition is likely due to additional environmental heat exposure exacerbating splanchnic hypo-perfusion, and hyperthermia upon the intestinal epithelium (Pires et al., 2016; Zuhl et al., 2014b) rather than the HIIT exercise pattern itself which under cold displays no difference to steady state. This additive effect at a mechanistic level is likely to reside with, a hyperthermia-induced (37 to 41°C) morphological disruption of enterocytes and their respective tight junction proteins (Lambert et al., 2002b; Ward et al., 2014; Zuhl et al., 2014). Pires et al. (2016) recently hypothesised a 'critical threshold' model whereby core temperatures close to or above 38.0°C 'may facilitate' increased GI permeability, whereas temperatures of above 39.0°C 'definitely induce' permeability. Core temperatures achieved within the HIIT and steady state exercise protocols fall within this physiological range. In line with the 'worst strain model' outlined above it is therefore probable that elevated GI temperatures are a significant co-contributor along with exercise mediated hypo-perfusion to augmenting increased GI permeability in the exercise trials and in particular the HIIT trial in the heat (Barberio et al., 2015; King et al., 2015; Vargas & Marino, 2016). The similarity in GI permeability between HIIT and steady state in the cold where external thermal load is minimised may support this idea.

A further confounding factor that may contribute to GI permeability is fluid loss. An approximation of whole body dehydration during the steady state and HIIT protocols was estimated using body mass reduction; after each trial, in spite of fluid replacement at 1 mL•kg⁻¹ every 15 minutes, the reduction in body mass indicates a small progressive dehydration. The 1 % observed here is unlikely to have impaired performance but it is interesting to note that despite extensive fluid replacement we still observed increased GI permeability (Edwards et al., 2007; Maughan et al., 2010). The idea that fluid replacement may attenuate

exercise induced GI permeability is not supported within the present study; unlike others (Lambert et al., 2008).

In epidemiological studies examining gastrointestinal complaints amongst athletes, symptomology is normally assessed via retrospective questionnaires assessing subjective symptom frequency and intensity whilst laboratory work tends to employ both subjective and objective markers of GI dysfunction (de Oliveira et al., 2014; Hoffman et al., 2016; Stuempfle & Hoffman, 2015; Wilson, 2017). In the present study, we prospectively assessed the subjective expression of gastrointestinal comfort (Pfeiffer et al., 2012) rather than a defined list of GI related symptoms (Pugh et al., 2017). However, despite this more generalist approach, we observed that subjective symptoms of GI distress were disassociated from the objective GI permeability measures (Figure 5.5). The low GI symptomology scores expressed (none to mild categorization zones) during all exercise conditions reflect a minimal perception of disruption to the GI tract. Pugh et al. (2017) describe a similar dissociation following high intensity interval exercise, present findings confirm that data in a different HIIT model. These observations are consistent with that reported more recently following acute exercise activities across a range of exercise intensities, modalities, and environmental conditions in both laboratory and field studies (Karhu et al., 2017; Morrison, et al., 2014; Pfeiffer et al., 2012; Pugh et al., 2017; van Wijck et al., 2011; Zuhl et al., 2014). The critical question of causality between permeability and symptomology remains unresolved.

5.7 Conclusions

The current study determined GI permeability in response to different exercise intensity patterns [steady state and HIIT] and their application under different environmental conditions. Data indicates GI permeability but not GI symptomology is elevated relative to resting values when an un-acclimated individual is exposed to acute periods of average equivalent intensity steady state and HIIT exercise. Further, it is shown that when exercise at the same relative intensity i.e. 70 % $\dot{V}O_2$ peak is undertaken in cold conditions the GI permeability response are independent of how the exercise is delivered i.e. either continuously or in an intermittent form. When the same protocols are repeated in the heat this relationship is abolished and HIIT would appear to elevate GI permeability more than continuous steady state exercise. A clear divergence between the objective and subjective markers of GI permeability and symptomology is noted which poses questions as the causal relationship between permeability and symptomology but also the usefulness of these subjective scales under such settings of laboratory based short duration steady state and HIIT based exercise.

Chapter 6

Effects of Acute High Intensity Intermittent Exercise on Gut Permeability Following Ibuprofen or Placebo Ingestion.

6.0 Effects of Acute High Intensity Sprint Exercise on Gut Permeability Following Ibuprofen or Placebo Ingestion.

This chapter develops from Chapter 5, in that it isolates and examines the relative impact of performing supra-maximal HIIT activity upon GI permeability and symptoms. In addition, it addresses the issue of whether the co-ingestion of NSAIDs widely used in athletic populations would accentuate exercise related GI permeability and symptomology.

6.1 Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely available over the counter agents used in the acute and chronic treatment of soft-tissue injuries as well as for analgesic purposes (Tscholl et al., 2016). Due to NSAIDs analgesic, anti-inflammatory, and antipyretic effects, they have become one of the most commonly used drug groups by recreational and high level athletes to ameliorate a plethora of musculoskeletal pathologies including post exercise muscle soreness (Da Silva et al., 2015; Holgado et al., 2017; Vaso et al., 2015). In particular prevalence data on the use of NSAIDs indicates team sports participants express high consumption rates of both officially prescribed and unofficially consumed NSAIDs (Holgado et al., 2017; Tscholl et al., 2012; 2015). The high prevalence rates of NSAID consumption for prophylactic purposes are often accompanied by limited awareness of the side effects of their use and more appropriately overuse particularly on a chronic basis (Didier et al., 2017; Gorski et al., 2009).

Clinically, NSAIDs induce GI mucosal damage in the form of mucosal erosion and ulceration, they increase GI permeability and GI inflammation all of which are well described adverse effects of their normal clinical usage (Marlicz et al., 2014; Blackler et al., 2014; Sostres et al., 2017). Significantly, NSAIDs such as Ibuprofen have previously been reported to increase gastrointestinal GI permeability and inflammation following prolonged, sub-maximal endurance exercise such as marathon and triathlons. No data exists on the effects expressed during other forms of exercise in particular during intermittent and supramaximal high intensity activity exist where NSAID use in conjunction with exercise is widespread (Jeukendrup et al., 2000; Küster et al., 2013; McAnulty et al., 2007; Nieman et al., 2006; Smetanka et al., 1999; Whatmough et al., 2017). Since the use of NSAIDs in a variety of sports and individual events is widespread, it is important to characterise the effect they have on the GI barrier function especially when they are ingested prior to exercise performance; a common occurrence in many sports (Tscholl et al., 2012). As such athletes may be particularly vulnerable to adverse GI symptoms and damage due to the effects of NSAIDs and exercise interacting to damage the GI system.

Several animal models have indicated a synergistic effect when both exercise and NSAIDs are combined leading to increased GI permeability and mucosal damage (Bradford et al., 2007; Lambert et al., 2007; Lambert et al., 2012). Empirical data on the effects of NSAIDs ingestion on GI permeability and damage in athletes is limited; having been described in endurance activity alone the effects following intermittent and high intensity intermittent exercise (HIIT) are undetermined (van Wijck et al., 2012). Given NSAIDs widespread use amongst invasion field sports (Tscholl et al., 2015) where exercise activity requires high intensity repeated bouts of activity, data on the interaction between exercise and NSAIDs would provide insight into potential effects on GI function.

The aims of the current study are therefore twofold; **1.** to assess the effects of repeated high intensity interval sprint exercise on gut permeability and symptomology relative to rest and **2.** Assess the effect of the co-administration of the NSAID [Ibuprofen] and exercise and compare to corresponding rest conditions. It is hypothesised that GI permeability will increase following HIIT exercise relative to rest, when combined exercise and ibuprofen will act synergistically further accentuating the exercise mediated increase in permeability. GI permeability is hypothesised to increase following ibuprofen ingestion at rest when compared to a placebo in the same condition.

6.2 Methods.

Participants. All participants were recruited from a physically fit, healthy male population of intermittent games players from the Liverpool John Moores University who were experienced in completing high intensity training. Initially 17 male participant's were recruited to participate, due to drop out for logistical, illness and failure to comply with inclusion criteria instructions in the final analysis, twelve male participants (age: 19.6 ± 2.3 years; height 1.78 ± 0.06 m); body mass 75.1 ± 5.9 kg) participated. None of the participants had any previous history of GI related diseases or other gastric problems and were not regularly consuming non-steroidal anti-inflammatory drugs (NSAIDs). Participants were asked to abstain from exercise and alcohol at least 24 h prior to experimental assessment and refrain from using NSAID during the study apart from that dispensed under the experimental allocation. Participant's confirmed verbally compliance with these requirements prior to experimental data collection. All experimental procedures and potential risks/discomforts were explained in detail and written informed consent was obtained prior to testing. The study was approved by the Liverpool John Moore's University Ethics Committee. Sample size estimates were determined a priori based upon data of Pals et al. (1997). Assuming a type I error of .05, a type II error rate (i.e. power of 80%) with an exercise to rest GI permeability ratio difference of 0.05 arbitrary units and an anticipated SD of 0.02. A total of 12 participants were estimated as required for this study.

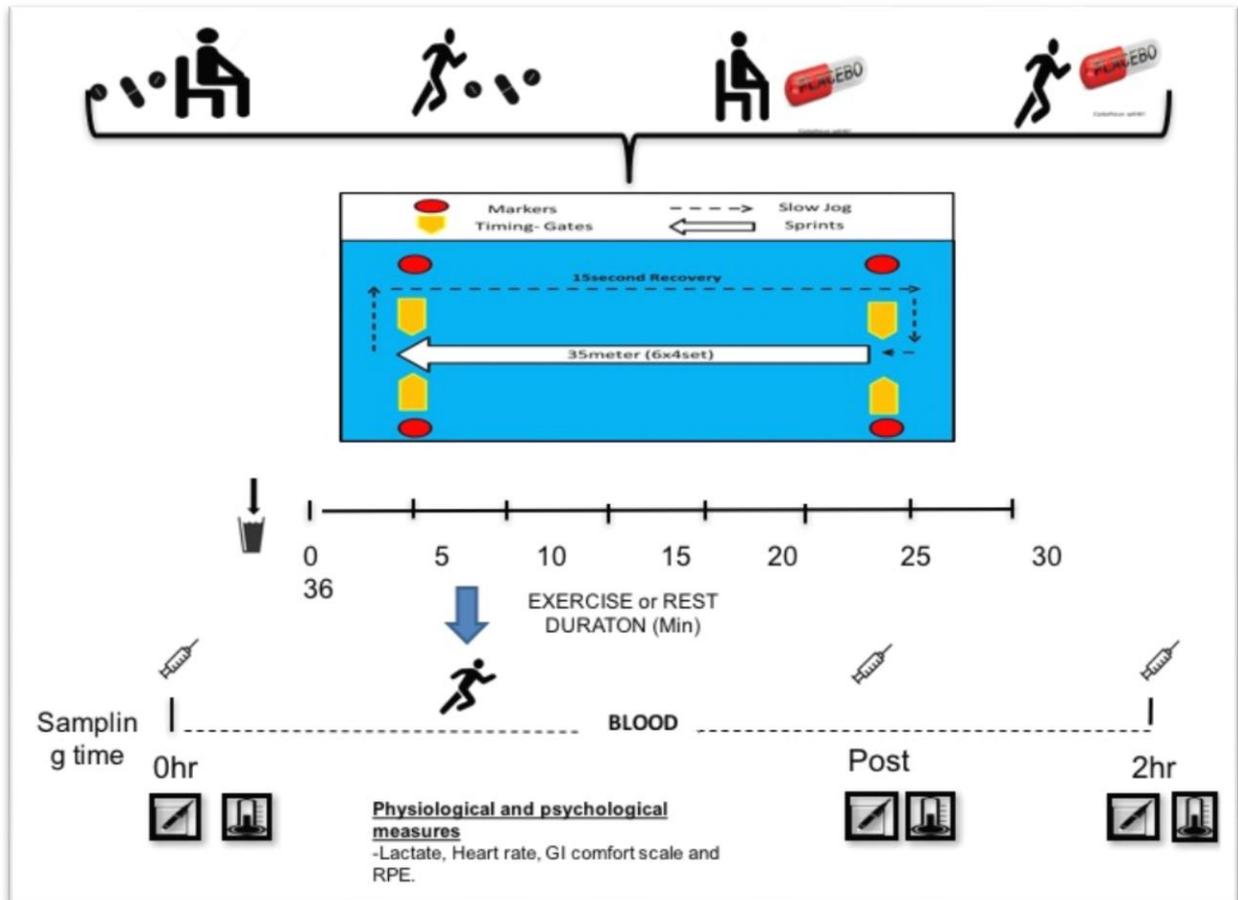


Figure 6.0 Schematic representation of the GI mediated responses to supramaximal HIIT exercise performed under Ibuprofen and placebo controlled conditions.

6.2.1 Experimental Design: Participants' completed a double blind placebo controlled counterbalanced repeated measure design separated by several days. Participants were required to complete four experimental trials at LJMU physiology Laboratory; **1)** ingestion of Ibuprofen prior to resting protocol, **2)** placebo ingestion prior to resting protocol, **3)** ingestion of Ibuprofen prior to repeated sprint protocol and **4)** placebo ingestion prior to repeated sprint protocol. All participants were asked to avoid strenuous exercise 24 h prior to both trials and were asked to consume either 800 mg of Ibuprofen or Placebo (400 mg the evening before and 400 mg on the morning of experiment) as detailed in section 6.3 below.

6.2.2 Pre-exercise arrangements: Two Ibuprofen tablets (200 mg, iso-butyl-propanoic-phenolic acid; GlaxoSmithKline, Brentford, Middlesex, United Kingdom) or two placebo tablets (maltodextrin) were ingested by the participants on the evening before the experimental protocol performance day and two [2 x 200 mg] on the morning of the experimental data collection day 60 min prior to the experiment. This procedure was to

utilised to enhance the ecological validity of the protocol to mimic athlete behaviour i.e. ingesting ibuprofen prior to training and/or competition. Participants fasted on the morning of experimental data collection; albeit they were allowed a digestive biscuit with the tablets to following prescribing recommendations (GlaxoSmithKline, Brentford, Middlesex, UK).

6.2.3 Experimental Protocol; Participants arrived at the Liverpool John Moores University (LJMU) laboratory at 10:00 am, on the four occasions as described above; 2 rest and 2 exercise conditions, separated by several days. Dosages of 800 mg (2x400 mg of ibuprofen or placebo pill) were given to each participant to take prior to bedtime (400 mg) the night before (e.g. 10.00 pm) and then one hour prior to protocol performance (400 mg) on the morning of the experiments. Participants were also asked not to ingest any additional form or dosage of NSAIDs during the course of the study and verbally confirmed this on each trial. On arrival at the laboratory, heart rate monitors (Polar FT1; Polar Electro, Tampere, Finland) were attached at approximately the V5 level around the chest. The heart rate monitor, worn around the participants' wrist, indicated their HR and continuously displayed heart rate throughout. A peripheral venous cannula (Nexivia, Becton Dickinson, Cambridge, UK) was inserted into an the antecubital vein prior to testing to allow procurement of blood samples and remained in-situ throughout exercise and post exercise period (Chapter 3, section 3.16). Samples were drawn during both rest and exercise trials at baseline (rest), post exercise (36 min) and 2 hrs post exercise. In addition, participants had their initial resting blood lactate levels analysed using a fingertip capillary blood lactate (Lactate Pro LT-1710, Arkray, Kyoto, Japan), analyser. Baseline psycho-perceptual data was collected to included RPE, GI discomfort and thermal comfort in line with procedures outlined in Chapter 3; Section (s). 3.7 and 3.8). After instrumentation was complete, participants ingested the GI sugar permeability probe solution for the determination of gut permeability [and thereafter followed by a 2 hour protocol collection period commenced (Chapter 3, section 3.10).

6.2.4 Experimental Test Protocols

6.2.4.1 Passive [Rest] Protocols. In line with the instrumentation process outlined above under the passive rest trial participants were required to be seated in the laboratory in a relaxed condition reading or watching TV for 120 minutes (Temperature $21 \pm 2.2^\circ\text{C}$ humidity $58 \pm 4\%$). Protocol commenced 15 min after ingestion and data was collected for 36 min, HR and RPE (6–20 scale; Borg, 1982) were monitored every minute. This time frame equated to the period of data collection during the alternate exercise protocol. Participants were given water following the schedule outlined (Chapter 3, section 3.11). Upon completion of the two hours' protocol i.e. time from drink ingestion the participants provided a final blood sample and the cannula was then removed. Psycho-perceptual data was collected as above (Chapter 3, Section 3.14).

6.2.4.2 Exercise Repeated Sprint Protocol [HIIT].

On a separate day, following several days' rest, the participants were asked to return to the LJMU laboratory (Temperature $19 \pm 1.1^\circ\text{C}$ humidity $52 \pm 7\%$) for the high intensity repeated sprint protocol under the alternate condition(s). Participants, following instrumentation and GI sugar ingestion commenced exercise. Each participant performed 4 sets of 6 x 35m sprints following a modified Running-based Anaerobic Sprint Test (RAST) sprint (Draper & Whyte, 1997). Track sprinting performance times were measured with wireless automated timing gates (Brower Timing Systems, Utah, USA) at start (0) and end point (35), to record sprint times (s) (Figure. 6.2). After each repetition, subjects reported their HR, perceived rate of exertion (RPE) and after 6 consecutive sprints with a 15 s jog back in between, blood lactate and thermal comfort were recorded as they engaged in a 5-min rest where water was supplied (Chapter 3, section 3.7). After four sets of high intensity sprints a post-test final lactate was collected along with bloods in line with the procurement schedule outlined.

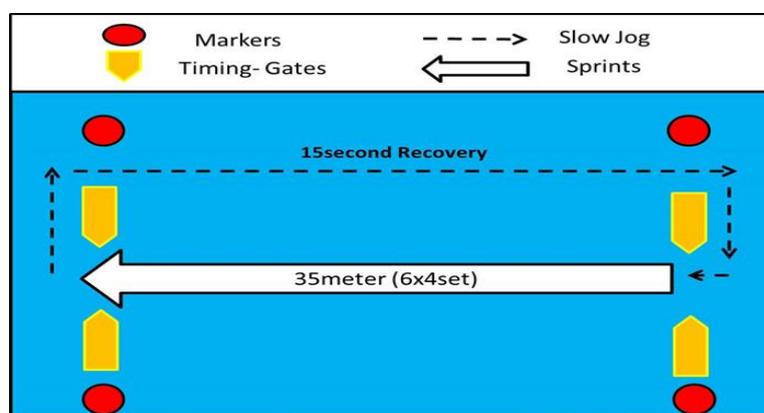


Figure 6.1: Schematic of 6 x 35 m x 4 sets repeated sprint protocol setup.



Figure 6.2 Wireless automated timing gates (Brower Timing Systems, Utah, USA) at start (0) and end point (35), to record sprint times (s)

6.2.6 Analytical Procedures;

Assessment of intestinal permeability: Intestinal permeability was assessed by analysing serum samples using a previously published method (Fleming *et al.*, 1996), with the modification of using L-rhamnose instead of mannitol as the monosaccharide probe. (Chapter 3, section 3.10).

6.2.7 Statistical Analysis. Descriptive statistics were produced for all data sets to check for normal distribution as indicated by Kolmogorov-Smirnov (accepted if $P > 0.05$). Data was examined utilising a two-way within subject design general linear model Mode: [Exercise vs Rest] and Condition [Ibuprofen vs Placebo] to determine the effects of discrete parameters of GI Permeability and GI Symptomology. Physiological and perceptual responses to rest, steady state and high intensity intermittent exercise over time for the parameters heart rate, RPE, thermal comfort, rectal temperature, were determined via the addition of a third main effect [time] (Three way) ANOVA. Where a significant main effect was determined, pairwise comparisons were analysed according to Bonferroni post hoc in order to locate

specific differences. If Mauchley's test of sphericity indicated a minimum level of violation, as assessed by a Greenhouse Geisser epsilon (ϵ) of ≥ 0.75 , data were corrected using the Huynh-Feldt ϵ . If Mauchley's test of sphericity was violated, data were corrected using Greenhouse Geisser ϵ . Paired t -test analysis was used where appropriate. Statistical significance was set at $p < 0.05$. Statistical analysis was performed using SPSS statistical software (SPSS 23.0, SPSS, Inc., Chicago, IL, USA). Visual representations of experimental data were produced using Microsoft Excel software package. Data are presented throughout as Mean \pm SD.

6.3 RESULTS.

6.3.1 GI Permeability: Lactulose/L-Rhamnose.

Lactulose-/L-Rhamnose: There was no significant main effect on drug or placebo treatment ($F_{1, 10} = .465, P > 0.05$) on GI permeability ratio. There was no significant main effect of activity ($F_{1, 10} = 2.24, P > 0.05$). There were no significant interaction effects ($F_{1, 10} = .465, P > 0.05$).

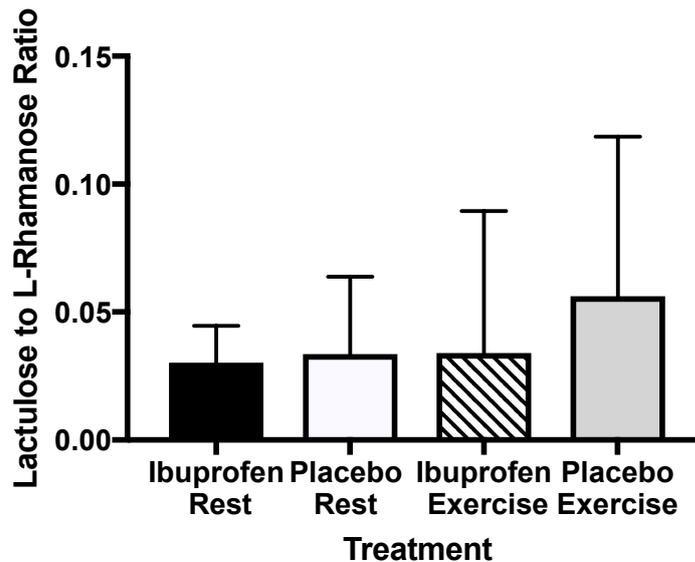


Figure 6.3 Lactulose/L-Rhamnose ratio (%) in the four exercise conditions; placebo rest, placebo exercise, ibuprofen rest and exercise.

6.3.2 Plasma Metabolite Responses to Exercise;

6.3.2.1 Lactate: There was no significant main effect for condition (placebo and ibuprofen) ($F_{1, 10} = 0.02, P = 0.88$). There was a significant main effect for activity rest vs sprints with significant increases in lactate concentrations from pre to post for both conditions ($F_{1, 10} = 801.2, P < 0.001$) (Figure 6.4). There was no significant interaction between activity and drug ($F_{1, 19, 8.94} = 0.01, P = 0.92$).

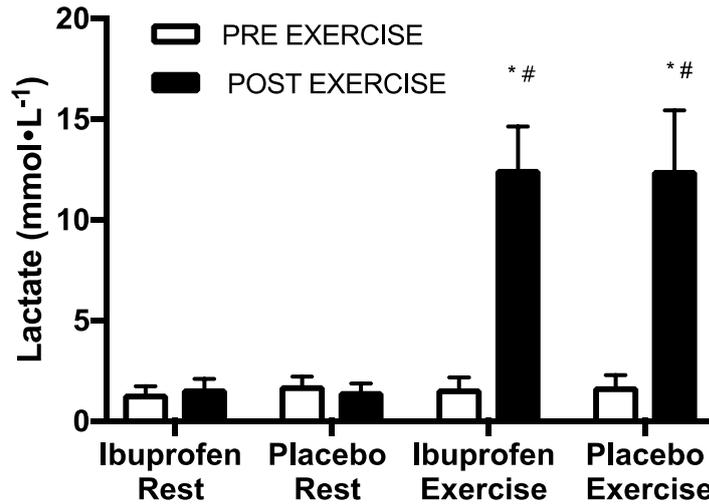


Figure 6.4 Lactate concentrations ($\text{mmol}\cdot\text{L}^{-1}$) pre-to post exercise across trials after rest and exercise ($4 \times [6 \times 35 \text{ m}]$) maximal intermittent sprints in the all four experimental conditions; * significantly different pre to post and # compared to passive experimental trials ($P < 0.001$).

6.3.3 Physiological Responses to Exercise.

6.3.3.1 Heart rate (HR); There was a significant main effect for condition (rest and exercise) ($F_{1, 10} = 450.22$, $P < 0.001$) upon HR. There was no significant main effect for condition (placebo and ibuprofen) ($F_{1, 10} = 3.10$, $P = 0.12$) on HR during exercise. There was a significant main effect for time with HR elevated from rest to exercise, with similar values between condition (placebo vs ibuprofen) (Figure 6.5). There was no significant interaction between placebo and ibuprofen ($F_{1, 10} = 2.61$, $P = 0.15$).

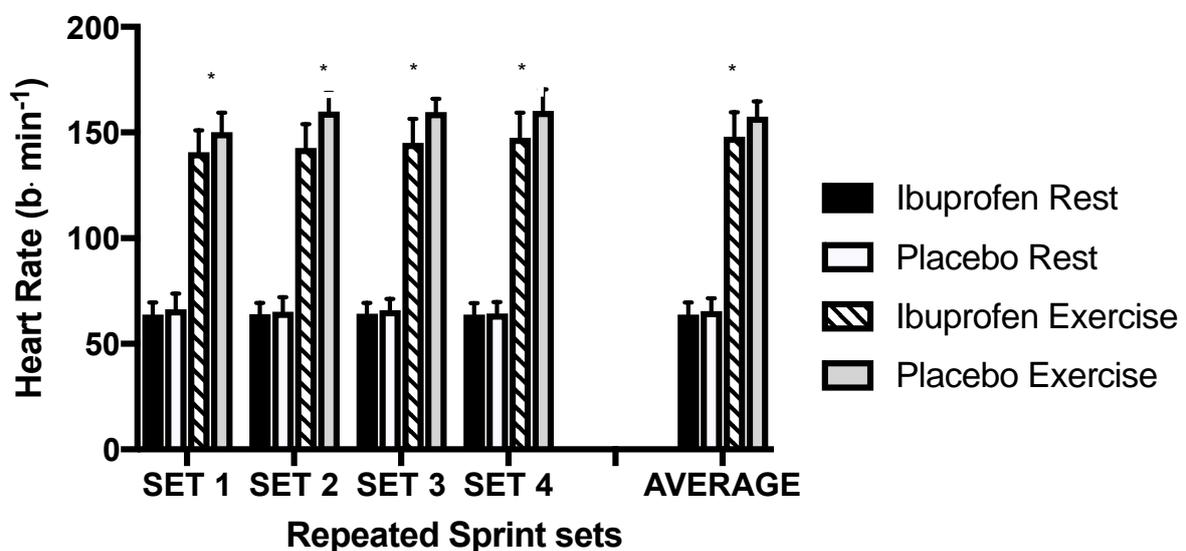


Figure 6.6 Heart rate ($\text{b}\cdot\text{min}^{-1}$) average at rest and exercise (4 x [6x35m]) maximal intermittent sprints in the all four experimental conditions; * Significantly different exercise compared to passive experimental trials ($P < 0.05$).

6.3.3.2 Rating Perceived of Exertion (RPE); There was a significant main effect of activity on RPE with exercise showing higher level than rest ($F_{1, 10} = 608.87, P < 0.001$) (Figure 6.6). There was a significant main effect for condition (placebo vs ibuprofen) with both exercise placebo and Ibuprofen showed an increase in RPE between set 1 and set 4 of exercise and a significant interaction effect between set 3 and 4 showed higher RPE in when Ibuprofen was ingested ($P < 0.05$).

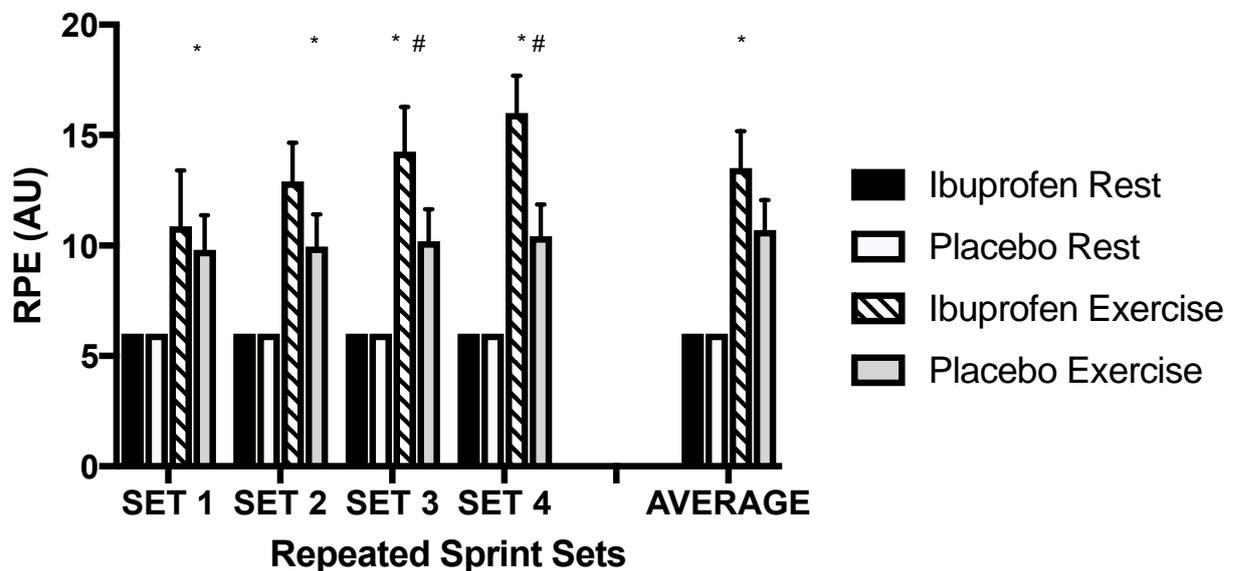


Figure 6.7 Ratings of Perceived Exertion (AU) averages at rest and exercise (4 x [6x35m]) maximal intermittent sprints in the all four experimental conditions; * Significantly different exercise compared to passive experimental trials ($P < 0.001$). # significantly different to alternate exercise condition.

6.3.3.3 Exercise Sprint Performance Time (PT). There was no significant main effect of treatment (placebo vs ibuprofen) upon sprint performance ($F_{1, 10} = 2.53, P > 0.05$) (Figure 6.8). There was no significant effect of time on average running speed from set 1 to set 4 ($P > 0.05$).



Figure 6.8 Sprint Performance (s) over the four sets (4 x [6 x 35 m]) maximal intermittent sprints during placebo and Ibuprofen ingestion.

6.3.3.4 Tympanic Aural Temperature. There was no significant main effect of treatment (rest vs exercise) or (Ibuprofen vs Placebo) ($P>0.05$) (Figure 6.9). ($P>0.05$) upon temperature ($P>0.05$). However values are below that reported for core temperature estimates.

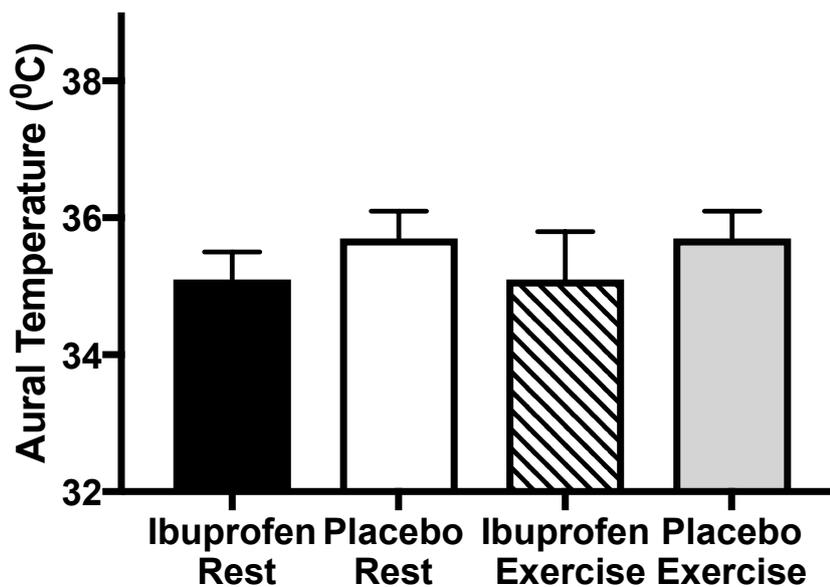


Figure 6.9 Tympanic (aural) temperature (s) over the treatments during placebo and ibuprofen ingestion.

6.4 Discussion.

The purpose of present study was to examine the effects repeated high intensity intermittent sprint exercise upon GI permeability and GI symptomology expression relative to rest and furthermore to evaluate the effect co-administration of the NSAID [ibuprofen] has on both these factors during both exercise and at rest. The primary findings suggest that an acute bout of repeated supramaximal HIIT exercise does not increase GI permeability or elevate GI symptoms. In addition, the expected adverse synergistic interactions between the exercise/rest protocols and NSAID consumption (800 mg Ibuprofen [2 x 200mg per dose]) does not increase GI permeability or GI symptomology above the control/placebo conditions.

Although the association between exercise and increased GI permeability has been previously examined; the exercise stress utilised in the majority of studies have been continuous, sub-maximal endurance efforts of running or cycling over a varied range of exercise durations (Costa et al., 2017b; Lambert, 2009; Snipe et al., 2017). The results of this current study do not agree with these previous findings, with data suggestive of a divergence in GI permeability between supramaximal HIIT exercise and the more continuous submaximal, variable duration models prevalent in the literature (Jeukendrup et al., 2000; March et al., 2017; Roberts et al., 2016). The primary factor differentiating the present data from these previous studies is the intensity and duration of the exercise undertaken. The HIIT task required very short durations of supramaximal (anaerobic) efforts for 5-6 s followed by 15 s of sub-maximal jogging to return to the start line for the subsequent sprint effort. This equated to about 120 s supramaximal and sub-maximal effort in each set followed by 5 min of rest between each set (1:2.5 work: rest ratio) delivered across 4 sets. In total, work time was about ~8 min over the course of the 36 min protocol, which is approximately in line with recommendations for other models of HIIT (Burgomaster et al., 2008; García-Pinillos et al., 2017; Jiménez-Pavón & Lavie, 2017). These factors of a short but intense exercise time and extended intra-session rest periods between sets when considered relative to sustained but moderate intensity exercise may have contributed to the differentiated i.e. lower the GI permeability and symptomology responses. Pals *et al.* (1997) demonstrated that increased GI permeability may be accrued by increasing exercise intensity in a dose response manner, with higher intensity exercise (80 % $\dot{V}O_2$ max), exhibiting a greater permeability than exercise performed at lower intensities of 40 % and 60 % $\dot{V}O_2$ max. Others have shown similar results, where exercise in excess of 70 % of maximal work/aerobic capacity occurs. However, common to both reports is the fact that exercise takes place over an extended

duration. Notionally, where exercise duration is greater than 50 min plus increases in GI permeability are observed (Costa et al., 2017; Pals et al., 1997; van Wijck et al., 2014;). As this is the first study to examine the effects of supramaximal HIIT on GI permeability, the reasons for the current findings must be considered in relation to those reported from endurance exercise. Several factors that may explain these differences in GI permeability responses include a reduction in splanchnic blood flow which is a function of exercise type, duration and intensity (ter Steege & Kolkman, 2012). Sustained exercise activity is acknowledged to decrease splanchnic blood flow up to 80 % (Otte et al., 2001; ter Steege & Kolkman, 2012; van Wijck et al., 2011); alternatively intermittent exercise and sprint activity splanchnic blood flow responses have been very poorly characterized (Cerný & Cvachovec, 2000; Kolkman et al., 2000). As such, it is uncertain the degree to which splanchnic blood flow is impaired, the period of time over which the reduction occurs and how long it is sustained during HIIT where the activity is short but anaerobic in nature. Speculatively, the oscillatory 'interval' activity pattern during HIIT may induce a rapid hypo-perfusion within each exercise set with the extended rest period between sets allowing splanchnic blood flow restoration thus facilitating higher levels of GI perfusion than would be expected under continuous exercise. In addition core temperature increases as function of exercise intensity and duration (Racinais & Sawka, 2015). Elevations in GI temperature brought on by exercise or passive heating have been recognised to compromise the integrity of the GI mucosal barrier leading to increased GI permeability (Dokladny et al., 2016; Vargas & Marino, 2016). Core temperature was not directly assessed in this study due the logistics of measuring it during the supramaximal HIIT trials thus the relative contribution of hyperthermia to the observed GI permeability was assessed with a surrogate measure. Aural [tympanic] temperature was utilized as a marker of core temperature; average values reported were lower than would be expected (Figure 6.8). It has been noted that this aural canal approach provides an underestimation of core temperature (Casa et al., 2007; Towey et al., 2017). It remains undetermined with current experimental methods whether the lack of change in permeability after HIIT exercise may be attributed to attenuation in splanchnic hypo-perfusion and/or GI temperature elevations.

Understanding why supramaximal HIIT does not increase GI permeability may come down simply to the brevity of stress exposure rather than intensity; the cumulative stress load from 'intensity x duration' relationship would seem to be insufficient to significantly elevate GI permeability in the present study. In conjunction with this observation gastrointestinal symptomology reported within the study was rated as not-present or very mild across all conditions a finding consistent with the findings reported during interval and steady state exercise activity (Karhu et al., 2017; Pugh et al., 2017) (Figure 6.10).

Given the prevalence of NSAIDs usage in sport and their known GI toxicity profile it was hypothesised that when the NSAID (Ibuprofen) were co-administered prior to exercise and passive rest there would be a synergistic effect increasing GI permeability above that noted with placebo; present data indicate that this outcome was not observed. These findings are contrary to previous studies that have examined the issue of NSAID ingestion following exercise over a range of dosing regimen, NSAID agents Cox 1-[aspirin] and COX 2 [ibuprofen], exercise duration and type (s) (Audet et al., 2016; Lambert et al., 2001; Lambert et al., 2007a; Lambert et al., 2012; McNulty et al., 2007; Smetanka et al., 1999; van Wijck, et al., 2012). These differences are not unsurprising given the contrasting exercise protocols applied; repeated sprint interval relative to continuous endurance activity.

The present HIIT sprinting protocol with ibuprofen co-administered demonstrated no difference in permeability relative to the same exercise activity without ibuprofen ingestion. Indeed at rest no increase in permeability was apparent which is contrary other reports on ibuprofen effects after passive ingestion (van Wijck et al., 2012). Mechanistically, studies have reported that after the consumption of NSAIDs and via the inhibition of cyclooxygenase (COX) isotypes 1 and/or 2 a reduction in nitric oxide production occurs. Physiological and tissue effects include reduced GI tissue perfusion, as well as mucosal cytoskeleton integrity impairment leading to elevated permeability and inflammation causing GI enterocyte damage and necrosis (Holgado et al., 2017; Iwamoto, 2013; Tscholl et al., 2016). Ibuprofen is a specific COX-2 inhibitor and is categorized as a weak acid although it is undetermined the exposure dose and frequency required to elicit these responses. These findings contribute to the idea that ibuprofen ingestion with a very short duration exercise protocols and adequate rest periods may not increase permeability when a normal conservative dosing regimen(s) on a single use basis are followed. It should be considered that the participants in this study were well trained intermittent games players thus one may assume some degree of training related adaption to HIIT type activity as well as possible GI training related adaption as well due to prior exposure to NSAIDs in the past (Costa et al., 2017; Miall et al., 2017). These considerations may limit the generalisability of the data beyond these strict delimitations. It is known that conservative dosing/usage of NSAIDs by recreational and elite athletes may not be adhered too (Gorski et al., 2011a; Tscholl & Dvorak, 2012; Vaso et al., 2015). Further work should consider longer duration dosing regimen and approximate the usage patterns reported in athletes more closely to determine if the present data can be replicated.

6.5 Conclusions

In conclusion, the current study is the first to assess the effects of HIIT intermittent maximal sprints on GI permeability and symptom expression. It was initially hypothesized that both exercise and ibuprofen would act synergistically accentuating their known individual adverse Gastrointestinal profile to increase GI permeability and elevate GI symptom expression. No increase in GI permeability or symptomology was present under both conditions (ibuprofen or placebo) either at rest or following exercise. The implications of the current findings, suggest that immediately preceding supramaximal HIIT exercise activity with the use of ibuprofen (400mg) will not adversely affect the GI permeability in participants who express no previous contraindications to their use. An important caveat, is that we must limit our findings to the ingestion of Ibuprofen in line with recommended UK prescribing guidelines. We also suggest that unlike longer duration steady state exercise, supramaximal HIIT does not seem to increase permeability or symptoms of GI distress. Given the popularity of HIIT exercise this may add a further advantage to its efficacy profile.

Chapter 7

**Effects of exercise modality (Running vs Cycling) on
Gastrointestinal Permeability, Symptomology and Damage in
Triathletes.**

7.0 Effects of exercise modality (Running vs Cycling) on Gastrointestinal Permeability, Symptomology and Damage in Triathletes.

This chapter develops the theme of activity and GI permeability to examine the relative impact of exercise modality i.e. running vs cycling upon GI permeability and damage. It is suggested that running will increase GI permeability relative to cycling and rest due to variances in mechanical loading on the GI system when relative exercise intensity and work completed are held constant.

7.1 Introduction

A loss in GI barrier integrity is considered to lead to intestinal permeability resulting in systemic inflammatory reactions and the occurrence of GI distress symptoms (nausea, vomiting, diarrhoea and abdominal cramps) (Haaf et al., 2014; Peters, 2001; ter Steege et al., 2008). The prevalence of exercise induced GI disturbance appear common in both male and female athletes, and often lead to impaired performance and the termination of exercise (Jeukendrup et al., 2000). Athletes perusing endurance-based sports appear susceptible to a greater frequency of GI distress with 25-70 % of elite endurance athletes experiencing such problems (Lambert et al., 1999; Riddoch & Trinick, 1988). Jeukendrup *et al.*, (2000) reported in Ironman distance triathletes that 43 % of competitors expressed serious GI symptoms with 7% having to abandon the race. Exact causality amongst symptomatic athletes appears to be multifactorial, although a reduction in splanchnic blood flow as well as hyperthermia and tissue hypoxia are suggested as a primary mechanism relative to exercise intensity, duration and putative environmental factors (ter Steege et al., 2012; van Wijck, et al., 2011). However, delineating the precise mechanism remains elusive between symptomatic and asymptomatic athletes (Karhu et al., 2017; Wright et al., 2011). As such other potential factors may be contributing to the patho-aetiology of both objective and subjective symptomologies of GI dysfunction noted in athletes.

Exercise modality and their associated movement mechanics may be potential factors responsible in modulating GI permeability and the development of GI symptoms. Epidemiological data report differential rates of GI symptomologies in running compared to other sports such as cycling or swimming where the body remains in a more stable position with running expressing higher GI symptom prevalence and severity scores (Peters, et al., 1999; Riddoch & Trinick, 1988; ter Steege et al., 2008). Rehrer et al. (1992) and van Nieuwenhoven *et al.* (2004) have reported triathletes to experience a greater proportion of GI symptoms during the running part of a triathlon in relation to swimming and cycling

components. The disparity in GI symptoms and GI permeability during different exercise modalities are thought to be as result of the repetitive high impact vertical and lateral oscillations that are transmitted during running to the GI tract (Rosado-Dawid et al., 2013; Rudzki et al., 1995; Stewart et al., 1984; Waterman & Kapur, 2012). Such observations may therefore help explain the diarrhoea and lower GI complaints prevalent amongst runners in comparison to cycling (Peters et al., 1999; Peters et al., 2002).

The exact underlying mechanisms that explain the higher prevalence of GI symptoms amongst runners remain to be elucidated (Costa et al., 2017; Pires et al., 2016). However, a direct comparison of exercise modality where increased loading and no loading is applied (i.e. running versus cycling) to examine GI permeability and symptomology has not been considered. Especially, where both exercise modalities are isolated to a single activity bout and matched for exercise intensity and total work load completed. The aim of the present study was therefore to examine isolated modality specific effects of loaded [running] vs unloaded [cycling] upon GI permeability, symptomology and tissue damage. It is hypothesised that GI permeability, as expressed by the serum appearance of lactulose to L-rhamnose ratio (L/R) will be elevated after running relative to cycling when matched for exercise intensity and total work completed. Second the cytosolic protein intestinal fatty acid binding protein (IFABP) will be elevated during running versus cycling as a marker of enterocyte tissue damage and third these will be associated with GI symptoms expressed.

7.2 Methods

7.2.1 Participants.

Initially 11 male participants were recruited to participate, due to drop out for logistical, illness in the final analysis, six male triathletes training in excess of six h per week and competing on a regular basis were recruited from triathlon clubs in the North West of England (Age: 29 ± 10 years; Body mass: 78 ± 10 kg; $\dot{V}O_2$ max cycle: 56.4 ± 5.0 mL·kg⁻¹·min⁻¹, $\dot{V}O_2$ max run: 62.7 ± 4.6 mL·kg⁻¹·min⁻¹). None of the participants had any previous history of GI related diseases or other gastric problems and were not regularly consuming non-steroidal anti-inflammatory drugs (NSAIDs). Participants were asked to abstain from exercise and alcohol at least 24 hours prior to experimental assessment and refrain from using NSAID during the study. Participant's confirmed verbally compliance with these requirements prior to experimental data collection. All experimental procedures and potential risks/discomforts were explained in detail and written informed consent was obtained prior to testing. The study was approved by the Liverpool John Moores University Ethics Committee. Sample size estimates were determined a priori based upon data of Pals et al. (1997). Assuming a type I error of .05, a type II error rate (i.e. power of 80%) with an exercise to rest GI permeability ratio difference of 0.05 arbitrary units and an anticipated SD of 0.02. A total of 12 participants were estimated as required for this study. However, failure to recruit to the study and dropout mean the final sample size reflects a convenience sample.

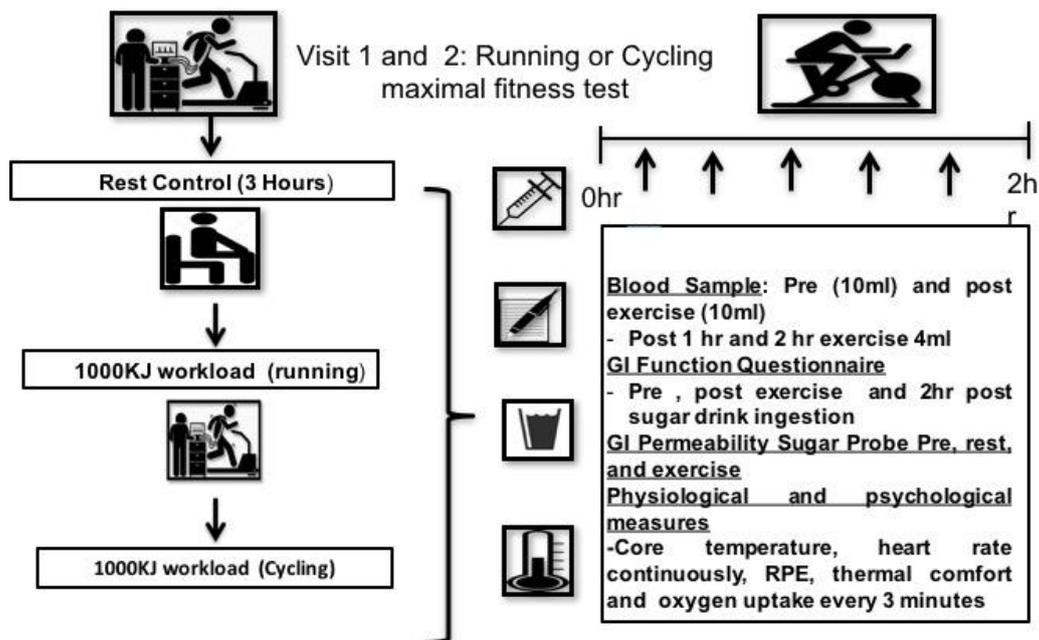


Figure 7.0 Experimental design summary for the effects of exercise modality [cycling versus running] upon GI permeability and biomarkers of GI damage, response in triathletes.

7.2.2 Experimental design and Exercise Protocol:

Each participant was required to attend the laboratory on 5 separate occasions (ambient conditions were similar between visits; temperature 22-24°C, humidity 45 %-52 %). The initial visits were to establish running and cycling $\dot{V}O_2$ max/peak and the final 3 visits to complete each experimental trial 'running' 'cycling' and 'rest'. All experimental trials were conducted in a repeated measures design, at least 4-h post-prandial with a minimum 4-day wash out period between each. We were unable to employ a randomization procedure, as the 1000 KJ workload had to be pre-determined first in order to set the subsequent workload during the run performance test; therefore, all participants performed the cycling trial first.

The exercise protocols consisted of a steady state cycling absolute 1000 KJ work test and an equivalent running workload test both performed at a fixed load eliciting ~70 % $\dot{V}O_2$ max. Performance of 1000 KJ of work was quantified in real time during cycling trials using the Lode Ergometry Manager Software (Lode Excalibur Sport, Groningen, and The Netherlands). Breath-by-breath indirect calorimetry was measured throughout (Oxycon Pro, Jaeger, The Netherlands) to determine total energy expenditure (EE). Total EE was then replicated during the running trial to ensure comparison of workload between the two conditions. The running protocol was performed at a 1 % gradient (Jones & DOUST, 1996) to reflect the metabolic and oxygen demands of running outdoors.

On arrival at the laboratory, participants were seated for 15 min and had a venous cannula inserted into an ante-cubital vein for serial blood sampling for the assay of GI permeability and damage following the time frame; pre protocol, immediately post protocol completion and 2 h post sugar probe ingestion (Chapter 3, section 3.7). Nude body mass was recorded (Seca 704, Birmingham, UK). Sample procurement and management and storage followed that outlined in (Chapter 3, section 3.9). Core body temperature was assessed via a rectal thermistor (Mini-thermistor; Grant instruments LTD, Shepreth,) and monitored throughout exercise using an electronic data logger (Grant Squirrel 1000 series, Grant Instruments, Cambridge, UK) (Chapter 3, section 3.5.1). A Polar FT1 heart rate monitor transmitter band was positioned across the chest (Polar Electro Oy, Kempe, Finland) and heart rate was recorded at 1 min intervals. Pre and post exercise haematocrit and haemoglobin was determined as outlined in (Chapter 3, Section 3.9.1). After instrumentation was complete, participants consumed the GI sugar permeability probe (Chapter 3, Section 3.7). During exercise, rate of perceived exertion (RPE; 6-20) and GI comfort scale (0-10) were recorded every 3 min (Chapter 3, Section 3.5 and 3.5.1). Participants consumed water at a rate of at least ~1 mL·kg⁻¹ every 15 minutes to alleviate fluid loss. Total fluid intake was recorded and post exercise nude body mass was obtained to determine estimated sweat rate. Upon

completion of exercise a further blood sample was immediately obtained (~5 min) at 2 h post drink ingestion.

7.2.3 Preliminary testing: Running and cycling maximal oxygen uptake ($\dot{V}O_2\text{max}$) were determined during two incremental exercise tests performed to volitional exhaustion. The maximal running and cycling tests were performed using the same motorised treadmill (H/P Cosmos Pulsar, Nussdorf-Traunstein, Germany) and electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) as used during each experimental trial. Briefly, participants $\dot{V}O_2$ peak was determined using a progressive incremental protocol on a motorized treadmill (h/p/cosmos pulsar, Nussdorf-Traunstein, Germany)(Chapter 3 Section 3.6); temperature of 22°C and 45 % relative humidity. The cycle ergometer test commenced at 125 w and increased by 25 w every 2 min thereafter until volitional exhaustion. The criteria of the British Association of Sport and Exercise Sciences (BASES) were used to classify attainment of $\dot{V}O_2\text{max}$ peak or max oxygen uptake criteria (Winter et al., 2007). Chapter 3, section 3.6) Expired respiratory gas fractions were analysed continuously using an online gas analysis system (Oxycon Pro, Jaeger, Netherlands) to allow for the subsequent determination (linear regression) of the workload required for the experimental trials (70 % $\dot{V}O_2\text{max}$) (Chapter 3, section 3.6).

7.2.4 Analytical Procedures:

7.2.4.1 Assessment of intestinal permeability

Intestinal permeability for the recovery of Lactulose and L-Rhamnose was assessed by analyzing serum samples using a previously published protocol (Fleming et al., 1996). Chapter 3, section 3.10)

7.2.4.2 Assessment of I-FABP

I-FABP was determined by analysis of plasma samples using an ELISA kit (Hycult Biotechnology, Uden, the Netherlands) according to the manufacturer's instructions. I-FABP concentrations (pg/mL) were measured in samples taken pre-exercise, immediately post-and 2 hrs post exercise. The intra-assay coefficient of variation was <8 %.

7.2.4.3 Serum Cortisol

Serum cortisol concentrations determined via automated Roche COBAS electro-chemiluminescence immunoassay procedure following the manufacturer's instruction. The CV was <10 %. The lower and upper limits of measurements were 0.5 and 1750 nmol·L⁻¹, respectively.

7.2.4.4 Statistical Analysis: Statistical analysis was conducted using the Statistical Package for the Social Sciences (version 22; SPSS Inc., Chicago, IL). All data were analysed with a standard 2-tailed paired t-test to determine differences between cycling and running trials. All data in text, figures and tables are presented as means \pm SD; a *P* value <0.05 was accepted as indicative of a statistical significance in the data.

7.3 Results

7.3.1 Physiological and Perceptual effort.

Heart rate was significantly elevated during the running trial when compared to cycling ($161 \pm 13 \text{ b}\cdot\text{min}^{-1}$ vs $153 \pm 11 \text{ b}\cdot\text{min}^{-1}$; $P = 0.035$). Oxygen uptake was similar during both the cycling and running experimental trials ($48.9 \pm 14.3 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ vs $52.2 \pm 9.7 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $P = 0.471$) respectively. Average core body temperature did not differ between the cycling and running experimental trials ($39.0 \pm 0.25^\circ\text{C}$ vs $39.4 \pm 0.59^\circ\text{C}$; $P = 0.118$). No significant differences in the ratings of perceived exertion during cycling and running (15.7 ± 2.4 vs 14.6 ± 2.1 ; $P = 0.721$) was noted. Absolute energy expenditure did not differ between cycling and running trials ($1374 \pm 412 \text{ kcal}$ vs $13757 \pm 359 \text{ kcal}$; $P = 0.209$). Average distances and time to completion for cycling and running experimental trials are shown in Table 7.1. The duration of the cycling trial was significantly longer than the running trial ($78.3 \pm 11.0 \text{ min}$ vs $62.4 \pm 11.8 \text{ min}$; $P = 0.014$). Total percentage loss in body mass did not differ between cycling and running experimental trials (1.8 ± 0.6 vs $2.1 \pm 0.6 \text{ kg}$; $P = 0.076$). Data are presented in table 7.1.

Table 7.1: Physiological, perceptual and performance data during running and cycling.

Parameter	Cycling	Running	P Value
$\dot{V}O_{2 \max}$ ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	58.2 ± 7.4	$65.4 \pm 10.0^*$	($P < 0.05$)
$\dot{V}O_2$ ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	48.9 ± 14.3	52.2 ± 9.7	($P > 0.05$)
Heart Rate ($\text{b}\cdot\text{min}^{-1}$)	153 ± 11	$161 \pm 13^*$	($P < 0.05$)
Energy Expenditure (Kcal)	1374 ± 412	1375 ± 355	($P > 0.05$)
Rectal Temperature ($^\circ\text{C}$)	38.9 ± 0.2	39.4 ± 0.5	($P > 0.05$)
RPE (AU)	15.7 ± 2.4	14.6 ± 2.1	($P > 0.05$)
Δ Body mass (kg)	1.8 ± 0.6	2.1 ± 0.6	($P > 0.05$)
Distance (km)	66.6 ± 0.5	$13.5 \pm 1.2^*$	($P < 0.0001$)
Time to complete (min)	78.3 ± 11.0	63.4 ± 11.8	($P > 0.05$)
GI Discomfort (AU)	0.7 ± 1.0	2.1 ± 1.3	($P < 0.05$)

7.3.2 GI permeability, Damage and GI comfort

Subjective severity ratings of GI discomfort (Table 7.1) recorded during the cycling trial was significantly lower than during running.

7.3.3 GI Permeability

Serum Lactulose-to-rhamnose ratios are presented in Figure 1. The mean serum ratio of lactulose to rhamnose was not different ($P = 0.252$) between cycling vs running (0.0318 ± 0.008 vs 0.0301 ± 0.006 ; $P = 0.252$) indicating no differences in small intestine permeability. Analysis wasn't performed on the rest condition due to sample size constraints ($n=3$).

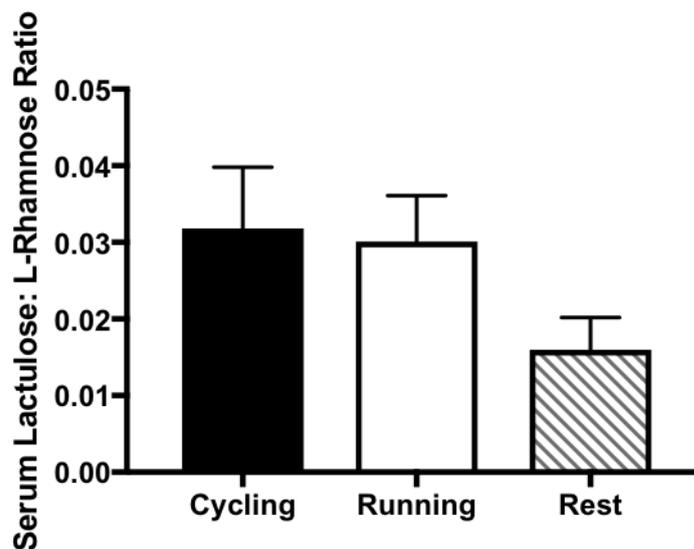


Figure 7.1 GI permeability as determined by lactulose: L-Rhamnose ratios following running and cycling ($n=6$) and rest ($n=3$).

7.3.4 Intestinal Fatty Acid Binding Protein

Serum Intestinal fatty acid binding protein concentrations are presented in Figure 7.2. The mean serum IFABP area under the curve was determined with concentrations not significantly different between cycling vs running, pre; post or 2-Hrs post indicating no significant differences in small intestine damage between conditions.

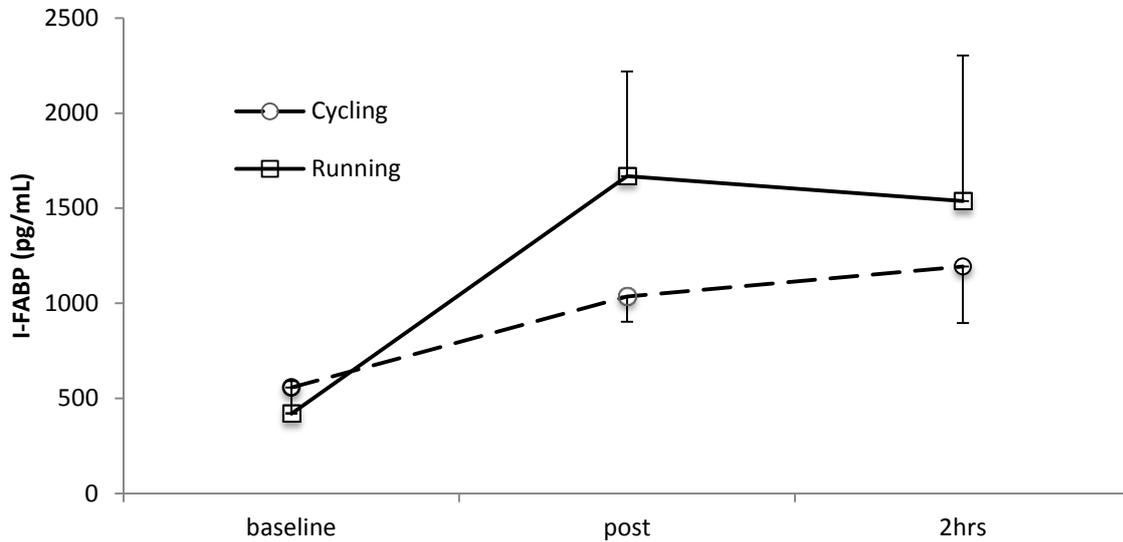


Figure 7.2 Plasma I-FABP response to cycling and running pre, immediately post and 2 h post exercise.

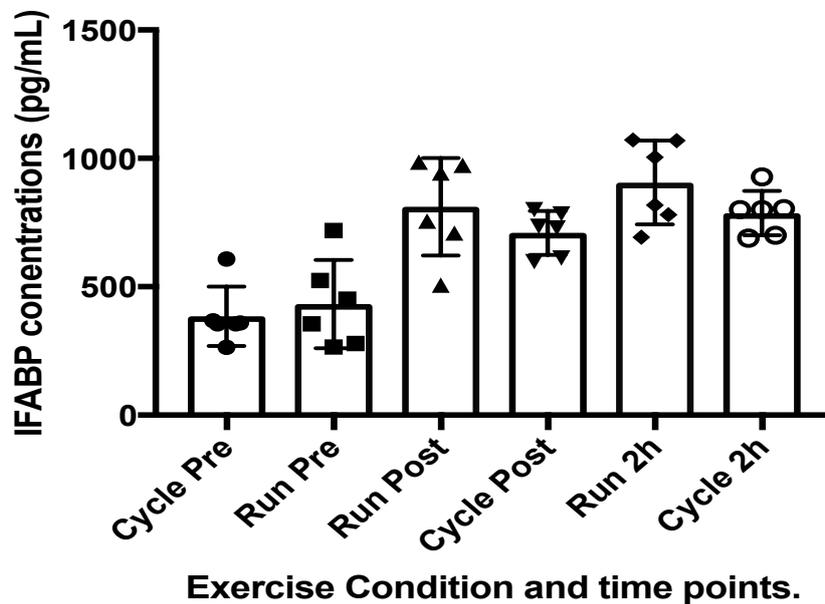


Figure 7.3 Individual plasma I-FABP response to cycling and running pre, immediately post and 2 h post exercise modality performance.

7.3.5 Cortisol Responses.

Serum cortisol release increased significantly pre-to post exercise and 2 h post exercise ($P < 0.05$). However no significant difference between running and cycling were observed ($P > 0.05$).

Table 7.2 Serum cortisol responses during running and cycling matched for energy expenditure and exercise intensity.

Condition	Time		
	Pre-Ex (nmol•L ⁻¹)	Post-Ex (nmol•L ⁻¹)	2h-Post EX(nmol•L ⁻¹)
Running Pre-Exercise (nmol•L ⁻¹)	385 ± 115.8	710 ± 85.9*	786.8 ± 86.8*
Cycling Post Exercise (nmol•L ⁻¹)	432.5 ± 172.1	812 ± 189.6*	906 ± 162*

Cortisol responses * significantly different from pre-exercise condition ($P < 0.05$).

7.4 Discussion

The aim of the present study was to quantify the difference in GI permeability and damage between the exercise modalities of running and cycling where each exercise was allocated to a single isolated session and matched for total energy expenditure and relative exercise intensity. Present data indicate that GI permeability and damage are not significantly affected by exercise modality. However, a clear disassociation between GI symptomology and markers of GI permeability and damage is apparent.

In evaluating these results, it is important to consider the potential mechanisms which may explain the observed outcomes. Firstly, tight junction proteins are essential to the maintenance of GI barrier integrity and the prevention of luminal antigen translocation via of paracellular pathways i.e. GI permeability (Bischoff et al., 2014; Shen 2012; Zuhl et al., 2014). During exercise, the structure and function of these tight junction proteins are modulated by various physiological stimuli including splanchnic hypo-perfusion, splanchnic hyperthermia and GI enterocyte hypoxia. These factors when expressed during exercise are subsequently followed by a loss in epithelial integrity and an increase in GI permeability in human and animal models (Dokladny et al., 2015; Pires et al., 2016; ter Steege & Kolkman, 2012; van Wijck et al., 2012). The magnitude of permeability would seem to reflect the duration and magnitude of splanchnic hypo-perfusion and hyperthermia (Derikx *et al.*, 2007; Otte et al 2001; ter Steege & Kolkman, 2012; van Wijck *et al.*, 2011). We have observed across both the running and cycling protocols hyperthermia at a level sufficient to induce increased permeability, however a tonometry wasn't available to measure GI perfusion we can't comment upon the level of hypoperfusion. However, it is accepted that exercise at similar level can impair GI perfusion by up to 80 % (Pires et al., 2016; ter Steege & Kolkman, 2012). However, others have observed that splanchnic haemodynamics between GI symptomatic and asymptomatic athletes do not differ (Wright *et al.*, 2011). As such it is postulated that other mechanisms associated with differences between the mechanics of running and cycling could be potential factors responsible in modulating GI permeability and the development of GI symptoms (Rudzki *et al.*, 1995).

Epidemiological data report differential rates of GI symptomology during running compared to other sports such as cycling or swimming where the body is subject to less mechanical loading. Running expresses higher GI symptom prevalence and severity estimates as well as increased clinical diagnosis of GI pathology (Choi et al., 2001; Halvorsen & Ritland, 1992; Heer et al., 1987; Peters et al., 1999; Riddoch & Trinick, 1988; ter Steege et al., 2008). Rehrer et al. (1992) and Lambert et al. (1999) have reported that triathletes experience a

greater proportion of GI symptoms during the running section of a triathlon relative to the swimming and cycling components. However, these observations are confounded due to the sequential nature of the triathlon where the stress during running is applied concurrently after cycling. Residual fatigue from the former may impact the latter element, thus negating differentiation between the relative effect of the different modalities on symptoms. Similarly, van Nieuwenhoven et al. (2004) has observed in a laboratory simulation amongst triathletes an elevated GI permeability and symptoms during running following cycling. Again, explanation may lie with the experimental design where the cycling and running exercise protocols were performed concurrently rather than separately thus differentiating between modality effects is confounded by their interaction.

These observations support the idea that repetitive high impact mechanical loading/forces are transmitted to and through the abdominal viscera that may contribute to the higher occurrence of symptoms observed during running. Rehrer *and* Meijer, (1991) have argued the abdomen is subject to vertical and lateral vibrations/oscillations due to the repetitive high impact ground reaction forces [2.5-3 body mass] which increase the rate of acceleration/deceleration loading imposed during running compared with cycling. This view being supported by others (Bahlsen & Nigg, 1987; de Oliveira et al., 2014; Simons & Kennedy, 2004; Waterman & Kapur, 2012). Increases in diaphragmatic pressure alter intra-gastric and intra-abdominal pressures mediating abdominal distention/bloating, cramps and the release GI hormones (MacLaren et al., 1995; O'Connor et al., 2006). Such mechanical loading has been noted to disrupt the enteric nervous system signalling and neuroendocrine communication patterns effecting an upregulation in local tissue inflammation (Chandrasekharan et al., 2013). Elevations in GI hormones and neural activity may further alter fluid balance in the intestine and induce abdominal cramps and diarrhoea (de Oliveira et al., 2014). Such observations may therefore help explain the gastroesophageal symptoms, diarrhoea and lower GI complaints prevalent amongst runners in comparison to cycling (Peters et al., 1999; Peters et al., 2002). Notionally support for a mechanical trauma effect may be seen in another analogous running related condition 'foot strike Haemolysis' that occurs as a result of increased loading during running relative to non-weight bearing activities (Telford et al., 2003).

In order to effectively evaluate the effect of exercise modality on GI permeability and the tissue damage surrogate IFABP, it was important that exercise intensity and absolute workload were matched. Data indicate no significant differences in energy expenditure, average oxygen consumption, RPE, core temperature, cortisol responses and percentage of

body mass lost between the experimental trials as such the two exercise modalities were appropriately matched as regards exercise intensity and energy expenditure. (Table 7.1 and 7.2). However, contrary to the initial hypothesis, no significant differences in GI permeability between cycling and running at ~70 % $\dot{V}O_2$ peak when matched for total energy expenditure was observed. GI permeability values are approximately comparable with previous studies illustrating a change in permeability after running at ~70 and ~80 % $\dot{V}O_{2\text{ max}}$ (Karhu et al., 2017; Lambert et al., 2008; Pals et al., 1997; Yeh et al., 2013). Indeed, when considered relative to rest both the running and cycling protocols induced a level of stress sufficient to compromise GI barrier integrity and increase IFABP in a manner indicated by others (van Wijck et al., 2014; Pugh et al., 2017). In order to match intensity and energy expenditure exercise time to completion was not off a fixed duration. The fact that our exercise trials varied in duration by approximately 20 min could explain the lack of difference in GI permeability between running and cycling protocols. The extended duration of the cycling activity relative to running may have imposed an additional period of 'stress' extending GI exposure to additional periods of splanchnic hypo-perfusion, hyperthermia and enterocyte hypoxia. The resultant effects could have mediated the equivalence in GI permeability seen between conditions.

IFABP is an enterocyte cytosolic protein that is released upon mechanical damage to the distal tip of the intestinal villi (van Wijck et al., 2011). Elevated circulating I-FABP have previously been correlated with both clinical and exercise induced splanchnic ischemia (Relja *et al.*, 2010; van Wijck *et al.*, 2011; 2014) and in patients with abdominal tissue trauma. As a secondary marker of tissue damage to the villus structure rather than the basement membrane, IFABP concentrations were determined over the time course of both running and cycling trials. In line with the initial hypothesis there is a clear but highly variable inter-individual GI tissue damage response within both modalities of exercise relative to pre-exercise samples. The running modality clearly induces a higher IFABP response overall but also more variable responses evidenced by the larger variances from the mean. It may be that IFABP is more sensitive to the mechanical loading differences between running and cycling. Although several of the post exercise IFABP concentrations are similar to pre-exercise baseline values (Figure 7.3). Costa et al. (2017) also noted IFABP resting values that exceed exercise values reported by others. The magnitude and variability in IFABP responses noted here are comparable to that reported by others across similar exercise modalities (Pugh et al., 2017; Snipe et al., 2017; van Wijck et al., 2011a; van Wijck et al., 2014). The variance observed in IFABP has been a particular problem for this biomarker across literature indicating that the efficacy of a clinical biomarker may not fully translate

through to different exercise related populations and applications.(Janssen Duijghuijsen et al., 2016; Janssen Duijghuijsen, et al., 2017; Pugh et al., 2017; van Wijck et al., 2011; van Wijck et al., 2014). Normative ranges for IFABP across athletic populations haven't been effectively established as yet, to benchmark the utility of this marker. As such, IFABP as a marker of GI damage requires further exploration. Subjective ratings of GI discomfort were elevated during the running relative to cycling. However, the GI severity values need to be contextualised as the ratings obtained are clustered around the 'very mild' discomfort rating [lower end of the scale]. This is again consistent with the trend emerging in the literature reporting disassociations between subjective and objective markers of GI function (Karhu et al., 2017; Pugh et al., 2017). Future work may need to seek alternate pathways to explain the observed epidemiological association between exercise, permeability and subjective symptomology.

7.5 Conclusion

In summary, this study indicates that there is no difference in GI permeability between an acute bout of running and cycling when the two exercise modalities are matched for total energy expenditure and exercise intensity. Despite this, the reported severity of GI discomfort was greater during running compared with cycling, a common phenomenon noted amongst endurance athletes. However, this observation must be tempered with the very mild nature of the symptom expressed. The mechanisms which lead to an elevated GI dysfunction amongst runners are complicated and multifactorial, it is however possible that a greater reduction in splanchnic blood flow, hyperthermia, and the possible mechanical/vibrational stimuli during running may compromise GI barrier function. We observe as with previous work a lack of association between subjective and objective markers of GI symptoms. However, current finding indicate that as GI permeability was increased to a similar level across both exercise modalities; as such the upregulation in GI permeability and IFABP related damage would appear unrelated to possible mechanical differences between modes of exercise.

Chapter 8 –
Synthesis of Findings

8.0 Synthesis of Findings

The purpose of this chapter is to integrate and interpret the findings obtained from the individual studies completed within this thesis. The realisation of the aims of the thesis as set out in each chapter will be confirmed prior to reviewing the initial hypothesis set out. The general discussion that follows will consider the main findings of each chapters in relation understanding the effects of exercise on GI permeability, symptoms and damage. The outcomes will then be presented prior to the development of conclusions.

8.1 Review of Hypothesis

A series of hypotheses were developed prior to conducting the studies described in the thesis

Hypothesis 1:

- **Chapter 4:** Soccer specific intermittent exercise [SSIE] in the heat will increase GI permeability and symptoms relative to rest and cold condition. It will also be expected that exposure to heat will increase passive GI permeability and symptomology relative to cold.

This hypothesis was partially accepted. Although this study indicates GI permeability is elevated relative to cold conditions when un-acclimated well-trained soccer players are exposed to acute period of both passive and SSIE exercise under hot conditions. Whilst GI permeability is increased, it fails to show under each condition a statistically significant elevation relative to rest. Present findings are supportive of the hypothesis linking additional thermoregulatory strain induced by heat exposure to the elevated GI permeability through what is suggested to be a possible exacerbation of splanchnic hypo-perfusion, intestinal ischemia, and hyperthermia of intestinal epithelium. We also reported a clear divergence between objective and subjective markers of permeability and symptomology which poses questions as to the usefulness of these scales under such settings of SSIE.

Hypothesis 2.

- **Chapter 5:** High intensity intermittent (HIIT) and continuous steady state exercise will increase GI permeability and symptomology relative to rest. High intensity intermittent relative to steady state exercise in the Heat (32 °c) relative to Cold (12°c) will express higher GI permeability and GI symptomology.

This hypothesis was partially accepted. This study indicates GI permeability is elevated relative to resting values when an un-acclimated individual is exposed to acute periods of both steady state and HIIT exercise in both the hot and the cold. Further, it is shown that

when exercise at the same relative intensity i.e. 70 % $\dot{V}O_2$ peak is undertaken in cold conditions the GI permeability response are independent of the exercise patterns i.e. either continuous or intermittent. When the same protocols are repeated in the heat this relationship is abolished and HIIT elevates GI permeability to a greater extent than continuous steady state exercise; however, this failed to attain statistical significance. A clear divergence between the objective and subjective markers of GI permeability and symptomology is again noted posing questions as to the usefulness of these scales under laboratory based short duration steady state and HIIT based exercise.

Hypothesis 3

- **Chapter 6:** GI permeability and symptomology will increase following supramaximal High Intensity Intermittent Exercise (HIIT) relative to rest. HIIT exercise and NSAID ingestion will act synergistically to augment this increase in GI permeability and symptoms relative to placebo and rest conditions.

This hypothesis was rejected. The current study indicated no increase in GI permeability or symptomology relative to rest where present after undertaking very short but intense sprint exercise on a repeated basis. Furthermore, after ingestion of Ibuprofen relative to a placebo no interaction between exercise and ibuprofen or rest and ibuprofen were apparent to elevate GI permeability or symptomology relative to the placebo. Preceding short but intense HIIT type exercise with the use of ibuprofen does not adversely affect the GI permeability in persons who express no previous contraindications to their use. Unlike longer duration steady state exercise, the present HIIT protocol not increases GI permeability or symptoms of GI distress. GI symptomology was reported as minor to mild and did not increase significantly with NSAIDS or exercise. In line with previous chapters a disassociation in objective and subjective measures is presented

Hypothesis 4

- **Chapter 7:** Indices of GI permeability and symptomology will be higher during running relative to cycling matched for absolute work load and relative exercise intensity.

This hypothesis was rejected. The current study observed indicates no difference in GI permeability or symptomology after performing acute bouts of running and cycling at 70 % Vo_2 peak, when the both modalities are matched for total energy expenditure and exercise intensity in separate sessions. Whilst elevations in symptomology during running vs cycling

were apparent, the magnitude of these were very mild. We observe as with previous work again the lack of association between subjective and objective markers of GI symptoms.

8.2 General Discussion

Epidemiological data report increased symptomology of gastrointestinal disturbance in both the upper and lower GI tract (nausea, regurgitation, wind, vomiting, diarrhoea, cramps, abdominal pain and bloating) in both male and female athletes (Haaf et al., 2014b; Lambert et al., 1999; Peters et al., 1999b; Riddoch & Trinick, 1988; ter Steege et al., 2008; 2012). In particular endurance athletes seem susceptible to this symptomology and frequently express gastrointestinal symptoms (Costa et al., 2017; Haaf et al., 2014; ter Steege et al., 2008). Gastrointestinal symptom severity presented across the studies ranged from mild (wind, bloating) to severe/clinically significant (acute colitis, faecal occult blood, chronic ischemia) the latter symptoms being expressed particularly at the extreme endurance event end of the scale (Cohen et al., 2009; Costa et al., 2016; Grames & Berry-Cabán, 2012; Jeukendrup et al., 2000; Pfeiffer et al., 2012; Roberts et al., 2016; Stuempfle et al., 2016; Stuempfle & Hoffman, 2015). In examining this literature association have seen sought between objective and subjective symptoms to provide explanation as to possible factors that contribute to the expression of these symptoms (Costa et al., 2017; Lambert et al., 2008). GI permeability and GI damage which although used interchangeably represent different patho-physiology; the former leading to translocation of molecules from luminal to systemic circulation whilst the later provide an indicator of loss of distal villus integrity (Grootjans et al., 2016; Grootjans et al., 2013). The central driving mechanism around these changes in gastrointestinal permeability, damage and symptomology are factors related exercise intensity, exercise pattern, modality and environment in which they are performed. A central theme in literature indicates that 70 % $\dot{V}O_2$ max/peak may be a critical threshold for permeability changes to occur (Pires et al., 2016). It is important to note that GI permeability in this thesis is considered relative to rest (within treatment effect) and relative to alternative treatment. In line with this model, the exercise intensity applied across the studies was designed to achieve approximately 70 % $\dot{V}O_2$ peak (chapter 4,5,&7) or to exceed it (chapter 6). In chapter 4 the impact of a SSIE protocol which can be classified as aerobic combined with periods of high intensity intermittent exercise was assessed for its impact on GI permeability. It was determined that performance of the SSIE protocol did not significantly increase GI permeability relative to rest; which may reflect the protocol not exceeding the 70% $\dot{V}O_2$ peak 'critical threshold' under cold conditions. However, when environment was considered as an additional stressor the SSIE in the heat achieved (>70 % $\dot{V}O_2$ peak) relative to the cold with increased GI permeability and symptoms also observed. Whilst we note increased GI

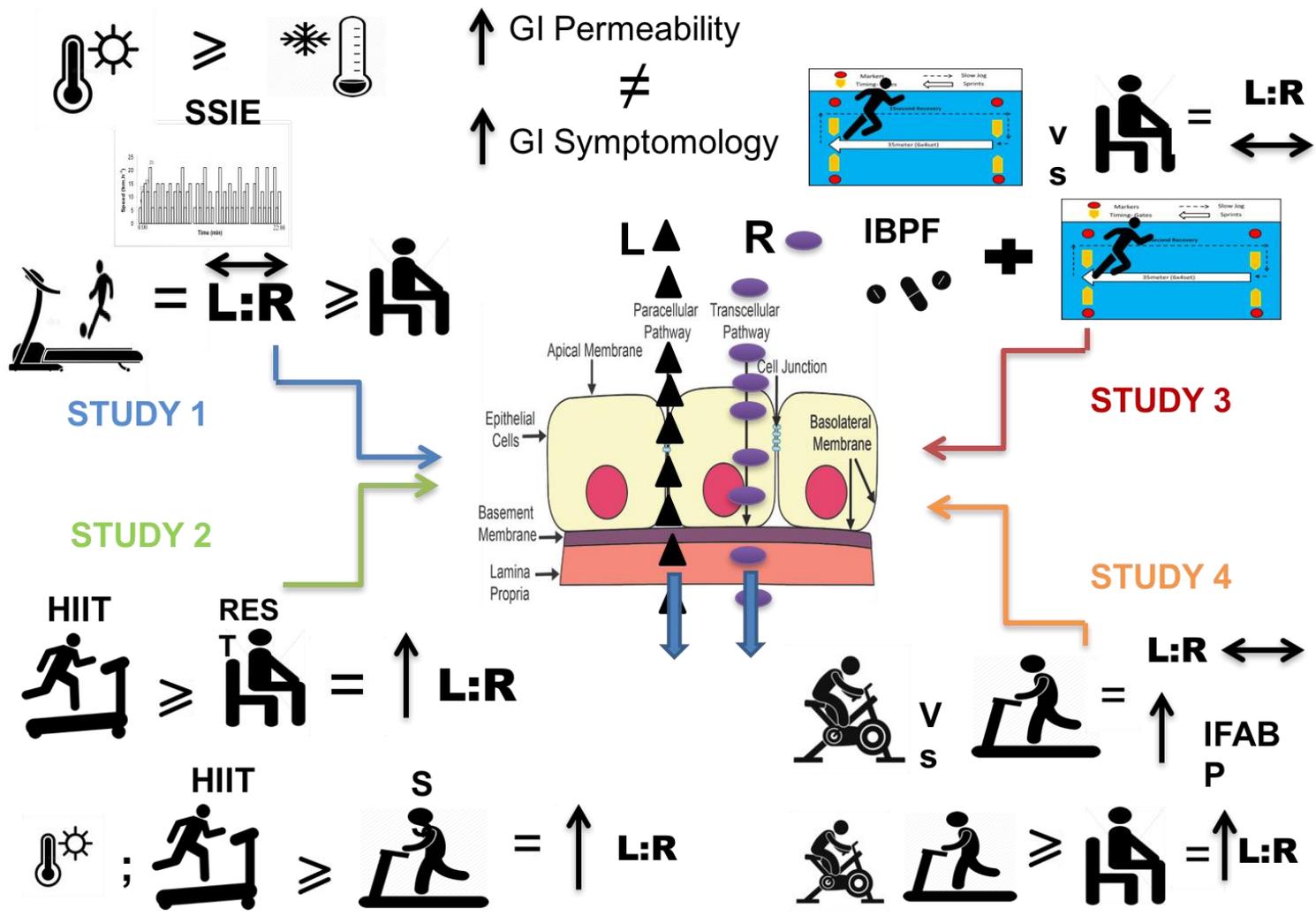


Figure 8.0 Schematic representation of the effects of exercise intensity, exercise pattern modality and pharmacological upon GI permeability and damage.

permeability in the heat the significance of this needs to be ascertained particularly in the context of soccer games played in hot environments (>32°C) and especially when the co-expressed symptomology scores are mild. Where permeability and heat stress are co-expressed there seems from present data to be a synergistic/additive effect present that elevates GI permeability without translating effectively to increased symptomology. It may be this GI permeability could lead to an increased risk of heat illness and heat stroke secondary to systemic inflammatory responses as a result of luminal antigen translocation from the gut (Selkirk et al., 2008; Selkirk et al., 2009). In studies 5 and 7 this theme of 'exercise intensity' being critical to GI permeability response was further explored. In chapter 5 relative exercise intensity was held at 70% $\dot{V}O_2$ peak during the performance of two separate protocols designed to determine how the exercise pattern i.e. continuous steady state vs intermittent exercise effects GI permeability and symptomology. Across both studies permeability was again increased relative to rest, with no change in symptom expression. In treadmill HIIT programmed at a 90%-50% work to active recovery ratio (~70 % $\dot{V}O_2$ peak) and steady state exercise as well as cycling performed at 70 % $\dot{V}O_2$ peak for minimum of 50 min will increase GI permeability. Importantly the magnitude of the increase in permeability does not appear to differ when undertaken in the cold for steady state exercise. However, as noted in chapter 4 the addition of heat stress to the HIIT protocol accentuated GI permeability; in this case relative to the cold. Based upon the observations from study 1 (chapter 4) and study 2 (chapter 5), exercise in the heat (32°C) is an important factor that provides an additive stress upon the GI tract contributing to increased GI permeability. In relation to how the exercise is patterned it is interesting to note that comparison of chapter 5 HIIT exercise consisting of long interval(s) (180 s) relative to study 3 (chapter 6) supra maximal sprint HIIT (short intervals <6 s x 6 [~36 sec]) indicates that supramaximal HIIT does not increase permeability (no environmental thermoregulatory challenge presented). The factors contributing to these differences can't be resolved within the current data set as no data on core temperature in the HIIT short intervals was determined. It is likely the short intensity bouts and long rest will attenuate heat load accumulation which would seem to have been an important factor elevating permeability in studies 1 and 2 (chapters 4 and 5). Further, when Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) are added to this HIIT model contrary to initial the hypothesis no further changes in GI permeability and symptoms are observed. These data are contrary to that reported in steady state exercise studies (Audet et al., 2016; van Wijck et al., 2012) and may thus differentiate HIIT from steady state NSAID effects observed in literature. In study 4 (chapter 7) maintaining the 'critical exercise intensity threshold' concept the first direct comparison of permeability after running and cycling was determined. It had been postulated that differences in GI symptomology between running and cycling could be explained largely by differences in mechanical/loading characteristics

applied to the GI tract (Gil et al., 1998). The direct comparison of running to cycling when closely matched indicates no difference in permeability as a result of mode of exercise during short term exposure (< 90 min).

In aligning a possible series of mechanism to explain the diverse results outlined above, GI permeability will be impacted by several key factors resulting from the protocols undertaken i.e. splanchnic hypo-perfusion, GI hyperthermia, perfusion-reperfusion related tissue hypoxia and oxidative stress (Costa et al., 2017; Lambert et al., 2002b; ter Steege et al., 2008; Ward et al., 2014). The onset of exercise is likely to bring about a redistribution of cardiac output from the splanchnic organs with reductions in splanchnic blood flow of up 80% dependent upon the exercise protocol applied and pattern of activity (Crandall & Gonzalez-Alonso, 2010; Knight et al., 2017; van Wijck et al., 2012b). In particular comparison of steady state activity and intermittent activity will result in different blood flow patterns with the former seeing a reduction and maintenance of that reduction in blood flow until exercise cessation. Conversely with HIIT exercise an oscillatory pattern of splanchnic blood flow will result as exercise intensity increases and decreases during progression through the protocol. This difference in splanchnic perfusion places significant stress on the gastrointestinal system in relation to managing thermal load and oxidative stress (Hayashi et al., 2012; Knight et al., 2017; Lambert et al., 2002; Perko et al., 1998). However, a sense of the likely impact of these pattern differences can be observed from the lack of difference in GI permeability responses under the HIIT vs steady state model in the cold (Chapter 5). Where exercise was under taken under different environmental conditions i.e. hot conditions heat accentuates permeability at rest (chapter 4) and with exercise (chapter 4 and 5). Figure 8.1 summarises the impact of the 4 individual studies upon GI permeability.

8.3 Achievement of Aims, Objectives

The primary aim of the present thesis was to examine the effects of exercise intensity, modality and environment upon gastrointestinal permeability, damage and symptomology. Initially, utilising an intermittent treadmill simulation protocol the effect of soccer related activity was evaluated to examine how GI permeability and symptomology is affected (Objective # 1). It was determined that performance of this SSIE simulation protocol did not significantly increase GI permeability. However, when environment was considered soccer performance in the heat relative to the cold increased GI permeability (Objective # 1). Study 2 [chapter 5] deconstructed the activity patterns typically experienced in soccer i.e. continuous running and intermittent exercise and directly compared how when matched for relative intensity the pattern of exercise impacts upon GI permeability and symptoms and whether this was modifiable by environment (Objective # 2). It was determined that GI

permeability increased under both continuous and intermittent exercise compared to rest. No differences between continuous and intermittent exercise patterns were observed when undertaken in the cold. However, a stepwise increase in permeability was noted after activity in the heat: Rest < SS < HIIT. Minimal expression of GI symptoms was noted and these were unrelated to the objective GI permeability markers (Objective # 2). Drawing upon this idea of deconstructing the elements of soccer related activity Study 3 [chapter 6] examined the effect of supra-maximal (sprint running) High Intensity Intermittent training (HIIT <6s) upon GI permeability and symptoms. It was observed that contemporary HIIT exercise consisting of, short duration repeated sprint (< 6s) performed on a repeated basis does not alter GI permeability significantly from rest. Further we find that when Non-Steroidal Anti-inflammatory Drugs (NSAIDS) are added to this model no further changes in GI permeability and symptoms are observed (Objective # 3). Finally, study 4 [chapter 7] addressed how modality of exercise impacted upon GI permeability, symptoms and damage (Objective # 4). Comparing running to cycling activity at the same relative intensity and with completion of similar absolute energy expenditures to assess possible effects high ground reaction force activity (running) to minimal-load bearing activity (cycling). No modality specific differences in GI permeability and symptom expression between running and cycling were noted.

8.4 Conclusions

This thesis has considered the effect of exercise intensity, modality and patterning and their environment interactions upon objective and subjective markers of GI function.

Based on the data presented the following conclusions are advanced:

1. Soccer specific intermittent exercise in the heat increases GI permeability with no corresponding increases in GI symptoms (chapter 4)
2. Continuous and Intermittent exercise increase GI permeability relative to rest, both patterns of exercise mediate similar GI permeability responses however this relationship is modified when the activities are undertaken in hot conditions. (chapter 5)
3. Exercising in the heat accentuates GI permeability responses (chapter 4 &5)
4. Contemporary HIIT exercise consisting of supra-maximal, short duration repeated sprints (<6 s) do not alter GI permeability and symptomology (chapter 6).
5. The duration and pattern of HIIT may mediate different GI permeability responses i.e. long HIIT (chapter 5) vs Short HIIT (chapter 6).
6. Acute NSAIDs ingestion in conjunction with HIIT exercise does not increase GI permeability (chapter 6).

7. Running relative to cycling activity performed at 70 % $\dot{V}O_{2 \text{ peak}}$ does not increase GI permeability (chapter 7).
8. A critical threshold of 70 % $\dot{V}O_{2 \text{ peak}}$ must be achieved for GI permeability to occur when exercise duration is relatively short in duration 60-90 min.

8.5 Limitations.

Participants in studies 1 and 2 were not acclimated to heat stress thus observations outlined are only relevant to those who have not yet acclimated to heat stress, whether variations in GI permeability vary as a function of heat acclimation are undetermined and require further clarification. It is unlikely the data would be altered by participant's transitioning from un-acclimated to acclimated as a result of the two heat exposures and has not been reported previously during similar activity (Barberio et al., 2015; Chalmers et al., 2014). Second, acquiring GI perfusion data was not possible; as such references to perfusion changes represent literature derived reports and may not fully reflect the magnitude and temporal pattern of splanchnic perfusion changes experienced during the SSIE and the population utilised. Third as noted, it remains to be determined whether the permeability is reflective of that observed if the physiological loads were more analogous to that experienced during 'actual' soccer match play, as such further work with more strenuous simulation protocols is required. It must be remembered that the responses in GI permeability and symptoms are likely to be specific to the chosen exercise intensities and work-rest ratios applied here thus other combinations may elicit different physiological, metabolic and thermoregulatory demands. GI permeability and symptoms may thus be a function of exercise stimulus (González-Alonso et al., 2008; Jiménez-Pavón & Lavie, 2017; Milanovic et al., 2015; Thum et al., 2017). Attribution of putative mechanisms of changes in GI permeability to splanchnic perfusion in this thesis were outlined upon the basis of comparative analysis to that observed in literature. No direct measure of splanchnic blood flow was possible in the present study i.e. via gastric tonometry as such this is a clear limitation in the exploration of mechanisms of action. Chapter 6 examined the effects of NSAID ingestion upon GI permeability. It should be considered that the participants in study 3 [chapter 6] were trained intermittent games players thus one may assume some degree of training related adaption to HIIT activity as well as possible GI training related adaption as well as prior exposure to NSAIDs (Costa et al., 2017; Miall et al., 2017). These factors may limit the generalisability of the data to other populations. Consideration in non-HIIT trained individuals is warranted given its widespread recommendation as a preferential mode of training. The reduced duration of exercise and extended rest periods present, coupled with possible attenuation in splanchnic hypo-perfusion and hyperthermia may have modified the effect ibuprofen had on the GI barrier compared to other longer duration endurance based studies outlined. In the

present work all studies were undertaken in a fasted state at least 8 hours post prandial. This period follows the models applied elsewhere in the literature (Davison & Diment, 2009; Playford et al., 2017; Pugh et al., 2017), but does differ from what normal practice in athletes would be, unless they were following a low carb fasted training regimen. Fasting for such periods of time in animal models from 4.5 to 10 hours has been shown to increase GI stress and affect intestinal morphology (Gilani et al., 2017; Higashizono et al., 2018). Therefore, it is important to consider that such factors may influence the degree of permeability likely to occur through non-exercise enterocyte stress priming the gut to be more permeable when exercise is undertaken.

The present thesis utilised two approaches to measure GI permeability a urine and a serum based protocol. The serum approach utilised in that latter studies of this work chapters (5,6 &7) differs from the urine used in chapter 4 alone. As such comparison of the soccer study where permeability was determined by urine recovery and the remaining studies may be confounded methodologically. Other have indicated that urine and serum L:R ratio samples collected at the same time provide different estimates of L:R ratios which are not associated (Pugh et al., 2017). This may be reflective of different kinetics of sugar absorption and transport. Whilst the method of serum recovery for L:R ratio has been validated previously (van Wijck et al., 2013), this validation was performed at rest. It is known that when exercise is undertaken the pharmacokinetics i.e. the rate at which a substance appears and is removed from circulation is altered (Boscarino et al., 2012) no data exists to support a similar 2 h time course during exercise as has been reported during the rest validation work. This is key limitation on these studies as the peak L:R ratio values may be delayed beyond this time frame.

Across all studies in the present thesis a clear limitation was the recruitment and retention of participants into the respective studies. Generally, each study was logistically complex and physically demanding spanning several weeks or more of time commitment and restraint upon physical activity and other lifestyle factors. As indicated in the individual chapters this impacted upon participant recruitment and retention, whether those that dropped out were in some way physiologically different to those that remained and completed the study is undetermined. Generally, lack of time and inability to meet testing schedules and pre-data collection restrictions was the predominant reason given rather than physical inability to meet the test requirements

8.6 Recommendations for future work.

In chapter 4 it was identified that the intensity of the soccer specific intermittent exercise treadmill protocol underestimated that observed during typical match play (Carling et al., 2012a). Recently free running over ground protocol(s) (Barrett et al., 2013) have been developed that provide strong associations with in game physical work rate and physiological responses it would be appropriate to undertake the determination of GI permeability and symptomology expression to determine if GI permeability is increased when work load provides for a more realistic level of work noted during soccer match play. This has important connotations especially for the examination of putative counter-measures whether nutritional or environmental that could impact upon recovery after exercise performance.

It is argued that the interaction of splanchnic perfusion and hyperthermia provide a synergistic stimulus to increase GI permeability and damage. To date splanchnic perfusion has been determined in a very restricted series of studies where GI permeability and markers of damage have been undertaken concurrently. None have been carried out in the past 5 years. It is critical therefore to determine the individual contributory role of hyperthermia in inducing increased GI permeability relative to splanchnic hypo-perfusion and examine which has a more important effect on permeability.

The determination of GI permeability relies on the ingestion and appearance of mono and disaccharide sugar probes in serum or urine to characterise the breakdown in GI barrier integrity. To date no consideration has been applied to key methodological issues that impact upon GI permeability assessment with L:R ratios or other sugar probe combinations. First, in order to determine the optimal time frame to detect GI permeability damage a ingestion timing study should be initiated that consider not only the timing of dose administration but also the influence of different types of exercise activity i.e. intermittent vs continuous on L:R ratio expression. Second, in order to determine the effects of a change in sugar pharmacokinetics upon peak determination of L:R sugars in serum exercise and rest comparative studies to evaluate pharmacokinetics should be performed. Third, Intestinal fatty acid binding protein is widely used as a marker of GI damage, however as a marker it currently presents no population specific normative ranges at exercise intensity dose response context. Further work should address these issues in athletic males and females, across a spectrum of sport and age ranges.

The impact of NSAIDs usage in conjunction with very high intensity intermittent exercise upon GI permeability was considered in chapter 6. It is known that the conservative usage of NSAIDs by recreational and elite athletes is not adhered with high levels of use and abuse within invasion field games acknowledged (Tscholl & Dvorak, 2012; Vaso et al., 2015). Understanding how GI permeability and GI symptoms are impacted in these populations is warranted. GI permeability changes with NSAID ingestion before undertaking a full intermittent field game simulation protocol that accurately represents work rate and physiological cost in these sports will provide insight into potential GI issues arising from the integration of exercise and drug where prescription dosing guidelines are not always adhered to. Further work should consider longer duration dosing regimen and approximate the usage patterns reported in athletes more closely. It would further be advantageous to consider GI permeability and symptomology expression in those athletes that classify themselves as habitual NSAID users relative to a NSAID naive group as an evaluation of chronic maladaptation the GI tract. A dose response profile for NSAID ingestion upon GI permeability and symptoms after the performance of a standardised exercise activity should be undertaken to understand threshold of toxicity and whether it varies when additional stressor i.e. physical activity and hyperthermia and oxidative stress are co-expressed.

Female participants were not assessed in this thesis given that GI symptoms appear to be more prevalent in female athletes with prevalence and severity higher than male athletes future studies need to determine female athlete responses to exercise markers of intestinal damage (Haff et al., 2014; de Oliveira et al., 2014).

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