
Gastrointestinal Function, Damage and Symptoms During Exercise and the Potential Therapeutic Role of Probiotic Supplementation

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the degree of Doctor of Philosophy

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Authors Declaration

I declare that the work in this thesis was carried out in accordance with the regulations of Liverpool John Moores University. Apart from the help and advice acknowledged, the work within was solely completed and carried out by the author.

Any views expressed in this thesis are those of the author and in no way represent those of Liverpool John Moores University and the School of Sport and Exercise Science.

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Dedication

For my family. My mum and dad who, from even from early in life, have pushed me to be the best I can be. My brothers, Michael and Luke, and my sister Danielle. And finally to Sarah-Jayne for putting up with me, moving across the country for me (again), and for making me laugh every single day.

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Abstract

Gastrointestinal (GI) symptoms are widely reported in athletes participating in prolonged endurance events including; cyclists, triathletes and marathon runners, although there is a large estimated range of between 4–96% of participants affected (Costa et al., 2017a). Two potential causes of exercise-associated GI symptoms are exercise-induced increases in GI permeability and damage, and the consumption of carbohydrate (CHO) during exercise (Costa et al., 2017b, de Oliveira, 2017, de Oliveira and Burini, 2014). To date, the association between markers of GI damage or barrier disruption and GI symptoms during endurance exercise has been equivocal. Additionally, while CHO consumption enhances exercise capacity and performance (Stellingwerff and Cox, 2014), ingestion also appears to increase the risk of GI symptoms, although data is again equivocal (Costa et al., 2017a). Given this prevalence of GI symptoms, probiotics may offer a convenient and practical strategy to reduce exercise-associated GI symptoms, with evidence showing their capability to alter CHO absorption and metabolism along with attenuating exercise-induced GI permeability and damage. The aim of this thesis is to investigate the relationship between markers of GI permeability and damage, measured in the blood, and GI symptoms during endurance exercise, up to marathon distance running. The role of CHO intake in GI symptoms will be examined, as well as the use of probiotics to reduce such symptoms. To realise these aims, the following objectives will be addressed:

- 1) Investigate the prevalence and severity of exercise-associated GI symptoms (Chapters 4 and 7)
- 2) Explore the role of different circulatory markers to measure GI permeability and damage, and their relation to GI symptoms (Chapters 5, 6, and 7)
- 3) Investigate the use of probiotics in reducing GI symptoms exacerbated by CHO during prolonged exercise (Chapter 6 and 7)
- 4) Investigate the efficacy of probiotics to reduce GI permeability and damage during exercise (Chapter 6 and 7)

The main findings from this thesis imply that a moderate prevalence of GI symptoms exists in recreational runners, with 27-58% of marathon participants having reported mild to severe GI symptoms during the race. Laboratory-based exercise protocols exhibited much lower symptom prevalence compared with marathon races. When markers of GI permeability and damage were assessed, there appeared to be no association between these and GI symptoms. One systemic measure that correlated to a number of GI symptoms during a marathon was CD14, a non-specific marker of monocyte activation. Probiotics had no measurable effect on markers of GI permeability and damage during a marathon race, however, probiotic supplementation had a small but significant effect on exercise metabolism during steady state cycling; with an increase in CHO oxidation and subsequent reduction in lipid oxidation. Probiotic supplementation was also associated with less severe GI symptoms during both a marathon race, and in the four weeks leading to it. Furthermore,

beneficial effects of probiotics on GI symptoms during the marathon were associated with better maintenance of running speed during the final third of the race.

Taken together, GI symptoms are prevalent in endurance athletes. Symptom aetiology can vary but probiotics appear to be a promising strategy that can be used to not only reduce GI symptoms, but may even indirectly improve exercise performance.

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Abbreviations

ANOVA - analysis of variance

CFU - colony-forming units

CHO - carbohydrate

ELISA - enzyme-linked immunosorbent assay

FODMAP - fermentable oligosaccharides, disaccharides, monosaccharides and polyols

GI - gastrointestinal

GSRS - gastrointestinal symptom rating scale

HIIT - high-intensity interval training

HR - heart rate

IBS - irritable bowel syndrome

I-FABP - intestinal-fatty acid binding protein

IL - interleukin

LR - lactulose:rhamnose ratio

NEFA - non-esterified fatty acid

NSAID - non-steroidal anti-inflammatory drug

PLC - placebo

PPO - peak power output

PRO - probiotic

RER - respiratory exchange ratio

RPE - ratings of perceived exertion

SD - standard deviation

SGLT - sodium-glucose transport proteins

TC - thermal comfort

TT - time trial

Chapter 1 - General Introduction

General Introduction

Gastrointestinal (GI) health has received considerable interest in the scientific literature (de Vos and de Vos, 2012, Bischoff, 2011). GI health has been defined as a state of physical and mental well-being in the absence of GI complaints that require the consultation of a doctor (Bischoff, 2011). The importance of GI health is demonstrated by the fact that GI complaints that cause an individual to consult a doctor are extremely common in the general population and can affect individuals chronically (Bischoff, 2011).

The canonical role of the GI tract is digestion and absorption. However, the GI tract has also been shown to contribute to our immune function and regulate systemic levels of inflammation and it has even been suggested to affect higher cognitive functions via the gut-brain axis (Mayer, 2011). Despite the size and multitude of functions of the GI tract, it has rarely been thought of as an athletic organ and comparatively less research has been conducted on GI structure and function in athletes compared with other physiological systems (e.g. the musculoskeletal system) and compared with the number of studies in clinical conditions (e.g. obesity, irritable bowel syndrome).

During exercise, and in particular in the elite sporting environment, the GI system is faced with a number of potential challenges to its function, including; reduction in blood flow, increased temperature, peri-exercise feeding and intrusion of pathogenic molecules (Lambert, 2009, van Wijck et al., 2012a, de Oliveira et al., 2014). Whilst faced with these challenges, there is potential for any of these factors to affect any portion or specific functioning of the GI system such as; gastroesophageal function, intestinal absorption, luminal barrier function, and colonic motility. Depending on the magnitude of any such challenges, should they occur, outcomes could include mild symptoms of discomfort, reduction in performance, or even clinical syndromes such as ischaemic bowel (Moses, 2005, Gil et al., 1998). In an early review, it was concluded that moderate exercise does not alter normal GI function (Stickney and Liere, 1960), however, strenuous or prolonged exercise has been found to lead to negative outcomes such as impaired gastric emptying, barrier function, and general GI discomfort (Gisolfi, 2000, Lambert, 2008). For example, marathon running has resulted in exercise-induced ischaemic colitis, requiring surgery (Cohen et al., 2009), although these cases are extremely rare.

More commonly experienced, and with less deleterious effects, mild-to-moderate GI symptoms typically include heartburn, nausea, bloating, abdominal cramps, vomiting, flatulence, the increased urge to defecate, and diarrhea. This wide range of symptoms, each with their own potential causative factor, makes it difficult to identify a single pathology. Changes in blood flow, hormonal alterations, neural affects, psychological stress, mechanical movement, altitude, nutrition, dehydration,

medications, and climate are all factors typically associated with elite sport environments and have been linked to GI symptoms (Lambert, 2008, de Oliveira et al., 2014, Haug et al., 2002, Rehrer et al., 1992a, Tielemans et al., 2013). Research into GI symptoms during exercise is further confounded by factors such as the technical difficulty of studying GI transit, diverse habitual diets, underlying GI disease states that may be undiagnosed, and differences in individual microbiota which may impact study results (de Oliveira et al., 2014, Lambert, 2008, Otte et al., 2001). Therefore, despite an increase in research interest to identify GI symptom aetiology, there is a paucity of specific causes for different symptoms.

One proposed mechanism for many GI symptoms during exercise is the exercise-induced reduction in splanchnic blood flow (van Wijck et al., 2012a, Peters et al., 1995). Such a reduction has been linked to disturbed GI motility, endotoxaemia, and reductions in intestinal absorption of water and carbohydrates (CHO), all of which can subsequently cause GI symptoms (Peters et al., 1995). These areas have therefore been suggested as targets for symptom reduction in future research (ter Steege and Kolkman, 2012).

The concept of reducing GI symptoms during exercise will form the basis of this thesis. The aim is to investigate the relationship between markers of GI permeability and damage, measured in the blood, and GI symptoms during endurance exercise (up to marathon distance running). Identifying a direct causal relationship would add rationale to the suggestion of attenuating GI barrier disruption to reduce GI symptoms in exercising individuals. Finally, the role of CHO intake in GI symptoms will be examined, as well as the use of probiotics to reduce such symptoms. Should probiotics be shown to reduce GI symptoms during endurance exercise, this would identify a simple strategy for athletes whom may experience GI symptoms, potentially leading to improved performance. To realise these aims, the following objectives will be addressed, as depicted in Figure 1.1:

- 1) Investigate the prevalence and severity of exercise-associated GI symptoms (Chapter 4 and 7)
- 2) Explore the role of different circulatory markers to measure GI permeability and damage, and their relation to GI symptoms (Chapters 5, 6, and 7)
- 3) Investigate the use of probiotics in reducing GI symptoms exacerbated by CHO during prolonged exercise (Chapters 6 and 7)
- 4) Investigate the efficacy of probiotics to reduce GI permeability and damage during exercise (Chapters 6 and 7)

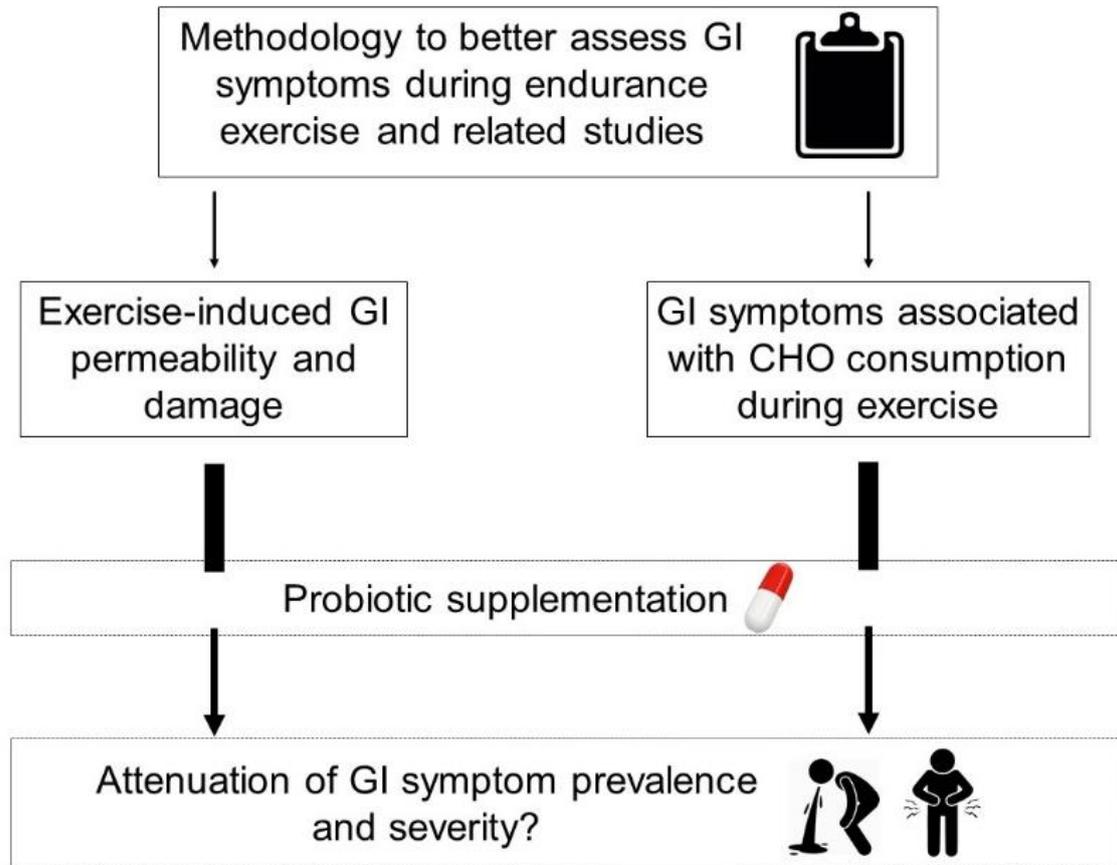


Figure 2.1. General overview of the objectives of this thesis. The prevalence and severity of GI symptoms will be assessed using appropriate methods. The relationship between circulatory markers and GI symptoms will be assessed, as will GI symptoms associated with carbohydrate (CHO) consumption during exercise. Finally, probiotic supplements will be investigated for their efficacy in attenuating exercise-induced GI damage and GI symptoms during endurance exercise.

Chapter 2 -Review of the literature

2.1 Exercise and GI symptoms

Under resting conditions, amongst other functions, the GI tract acts to digest, absorb and excrete matter from our diets. Parasympathetic activity is relatively high, as is relative splanchnic blood flow, and there is a well-balanced control of neural and hormonal stimulation (Schwellnus and Wright, 2008). Exercise therefore offers a homeostatic challenge whereby splanchnic blood flow and parasympathetic activity are reduced, and there may be altered neural and hormonal stimuli (Schwellnus and Wright, 2008, Brouns and Beckers, 1993). Due to the varying nature of sport and exercise (e.g. intensity, duration, patterns of movement), the effects of 'exercise' on the GI system are not universal. For example, relatively moderate volumes of exercise and physical activity have been shown to have potential benefits to cancer risk, inflammatory bowel disease, and GI haemorrhage, but strenuous exercise can often induce GI symptoms (Figure 2.1) (Peters et al., 2001). Symptoms of pain or discomfort attributed to the GI tract have been of interest to researchers for a number of decades. GI discomfort encompasses a number of different symptoms such as bloating, belching, flatulence, vomiting and the sudden urge to defecate, all of which have been found to be widely seen in the general populations and can negatively impact quality of life (Tielemans et al., 2013, Halder et al., 2004).

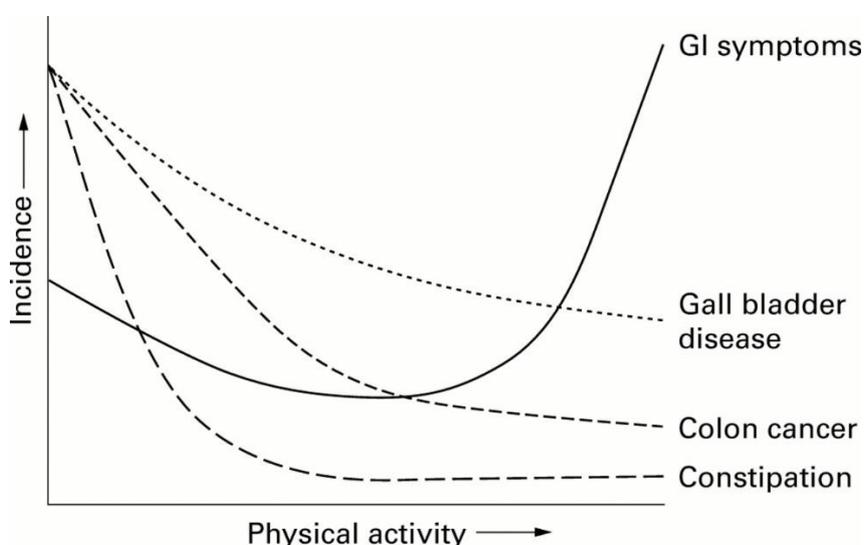


Figure 2.1 Relationship between the incidence of some gastrointestinal diseases/symptoms and amount/intensity of physical activity (Peters et al., 2001).

The general public could therefore theoretically adopt a strategy of only performing a small amount of physical activity to improve health, whilst avoiding the deleterious effects of large volumes of physical activity. Whilst this view is grossly oversimplified participating in competitive sport often means performing over 10 hours of specific exercise a week (Torstveit and Sundgot-Borgen, 2005). Given that a significant proportion of this exercise will be at a relatively high intensity, it is also of interest that as well as the total exercise volume, increases in exercise-intensity has also been linked

to an increase in symptoms (Riddoch and Trinick, 1988). Given the large training volumes and high intensity of exercise, it should be expected then that GI symptoms are prevalent in competitive sport. Consequently, minimisation of symptoms must be sought through alternate means rather than reductions in exercise volume or intensity.

2.1.1 Prevalence of GI symptoms in sport

GI symptoms have been widely reported in athletes participating in prolonged endurance events (including cyclists, triathletes and marathon runners) (Rehrer et al., 1992a), with minimal information for sports involving intermittent activity such as football, hockey or rugby. There is also a lack of research investigating the prevalence of symptoms in elite athletic populations, including endurance sports, where the research has primarily focused on mass participant events (Stuempfle and Hoffman, 2015, Pfeiffer et al., 2012). This is surprising given that many of the factors associated with GI symptoms are commonly seen in elite sport such as: use of non-steroidal anti-inflammatories, GI ischemia during high intensity exercise, different dietary habits, varying intake of fluid and carbohydrate during exercise, use of buffering supplements, and mechanical effects such as oscillations of the GI organs (Oliveira, 2017, Costa et al., 2017a). Given the lack of data on elite athletes, it is difficult to infer how prevalent GI symptoms are across elite sport and examine their specific aetiology.

The prevalence of GI symptoms in endurance based sports varies greatly, with a reported range of between 30-90% of participants effected (de Oliveira et al., 2014). When investigated within the same study, there was a difference in the prevalence of total GI symptoms between runners, cyclists and triathletes, as well as differences in the types of symptoms experienced by each group, which may reflect different sport specific aetiologies of symptoms (Peters et al., 1999). This likely reflects a real difference in symptom prevalence between these sports, attributable to consistent methodologies. Conversely differences may exist between studies due to differences in methods, such as the quantification of symptoms in regards to the scale, for example, 4, 7, 9, and 10 point scales have all been used, each of which also using different descriptor terms (Riddoch and Trinick, 1988, Pfeiffer et al., 2009, Svedlund et al., 1988b, Nieman et al., 2006). Additionally, depending on the study, a positive response of any magnitude, including those that do not effect performance, have been reported, thus overestimating the prevalence of symptoms and leading to erroneous conclusions as to the severity of the issue. For example, one study in ultramarathon participants concluded that of all respondents to a post-race questionnaire, 96% experienced at least one GI symptom. However, on a 5-point Likert scale, many of these were classified as 1 ('mild') and were symptoms which may not effect performance such as flatulence and belching. Despite these issues, it has been consistently shown that endurance based sports are often associated with GI symptoms (Stuempfle and Hoffman, 2015).

Nonetheless, it is crucial that future studies evaluating GI symptom prevalence using well-designed methodology which clearly defines and reports both the prevalence *and* severity of a number of GI symptoms.

2.1.2 Potential causes of GI symptoms in sport

Due to both the complexity of the GI system and its functions, and the wide variety of GI symptoms, there is no single aetiology, but instead there are complex and interactive pathways involved that may cause a particular symptom (Peters et al., 1995, Bischoff, 2011, van Wijck et al., 2012a, Schwellnus and Wright, 2008) (Fig.2.2). Available data suggest that GI microbiota could contribute to symptoms in multiple ways, including effects on GI immune system activation and inflammation, membrane permeability, intestinal motility, gut-brain communication, and gas production (Stern and Brenner, 2018). The repetitive movement and mechanical damage of the GI system has been suggested to be a possible cause of GI symptoms and it has been shown that, consequently, runners typically report more GI symptoms than cyclists (Pfeiffer et al., 2012, Peters et al., 2000). In the general public, persistent GI symptoms are associated with psychological traits such as stress and anxiety (Hauser et al., 2014, Koloski et al., 2000). In a group of triathletes, GI symptoms were perceived to be worse when psychological stress was present (Sullivan, 1987). Athletes have also reported GI symptoms directly before competition, believed to be from psychological stress (Worobetz and Gerrard, 1985). In regards to GI digestive and absorptive function, gastric emptying appears to be unaffected by moderate exercise, but may be delayed when exercise intensity exceeds 80% $\dot{V}O_{2max}$ (Gisolfi, 2000). Studies examining the effect of exercise on small bowel transit are limited and technically difficult to perform. It has been shown that exercise can delay *or* can speed up small bowel transit depending on the exercise modality and the method of assessment (Rao et al., 2004, van Nieuwenhoven et al., 2004). There are also non-exercise causes of symptoms. For example, GI illnesses such as acute gastroenteritis are often one of the most commonly diagnosed during major international sporting events (Engebretsen et al., 2013). From these many potential causes of GI symptoms, it is difficult to draw definitive conclusions, and therefore make recommendations to athletes. Future studies must therefore consider this wide range of potential mechanisms of GI symptoms and, where possible, control, or at least acknowledge these confounding factors.

Two of the potential causes of GI symptoms during sport and exercise that have received the most research attention in recent years are 1) increased GI permeability and damage and 2) the use of sport nutrition products, particularly the ingestion of carbohydrates during exercise. There is emerging evidence about the specific mechanisms whereby these could cause GI symptoms, as well as potential

strategies to prevent them (discussed below). However, there are still gaps within the research knowledge, and particular areas of information remain unclear or require further investigation.

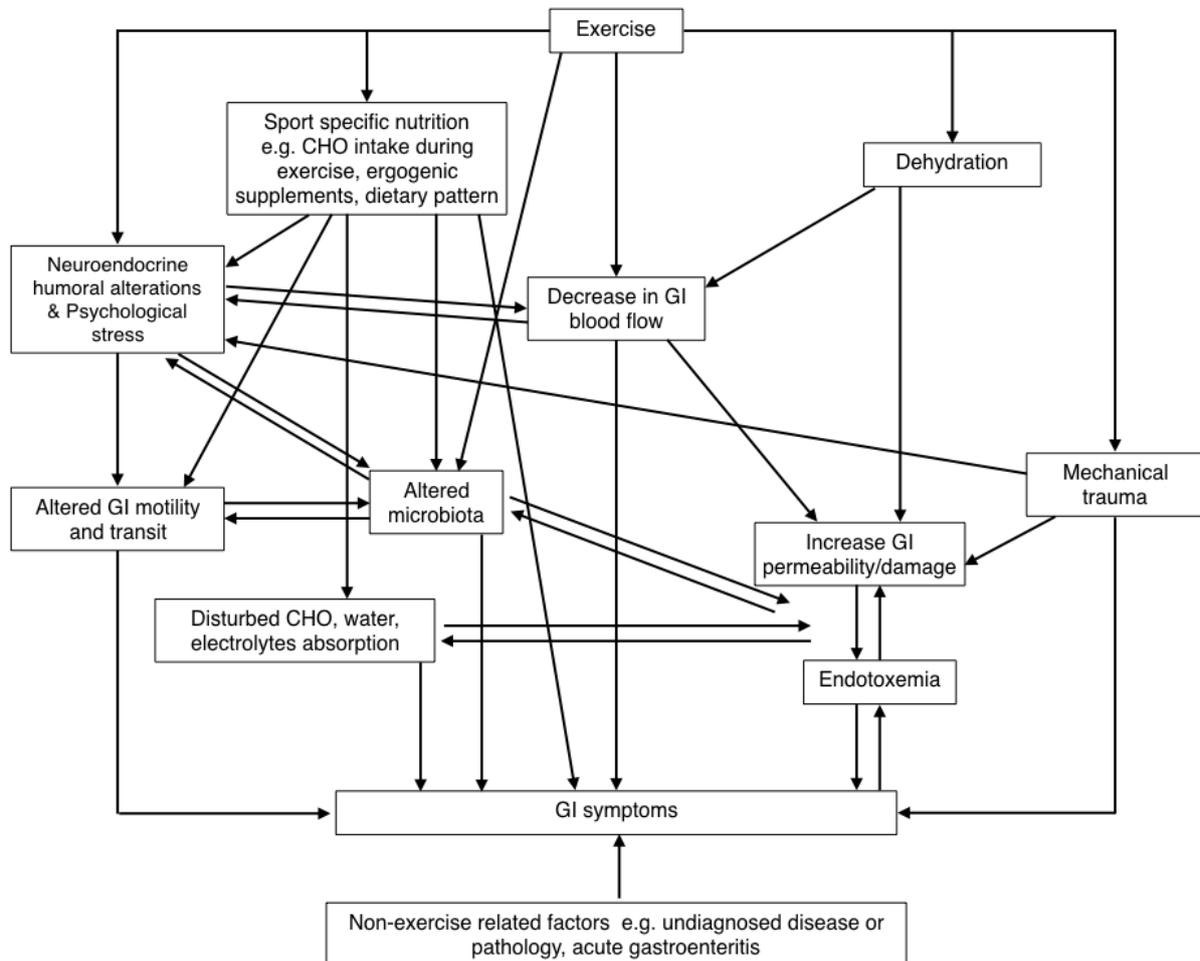


Figure 2.2. Potential causes of GI symptoms in sport. Adapted from Peters et al. (1995)

2.2 Exercise, GI permeability and symptoms

2.2.1 The Intestinal Barrier

The intestinal barrier separates the body's internal and external environments and helps protect the inside of the body against the ingress of harmful substances, and protect against a reaction to omnipresent harmless compounds (Konig et al., 2016). The intestinal barrier has two primary functions, the first of which is to prevent the transport of harmful substances such as dietary antigens, digestive enzymes, and both commensal and foreign bacterial pathogens from the lumen to the internal environment (Groschwitz and Hogan, 2009). Secondly, the intestinal barrier must be semi-permeable to allow the selective translocation of essential dietary nutrients, electrolytes and water from the intestinal lumen into the circulations (Groschwitz and Hogan, 2009). The physical intestinal barrier is comprised of a continuous layer of epithelial cells (Fig 2.3) sealed by intercellular junctional

complexes on the apical end of the lateral surface of the epithelial cells (Van Itallie and Anderson, 2006).

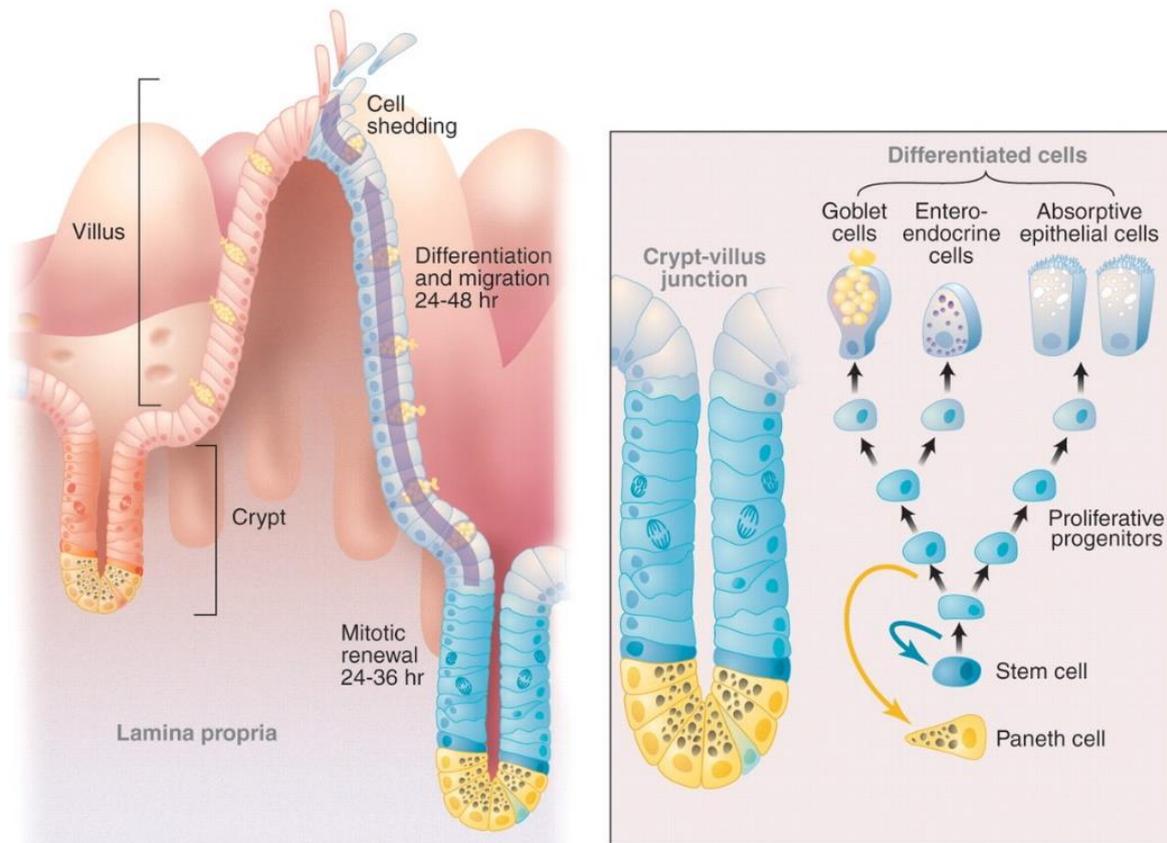


Figure 2.3. The anatomy of the small intestinal epithelium. The epithelium is shaped into crypts and villi (left). The lineage scheme (right) depicts the stem cell, the transit-amplifying cells, and the two differentiated branches. The right branch constitutes the enterocyte lineage; the left is the secretory lineage. Relative positions along the crypt-villus axis correspond to the schematic graph of the crypt in the centre. (Radtke and Clevers, 2005)

Tight junctions (TJ) are formed by transmembrane sealing proteins, that include members of the claudin family, occludin and junctional adhesion molecules that interact in the paracellular space with proteins from the adjacent cell (Fig 2.4). TJ function as a selective semi-permeable barrier that allows the passage of ions and solutes through the paracellular space while prohibiting the translocation of luminal antigens, microorganisms and their toxins into circulation (Groschwitz and Hogan, 2009). Occludin was originally thought to be the primary transmembrane protein involved in the TJ formation (Furuse et al., 1993). However, occludin depleted mice have structurally and functionally normal TJ (Saitou et al., 2000). It has since been postulated that occludin plays a regulatory role in the TJ formation, while it is the claudin proteins (claudin-1, claudin-2 and claudin-3) that are the primary sealing proteins (Zuhl et al., 2012), an assertion supported by the observation that the overexpression of claudin proteins result in a greater TJ resistance (Furuse et al., 2002). Disturbances or changes in any of the TJ proteins or their phosphorylation can cause an increase in intestinal permeability (DeMeo

et al., 2002). This is clinically defined as the non-mediated diffusion of large, normally prohibited molecules (>0.15 kDa molecular mass), from the intestinal lumen to the blood (Lambert, 2009, Travis and Menzies, 1992). Several situations can disturb the normal physiology of the intestine and increase permeability. It has been observed that exercise (Jeukendrup et al., 2000, Pals et al., 1997, Marchbank et al., 2011), exercise in heat stress (Zuhl et al., 2014), certain types of medication such as non-steroidal anti-inflammatory drugs (NSAIDs) (Playford et al., 2001), and excessive alcohol intake (Bode and Bode, 2003) can change intestinal permeability. It is also believed that psychological stress can result in changes in intestinal permeability (Mayer, 2011). While intestinal permeability has been associated with chronic inflammatory conditions and a number of disease states, the effects of acute increases in GI permeability following exercise in healthy adults, and the consequences of such increases are still poorly understood.

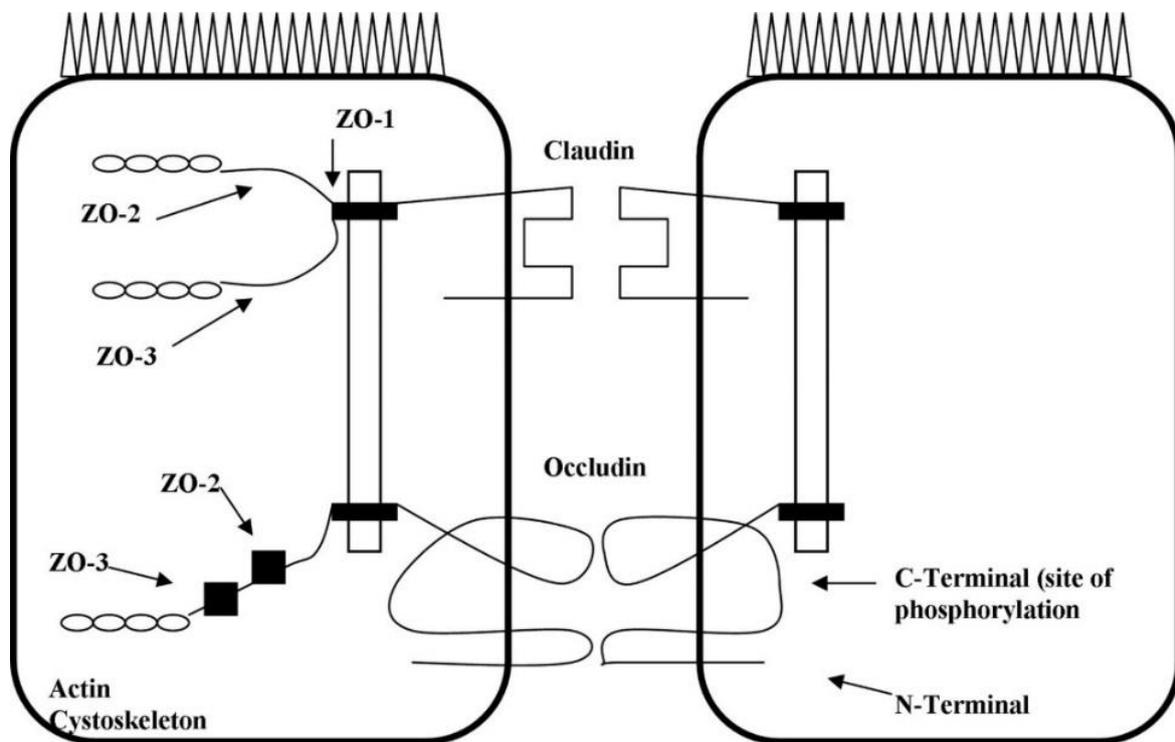


Figure 2.4. The tight junction barrier is composed of tetraspanning membrane proteins claudins and occludin, and the regulatory proteins ZO-1, ZO-2 and ZO-3. (Zuhl et al., 2012).

2.2.2 Barrier disruption and symptoms of GI discomfort

The GI tract contains large quantities of potentially highly toxic lipopolysaccharides (LPS, or endotoxin) sloughed from the walls of gram-negative bacteria. However, LPS causes no harm when confined to the intestinal lumen and do not cross the GI barrier at a rate greater than the ability of the liver to remove them from the circulation. Changes in intestinal permeability can result in LPS entering the central circulation, a condition known as endotoxaemia (Munford, 2016, Lambert, 2008). Following the translocation of LPS, it attaches to lymphocyte toll-like receptor 4 (TLR4), and cluster of

differentiation 14 (CD14) receptors, triggering the transcription and release of pro-inflammatory cytokines such as tumour necrosis factor α (TNF- α), IFN- α , IL-1 β or IL-6 (Zuhl et al., 2012). Subsequently, this immune signalling can lead to a number of GI symptoms. This inflammatory cascade has been shown to induce fluid accumulation in the small intestine, and subsequent diarrhea, with TNF- α in particular being shown to be a key moderator (Musch et al., 2002). LPS administration has been shown to affect rectal pain perception whereby both painful stimuli become more painful and the sensitivity to non-painful stimuli is increased (Benson et al., 2012). LPS mediated immune signaling also exacerbates symptoms of nausea and sickness (Dantzer et al., 2008). This has made LPS a marker of intrigue in regards to exercise-induced GI symptoms.

In exercise studies, circulatory LPS has been measured in numerous field and laboratory based studies to identify links between LPS and GI damage, systemic inflammation and GI symptoms, although values differ between studies (Barberio et al., 2015, Bosenberg et al., 1988, Camus et al., 1997, Jeukendrup et al., 2000, Roberts et al., 2016, Yeh et al., 2013). One methodological issue was seen in the study by Bosenberg et al. (1988) where values reported were 15 times that of the upper detection limit for the assay used, and higher than values typically seen in sepsis patients (Hurley, 2014, Jeukendrup et al., 2000). This has made interpretation of the associations between circulatory LPS and exercise-induced GI symptoms difficult. There has also been highly variable values reported in clinical research involving healthy participants, with differences of up to 600 fold reported between studies (Boutagy et al., 2016). This may be due to differences in pre-treatment of samples, which has shown to significantly influence the levels of LPS detected (Gnauck et al., 2016). As such, there have been questions of validity in measuring plasma LPS and the assays used in this process (Gnauck et al., 2016, Gnauck et al., 2015, Munford, 2016). Therefore, LPS may not be a valid method to assess acute changes, or moderate differences in immune activation due to GI damage or barrier disruption. As such, alternative measures have been used such as intestinal-fatty acid binding protein and the indirect assessment of GI permeability using orally consumed sugar probes.

2.2.3 Exercise-induced GI permeability and symptoms

Intestinal permeability can be assessed non-invasively by the urinary excretion of an orally administered, non-metabolised, non-toxic water-soluble probe and measuring its recovery in urine. Initially, single test substances such as lactulose, various polymers of polyethylene glycol or ⁵¹Cr-labeled ethylenediaminetetraacetic acid (EDTA) were used, although there were inherent sources of error due to the individual variations in non-mucosal factors such as GI transit time, body fluid distribution, renal clearance and their influence on test results (Bjarnason et al., 1995, Travis and Menzies, 1992). To address this limitation, the comparison of the ratio of urinary concentration over a defined interval (usually 5h) between a larger molecule, such as lactulose, and a smaller molecule,

such as rhamnose or mannitol against the amount that has been orally administered is used to give an index of intestinal permeability (Camilleri et al., 2010). The dual-sugar probes are believed to follow different pathways (Bjarnason et al., 1995). The larger molecule crosses the intestinal barrier paracellularly through the TJs whilst the smaller molecule can pass both paracellularly and transcellularly through the aqueous pores of the enterocyte cell membrane or the lipid soluble brush border (Travis and Menzies, 1992) (Fig 2.5). Consequently, permeation of the smaller molecule is not thought to be affected by the changes in intestinal permeability and is affected in the same way as the larger molecule by individual variations in non-mucosal factors (Fig 2.6). Therefore, the ratio between the two administered molecules is a better indicator of changes in small intestinal permeability (Camilleri et al., 2010, Travis and Menzies, 1992).

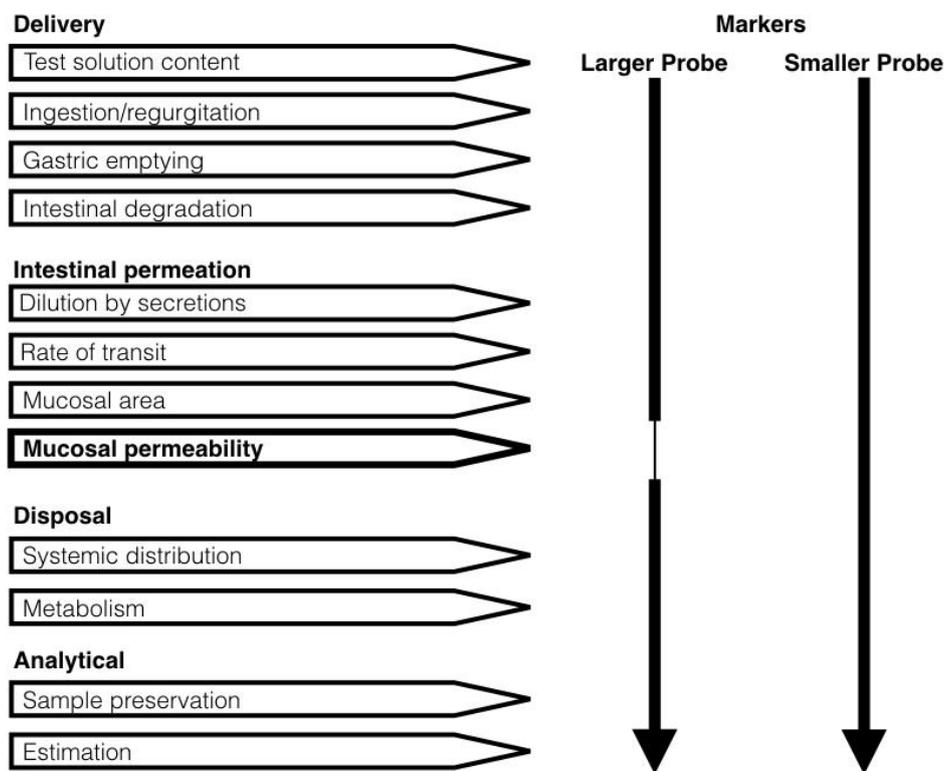


Figure 2.5. Principle of differential tests of permeability. The diagram illustrates the many factors that influence the concentration of marker probes. Mucosal permeability is the only point of difference between the handling of two differently sized marker probes. The other influences cancel out when excretion of the markers is expressed as a ratio. Adapted from (Travis and Menzies, 1992)

The urinary ratio of lactulose and rhamnose (LR) was first used in clinical settings as a marker to assess changes in intestinal permeability as a consequence of a number of clinical conditions (Keating et al., 1995, Sanderson et al., 1987). The use of LR test has also extended to exercise trials to give an indication of changes in permeability within-participants following interventions by comparing changes from baseline (Marchbank et al., 2011, Mahmood et al., 2007), or between participants of different groups (Playford et al., 2001). Furthermore, LR has been widely used to assess changes in

intestinal permeability following exercise in both a laboratory setting (Lambert et al., 2001, Pals et al., 1997, van Wijck et al., 2014) and following strenuous athletic competition in the field (Smetanka et al., 1999). It is important to acknowledge variances in the method that investigators have employed when adopting the urinary LR test to assess intestinal permeability changes during and following exercise. There have been differences between the dose of both lactulose and rhamnose (possibly effecting osmolality) administered between exercise studies (Table 2.1). There are also differences in the timing of when the probe was administered with some investigators choosing to administer the LR probes immediately prior to exercise (van Nieuwenhoven et al., 1999, van Nieuwenhoven et al., 2004), 20 minutes into an exercise bout (Zuhl et al., 2014), 30 minutes into an exercise bout (Pals et al., 1997, Van Wijck et al., 2011, van Wijck et al., 2013b), or post-exercise (Smetanka et al., 1999). As such, it is often difficult to compare absolute LR values between different studies (Table 2.1). Differences in the dose of sugar probes may in part be due to differences in analytical methods and the sensitivities to measure small concentrations.

While markers of GI permeability are able to infer deleterious effects of exercise on the GI tract, the primary outcome in the majority of endurance-exercise studies should be GI symptoms, although only a limited number of studies have investigated the link between GI permeability and exercise-induced GI symptoms (Table 2.1). Studies have shown that moderate endurance exercise can elicit measurable increases in GI permeability, yet many of these studies have failed to report GI symptoms (Marchbank et al., 2011, Zuhl et al., 2014). Those studies that have reported symptoms have shown only mild, or in many cases no GI symptoms at all, but have seen an increase GI permeability following exercise (Pals et al., 1997, Van Wijck et al., 2011, Lambert et al., 2008). GI permeability has been shown to be increased following running a half and full marathon, but without correlation to any GI symptoms (Smetanka et al., 1999, Oktedalen et al., 1992) suggesting that there may be no causal relationship, or that there are other confounding factors not accounted for. Costa et al. (2017b) reports an inverse correlation between exercise-induced GI permeability, where those with the highest GI permeability reported the lowest symptoms. Therefore, future studies should report both GI permeability and GI symptoms and report confounding factors (e.g. dietary intake during field-based studies) that may be leading to symptoms.

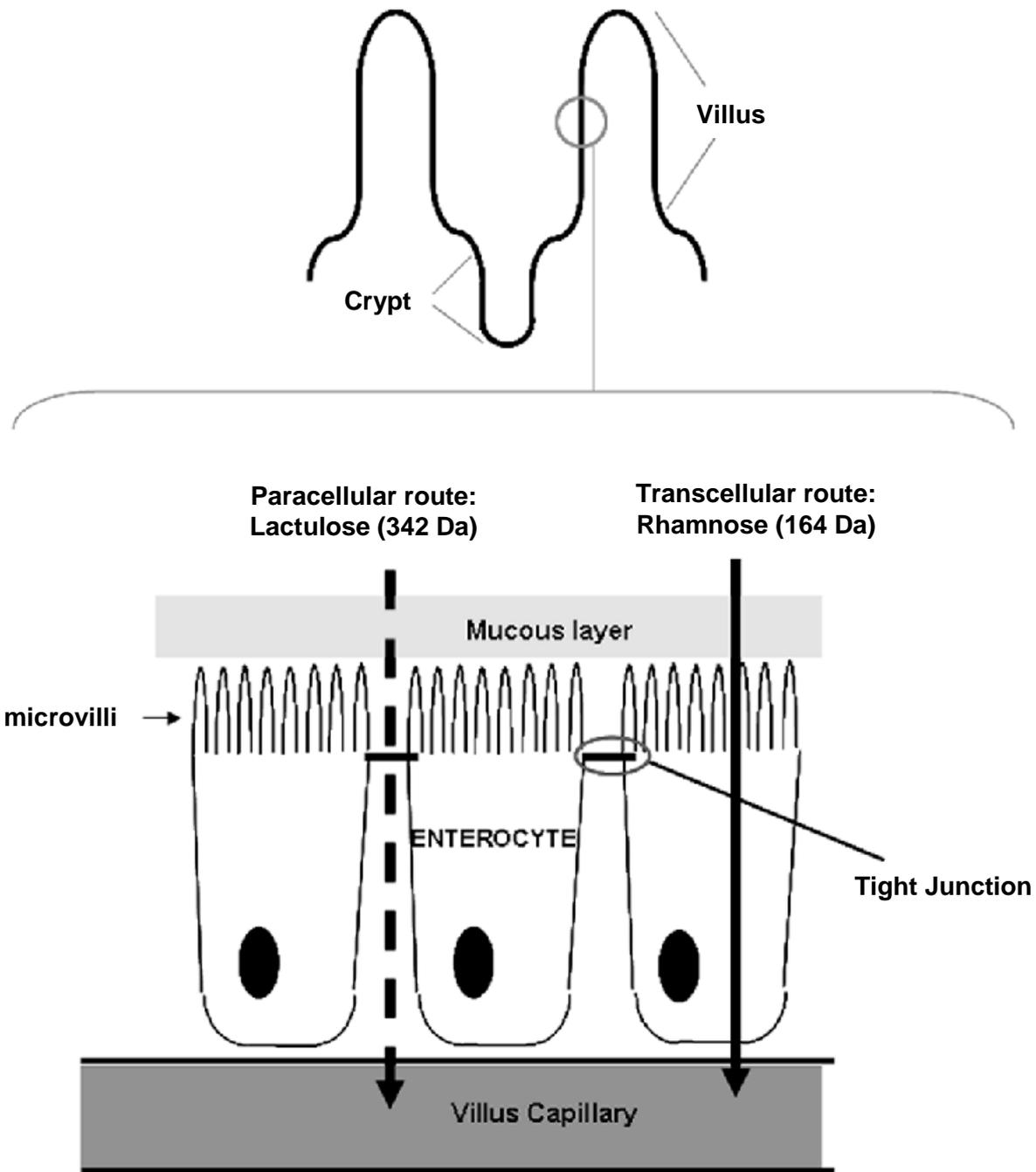


Figure 2.6. Depiction of the suggested transcellular (through the cell) and paracellular (in between cells) routes of permeation of lactulose and rhamnose. The transcellular route is indicative of small intestinal surface area, whilst tight junctions strictly regulate the paracellular pathway. Adapted from Tooley et al. (2009)

2.2.4 I-FABP and GI symptoms

Intestinal-fatty acid binding protein (I-FABP) is part of a family of nine different FABP types, each named after the tissue of its first detection (Glatz and van der Vusse, 1996). I-FABP is a cytosolic water-soluble protein which appears to be present in mature enterocytes of only the small and large intestine and is rapidly released into circulation upon injury and damage to mature enterocytes

(Pelters et al., 2003b, Grootjans et al., 2016). As such, the use of I-FABP has also emerged as an early and sensitive marker of small intestinal injury (Pelters et al. 2003; Derikx et al. 2008) with increases in I-FABP correlating with splanchnic hypoperfusion (Van Wijck et al. 2011). Whilst multiple models of exercise alter plasma I-FABP concentrations, many studies have failed to evaluate the association of I-FABP concentrations with the onset or severity of GI symptoms (Table 2.2). Future studies must therefore consider and report GI symptoms when assessing I-FABP concentrations during endurance exercise studies. Pre-exercise I-FABP concentrations may help identify individuals most at risk of developing symptoms, as with celiac disease (Oldenburger et al., 2018), and post-exercise values could help establish whether a correlative relationship, or potential threshold, with GI symptoms exists.

Table 2.1. Studies reporting exercise-induced GI permeability, methods of assessment and links to GI symptoms. LR data is mean \pm SD unless otherwise stated.

Reference	Population	Exercise protocol	Dual-sugar details	Dual-sugar ratio	GI symptoms
(Buchman et al., 1999)	15 male and female marathon runners	Road marathon competition.	L 5 g, M 2 g post-race. Measured in urine over 6h. Resting control obtained 14 d pre-race	Rest 0.03 \pm 0.02 Exercise 0.07 \pm 0.10	Multiple symptoms measured but not compared with L:M
(Lambert et al., 2008)	20 trained runners	60 min running 70% $\dot{V}O_{2max}$	L 5g, R 5 g immediately pre-exercise. Measured in urine over 5h post ingestion	Rest 0.035 (0.01-0.10) Exercise 0.063 (0.02-0.17)*†	Visual analogue scale used but scores were low and not compared with L:R
(March et al., 2017)	18 healthy male participants	20 min running 80% $\dot{V}O_{2max}$	L 5g, R 1 g immediately post-exercise. Measured in urine over 5h post ingestion	Rest 0.35 \pm 0.06 Exercise 0.95 \pm 0.12	Not systematically measured
(Pals et al., 1997)	6 active male & female participants	60 min running 40%, 60% and 80% $\dot{V}O_{2peak}$	L 5 g, R 2 g 30 min into exercise. Measured in urine over 5h post exercise	Rest 0.048 \pm 0.01 40% 0.056 \pm 0.01 60% 0.064 \pm 0.01 80% 0.107 \pm 0.02*	Not systematically measured
(Smetanka et al., 1999)	8 marathon runners 6 resting controls	Road marathon competition.	L 5 g, R 2 g ~30 min post-race. Measured in urine over 5h post exercise	Runners 0.019 \pm 0.01 Controls 0.022 \pm 0.01	Symptoms measured but not compared with L:R
(van Nieuwenhoven et al., 1999)	10 asymptomatic runners	90 min cycle 70% W_{max}	L 5 g, R 0.5 g immediately pre-exercise. Measured in urine over 5h	Rest 0.02 (0.01–0.27) Cycling 0.01 (0.00–0.01)†	Not systematically measured

(van Nieuwenhoven et al., 2004)	10 symptomatic runners	90 min run 70% $\dot{V}O_{2max}$ 90 min cycle 70% W_{max}	L 5 g, R 0.5 g immediately pre-exercise. Measured in urine over 5h	Rest 0.02 (0.01–0.04) Cycling 0.03 (0.01–0.04) Running 0.04 (0.02–0.05)*†	Not systematically measured
(Van Wijck et al., 2011)	6 healthy male participants	60 min cycling 70% W_{max}	L 1 g, 0.5 g R 30 min into exercise. Measured in urine and plasma	Rest Urinary 0.022 ± 0.005 Plasma 0.002 ± 0.001 Exercise Urinary 0.042 ± 0.04 Plasma 0.006 ± 0.004	Not systematically measured
(Van Wijck et al., 2012b)	9 male cyclist and triathletes	60 min cycling 70% W_{max}	L 1 g, 0.5 g R 30 min into exercise. Measured in urine over 2h	Rest 0.01 ± 0.01 Exercise 0.03 ± 0.02	Not systematically measured
(Zuhl et al., 2015)	8 endurance trained male and female participants	60 min running in the heat 70% $\dot{V}O_{2max}$	L 5 g, R 2 g 30 min into exercise. Measured in urine over 5h	Rest 0.022 ± 0.008 Exercise 0.06 ± 0.047*	Not systematically measured

L = lactulose, R = rhamnose, M = mannitol, * significant difference, † data is median (range). Where studies had multiple research interventions, data from the placebo arm of each study is shown.

Table 2.2. Studies reporting exercise-induced increases in I-FABP and links to GI symptoms. Data is mean \pm SD unless otherwise stated.

Reference	Population	Exercise protocol	I-FABP values (pg.mL ⁻¹)	GI symptoms
(Barberio et al., 2015)	n=8 endurance trained male participants	Running at 78% $\dot{V}O_{2max}$ (4 mMol.L ⁻¹ blood lactate) until Tc increases 2.0°C or volitional exhaustion (~24 minutes) in 40°C	Pre-exercise 640 \pm 125 Post-exercise 937 \pm 149*	Not systematically measured
(Karhu et al., 2017)	9 asymptomatic runners (5M, 4F) 8 symptomatic runners (4 M, 4F)	90 min run at 80% 10 km PB	Symptomatic Pre-exercise 389 \pm 327 Post-exercise 961 \pm 949* Asymptomatic Pre-exercise 314 \pm 152 Post-exercise 804 \pm 599*	Measured but not compared to I-FABP values.
(Lis et al., 2015)	n=13 male and female competitive cyclists	45 minutes steady state cycling at 70% W_{max} & 15 min TT	Pre-exercise 94 \pm 83 Post-steady state 233 \pm 188* Post-time trial 304 \pm 191*	Measured but not compared to I-FABP values.
(Morrison et al., 2014)	n=7 trained male participants	15 min cycling at 50% HRR, 30 min running 80% HRR, 30 min running TT) + 15 minutes cycling at 50% HRR in 30°C	Pre-exercise 143 \pm 59 Post-exercise 949 \pm 423*	Measured but not compared to I-FABP values.
(Sessions et al., 2016)	n=7 endurance trained male and female participants	60 minutes running at 70% $\dot{V}O_{2max}$ in 30°C	Pre-exercise 261 \pm 160 20-min post-exercise 337 \pm 207	Not systematically measured
(Van Wijck et al., 2012b)	n=9 male cyclists and triathletes	60 minutes cycling at 70% W_{max}	Pre-exercise 295 \pm 46 Post-exercise 474 \pm 74*	Not systematically measured
(van Wijck et al., 2013a)	n=12 recreationally trained male participants	30 minutes resistance exercise	Pre-exercise 254 \pm 31 Post-exercise 344 \pm 53*	Not systematically measured

(Karhu et al., 2017)	9 asymptomatic runners (5M, 4F) 8 symptomatic runners (4 M, 4F)	90 min run at 80% 10 km PB	Symptomatic Pre-exercise 389 ± 327 Post-exercise 961 ± 949*	Measured but not compared to I-FABP values.
			Asymptomatic Pre-exercise 314 ± 152	
			Post-exercise 804 ± 599*	

TT = time trial, HRR = heart rate reserve, PB = personal best. * Significant difference. Where studies had multiple research interventions, data from the placebo arm of each study is shown.

2.3 Exercise, symptoms of GI discomfort and sports nutrition

Both habitual daily nutrition and specific sports nutrition can have both beneficial and deleterious effects on GI symptoms. For example, some supplements that have been shown to improve exercise performance can also increase the risk of GI symptoms. Caffeine intake has been seen to correlate with lower GI symptoms during a triathlon (Wilson, 2016), possibly due its effects of increasing intestinal secretion and colonic motility (Brown et al., 1990, Wald et al., 1976). Sodium bicarbonate intake has been associated with vomiting and diarrh

ea (Burke and Pyne, 2007). However, the area of sports nutrition that has received the most research attention in regards to performance and GI symptoms is CHO ingestion during exercise. A body of literature now exists which shows that, despite CHO ingestion during endurance exercise clearly enhancing performance (Stellingwerff and Cox, 2014), CHO consumption during exercise can lead to GI symptoms. Even if GI symptoms associated with CHO consumption are 'mild' there is evidence to suggest that they are associated with impaired endurance performance (Rowlands et al., 2012, O'Brien and Rowlands, 2011, Triplett et al., 2010). However, this has not always been shown (Pfeiffer et al., 2012). The following section will examine the current body of literature in regards to CHO ingestion during endurance exercise, the reported associations with GI symptoms, and the potential dose-response effect.

2.3.2 CHO intake and GI symptoms

Total CHO intake during endurance competitions varies not only between sports but even between individuals within a sport, with ranges reported of between 0-136 g·hr⁻¹ (Pfeiffer et al., 2012, Burke et al., 2011, Kruseman et al., 2005). There is conflicting evidence between total CHO intake and GI symptoms, although greater CHO intakes during exercise have typically resulted in an increase in GI symptom prevalence and/or severity. For example, during a 16 km run, participants ingesting 90 g·hr⁻¹ CHO reported increased ratings of nausea compared to when they consumed 60 g·hr⁻¹, as well as reporting a higher incidence of moderate symptoms (a score of 4 or more on a 9 point scale), although overall incidence of symptoms was low (Pfeiffer et al., 2009). During a competitive 18 km running race, incidence of flatulence, reflux and intestinal cramps were higher when consuming CHO (~40 g·hr⁻¹) compared with water (van Nieuwenhoven et al., 2005). Incidence of bloating, urge to defecate, nausea and flatulence were all higher when participants consumed 108 g·hr⁻¹ CHO compared to 72 g·hr⁻¹ during 120 min cycling 63% $\dot{V}_{O_{2max}}$ (Jentjens et al., 2004a). The range of GI severity scores between participants within studies does though suggest individual tolerances, whereby individuals may be able to absorb more CHO during exercise. Despite potential individual differences, one of the more robust findings is the prevalence of GI symptoms when large amounts (>60g) of glucose are ingested during exercise (Table 2.3). There is therefore an exercise:performance paradox by which CHO consumed during

exercise has the potential to enhance performance however the consumption of CHO could also cause GI distress and decrease performance. It is therefore crucial that studies are now performed to examine strategies to attenuate GI symptoms associated with CHO intake during endurance exercise.

2.3.2 GI symptoms and glucose intake

Consumption of large amounts of glucose, or glucose polymers can lead to GI symptoms via a number of mechanisms. Exercise above 70% $\dot{V}O_{2max}$ and consuming higher concentrations of glucose drinks can both reduce gastric emptying (Costill and Saltin, 1974, Horner et al., 2015, Maughan et al., 1990). However, many exercise studies have utilised lower exercise intensities. A factor that has received more attention is the maximal rate of absorption and uptake of exogenous glucose during exercise. It has been shown that even when more than twice as much glucose is consumed, glucose appearance in circulation from the GI system appears to be limited at around $1.0 \text{ g}\cdot\text{min}^{-1}$ (Jeukendrup et al., 1999b). Table 2.3 shows a number of studies where participants have consumed different amounts and sources of carbohydrate during exercise. In those where total CHO intake, either as glucose or as a hydrolysed maltodextrin, is around $1.2 \text{ g}\cdot\text{min}^{-1}$ or more, there are more frequent or severe GI symptoms compared to water only trials or trials feeding multiple transporter carbohydrates. Glucose transport across the brush border occurs by sodium-dependent glucose transporter (SGLT1), whereas fructose is absorbed by GLUT5 (Wright et al., 2003). During exercise, consumption of glucose + fructose solutions appear to be absorbed and oxidised at the higher rate of $1.5\text{-}1.8 \text{ g}\cdot\text{min}^{-1}$ (Wallis et al., 2005, Jeukendrup and Moseley, 2010). Comparatively when glucose only is consumed in amounts greater than that which it can be absorbed, malabsorption may then be a contributing factor to GI symptoms. Studies are therefore required to investigate strategies to increase the maximal rate of glucose absorption during exercise and the potential attenuation of GI symptoms that this may cause.

Table 2.3 GI symptoms and intake of different sources and amounts of carbohydrates.

CHO Intake	Subjects	Exercise Protocol	GI Symptoms	Reference
2.4 g·min ⁻¹ GLU 2.4 g·min ⁻¹ GLU,FRU, SUC (2:1:1) 600 mL at start, 150mL every 15 min.	8 trained male cyclists	150 min cycling 50% PPO	More severe symptoms with GLU. One subjected vomited, two could not consume all GLU drinks	(Jentjens et al., 2004a)
1.8 g·min ⁻¹ GLU 1.2 GLU + 0.6 SUC g·min ⁻¹ 1.2 GLU + 0.6 MAL g·min ⁻¹ Water 600 mL at start, 150 mL every 15 min.	9 trained male cyclists	150 min cycling 50% PPO	More severe and non-severe symptoms during GLU and GLU + MAL than water and GLU + SUC	(Jentjens et al., 2004c)
1.8 g·min ⁻¹ GLU 1.2 g·min ⁻¹ GLU 1.2 GLU + 0.6 FRU g·min ⁻¹ Water 600 mL at start 150 mL every 15 min.	8 trained male cyclists	120 min cycling 50% PPO	More severe and non-severe symptoms during 1.8 g·min ⁻¹ GLU than all other trials	(Jentjens et al., 2004b)
1.8 g·min ⁻¹ MAL 1.2 MAL + 0.6 FRU g·min ⁻¹ Water 600 mL at start, 200 mL every 15 min.	8 trained male cyclists	150 min cycling 55% PPO	More severe symptoms during MAL than MAL + FRU	(Wallis et al., 2005)
1.5 g·min ⁻¹ GLU 1.0 GLU + 0.5 FRU g·min ⁻¹ Water 600 mL at start, 270 mL every 20 min.	8 endurance trained males	5 hrs cycling at 50% PPO	Increased scores of 'fullness' during GLU than both other trials	(Jeukendrup et al., 2006)

2.4 g·min ⁻¹ GLU 1.2 GLU + 1.2 FRU g·min ⁻¹ 250 mL to start, 250 mL every 15 min.	9 trained male cyclists	Simulated 100 km time trial with intermittent 1 km and 4km sprints	Severe GI distress during GLU including two episodes of diarrhea, one episode of vomiting, and one incident of “sour stomach”. 7 out of 9 reported fullness during GLU. None during GLU + FRU	(Triplett et al., 2010)
~1.0 MAL + ~0.5 g·min ⁻¹ GLU ~1.0 MAL + ~0.5 g·min ⁻¹ FRU	(race) 7 male, 3 female mountain bikers (lab) 16 male cyclists	Competitive mountain bike race ~3 hr cycling with high intensity intervals and sprint work load test	Increased abdominal cramps (race) and nausea (lab) during MAL + GLU Increased performance was associated with lower abdominal cramps	(Rowlands et al., 2012)
1.8 g·min ⁻¹ GLU 1.2 GLU + 0.6 g·min ⁻¹ FRU 0.6 GLU + 1.14 g·min ⁻¹ SUC Water	10 trained male cyclists	180 min cycling 50% PPO	Increase in upper GI symptoms during GLU only compared to all other trials	(Trommelen et al., 2017)
~1.3 g·min ⁻¹ MAL ~0.7 g·min ⁻¹ MAL + ~0.6 g·min ⁻¹ FRU	14 male and 6 female recreational runners	120 min running at 90% pace of most recent marathon + 4 mile Time Trial	Increase in total number of GI symptoms and moderate GI symptoms during MAL compared to MAL + FRU	(Wilson and Ingraham, 2015)

2.3.3 Glucose malabsorption

There are a number of studies showing that glucose consumption above 1.0-1.2 g·min⁻¹ can lead to GI discomfort (Table 2.3), although there is a paucity of studies to explain the precise mechanisms. During exercise, glucose malabsorption can occur and appears related to a number of GI symptoms expressed (Peters et al., 1993). Consuming large amounts of glucose during exercise leads to saturation of the glucose transporter SGLT1, leaving residual glucose within the intestinal lumen (Peters et al., 1993, Oliveira, 2017, Lang et al., 2006). This residual glucose may then lead to GI symptoms via a number of mechanisms (Fig 2.6). Excess glucose may delay gastric emptying and oro-caecal transit via the 'ileal brake' (Shin et al., 2013). Animal (Azpiroz and Malagelada, 1985) and human data (Layer et al., 1990) have shown that ileal perfusion with macronutrients can reduce gastric emptying, duodenal motility and GI hormone release, while glucose confined to the most proximal region of the small intestine has no effect (Lin et al., 1989). Large boluses of glucose can result in an increase in fermentation of glucose by bacteria in the distal half of the small intestine, leading to gas-like symptoms (Urita et al., 2006, Murray et al., 2014). Magnetic resonance imaging shows consumption of a glucose drink increases colonic gas compared to fasting levels in healthy volunteers (Major et al., 2017, Murray et al., 2014). Colonic distension may also be caused by glucose malabsorption and be a cause of GI symptoms. Distension can be caused by increasing gas, as already described, and an increase in bowel water content, or both. Again, MRI studies have shown that water content of the colon increases, as does colonic volume, in the hours after consuming a bolus of glucose, although relatively less than other mono- or oligo-saccharides (Major et al., 2017, Murray et al., 2014). Given the transit time required for glucose to reach the colon, colonic distension from glucose ingestion is most likely of relevance only to ultra-endurance sports, or in the hours after exercise lasting ≤2 hr.

The studies investigating these potential causes of glucose-mediated GI symptoms have been performed outside of the exercise environment, and some have looked at IBS patients. However, the mechanisms are plausible, particularly given the small number of studies showing positive hydrogen breath tests during exercise after glucose consumption. Strategies to minimise the potential of glucose malabsorption may then well lead to a reduced risk of GI symptoms during exercise, where high CHO consumption is required.

Intestinal lumen Glucose $\sim 1.5 \text{ g}\cdot\text{min}^{-1}$

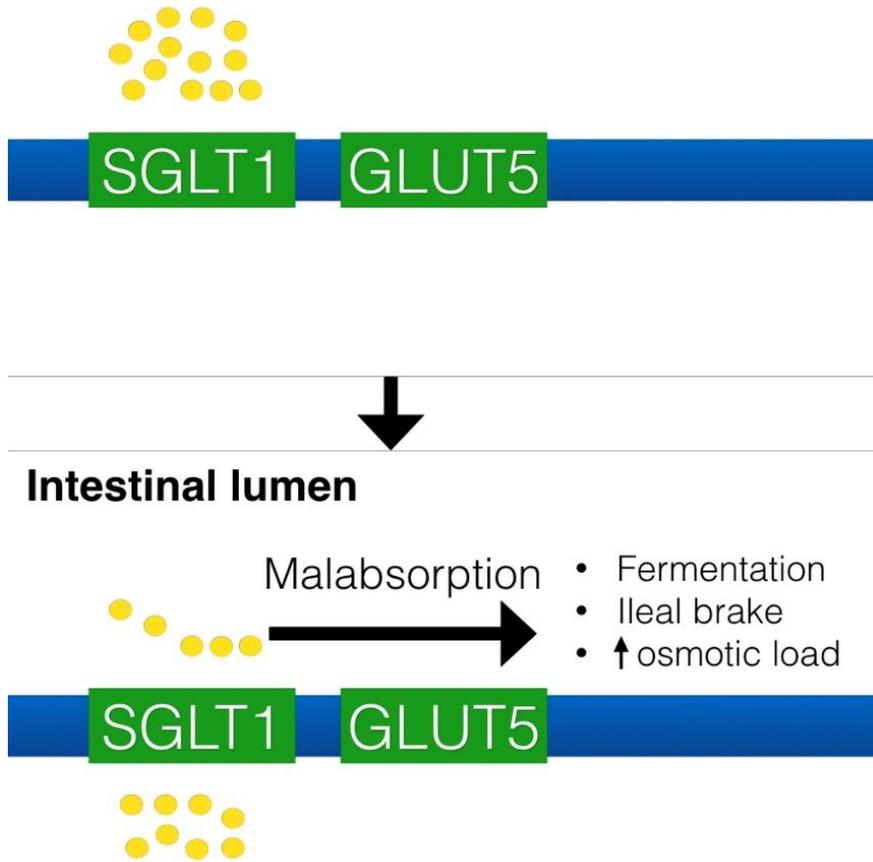


Figure 2.7. The potential consequences of glucose malabsorption

2.4 Probiotics

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al., 2014). They consist of bacteria, and are commonly available as concentrated capsules, as a powder, or in various dairy or fermented food products. Care is needed when evaluating studies, as benefits can often be specific to the bacterial strain studied, and thus benefits are not universal to all probiotic products (Allen et al., 2017, Sanchez et al., 2017). There is some limited research suggesting that probiotic supplementation can reduce either the duration, frequency or severity of upper respiratory tract infections in athletes (Pyne et al., 2015). However, to date, there is only limited research investigating the effect probiotics have on GI symptoms in athletes. There are some studies suggesting that they are beneficial in the general public, or in some clinical conditions, and the potential mechanisms have begun to be described (Hungin et al., 2013, McKenzie et al., 2016). For example, there is promising results regarding the use of probiotics in the treatment of acute gastroenteritis, Clostridium difficile-associated diarrhea, colitis, irritable bowel syndrome, necrotizing enterocolitis, and others (Thomas and Versalovic, 2010). Probiotics have also been shown to reduce the relative risk of traveller's diarrhea, and so may be of particular benefit to the traveling

athlete. Probiotics may therefore be effective in attenuating exercise-induced GI symptoms, although research to date is limited.

2.4.1 Probiotics and exercise-induced GI symptoms

A small number of studies have evaluated the effect of probiotics on GI symptoms in athletes, although methodological issues persist with many studies reporting only the frequency or duration of symptoms, and not the severity. A group of runners participating in a marathon race supplemented for 3 months prior to the race with *Lactobacillus rhamnosus* but had no difference in frequency or duration of GI symptoms either during the 3 months, or in the 2 weeks following the race, compared with the placebo group (Kekkonen et al., 2007). Similarly, *Lactobacillus fermentum* had no effect on GI symptom prevalence during training in cyclists and triathletes during an 11 week supplement period (West et al., 2011). A multi-strain probiotic had no significant effect on symptoms compared with placebo during a 150 day intervention in active individuals, although this may be due to criteria to classify a symptom, and the subsequent low frequency of symptoms reported (West et al., 2014). The lack of an effect may be due to the inefficacy of probiotic supplementation or specific strains used, or it may be in part due to methodological issues. In the studies described above, GI symptoms were not the primary outcome, and methods to quantify GI symptoms would have required probiotics to completely alleviate a symptom for a benefit to be shown to be significant. During a 12-week supplementation period of a multi-strain probiotic in recreational triathletes, GI symptoms were significantly lower at weeks 4, 8 and 12 compared to placebo (Roberts et al., 2016). Detailed questionnaires were adapted and used from previous studies investigating exercise-related GI symptoms that may have been more sensitive to change differences in symptoms. In practice, it could be argued that an attenuation of symptoms, of any magnitude, would have a real-world impact on quality of life, and potentially sporting performance. Future studies should therefore ensure that GI symptoms are systematically evaluated both during the probiotic supplement period as well as during endurance competition in order to assess their effectiveness at attenuating GI symptoms.

2.4.2 Probiotics and GI permeability

Both *in vivo* and *in vitro* work has shown that there are a number of potential mechanisms by which probiotics can reduce intestinal permeability, or confer protective effects against potential disruptions to TJ. There are a number of studies that have shown that culturing epithelial cells in medium in which probiotic strains have previously been cultured, can increase TJ protein expression, as well as their redistribution from the cytosol to the boundaries (Zyrek et al., 2007, Ewaschuk et al., 2008, Anderson et al., 2010). *In vivo*, probiotic infusion directly into the duodenum for 6 hours, via a nasogastric tube, resulted in an increase in mobilisation of occludin and zonulin proteins to the apical surface of

epithelial cells from biopsy samples (Karczewski et al., 2010). This change in TJ protein expression and distribution has been shown to correlate with baseline transepithelial resistance *in vitro* and confer protection against threats to TJ permeability. For example, *L. salivarius* prevented the H₂O₂-induced redistribution of occludin and claudin-1 in Caco-2 cells (Miyachi et al., 2012). Probiotic bacteria can also improve GI barrier function beyond their effects on TJ protein expression and mobilisation. *In vitro* studies have shown that probiotic supplementation can prevent epithelial apoptosis, increase mucin secretion, inhibit pathogenic bacteria attachment, and decrease secretion of pro-inflammatory cytokines (Mennigen and Bruewer, 2009, Caballero-Franco et al., 2007, Allen et al., 2017). This multitude of mechanisms suggests that probiotics may be effective in attenuating the exercise-induced increases in GI permeability that have been reported.

Studies that have investigated the influence of probiotic supplementation on GI permeability in athletes are limited. Four weeks supplementation with a different multistrain probiotic had no effect on urinary LR post exercise (Shing et al., 2014), however, without a resting control, it is impossible to assess if the exercise bout lead to an increase in GI permeability. Conversely, 12-week supplementation with probiotics attenuated intestinal permeability assessed by LR, six days after a long distance triathlon (Roberts et al., 2016). A multi-strain probiotic supplement for 14 weeks reduced stool zonulin concentrations (Lamprecht et al., 2012), although this cannot be used as an acute marker to assess the effect of exercise-induced GI permeability. Taken together, it is difficult to ascertain the effects of probiotic supplementation on GI barrier disruption and so more studies are required.

2.4.3 Probiotics and CHO absorption and oxidation

There is mechanistic evidence that probiotic supplementation could increase CHO oxidation during exercise. Previous animal work in race horses showed that probiotic supplementation during a training phase prevented the decline in respiratory quotient observed in a control group, with the authors suggesting that this related to an improvement in the CHO aerobic enzymatic capacity, CHO utilization, or both (Art et al., 1994b). Probiotics may also increase absorption of consumed CHO during exercise *In vitro*, Caco-2 cells show increases in expression of glucose transporters and subsequently increased glucose uptake when cultured with supernatants prepared from anaerobic culture of *Lactobacillus* strains (Rooj et al., 2010b). Additionally, both *Bifidobacterium bifidum* and *Lactobacillus casei* supplementation for 28 days stimulated SGLT1 expression in mice models, as well as increasing maltase activity (Barrenetxe et al., 2006). The pro-inflammatory cytokine TNF α has been shown to downregulate both the expression and activity of SGLT1 in Caco-2 cells, reducing glucose transport (Barrenetxe et al., 2013). This may be another mechanism by which probiotics could alter exercise metabolism as probiotics have been shown to augment the secretion of TNF α by epithelial

cells (Hardy et al., 2013). Studies investigating exercise metabolism following probiotic supplementation are now needed to assess their efficacy and investigate if these potential mechanisms translate to a measurable effect.

2.4.4 LAB4

The purported benefits of probiotics are not universal and will differ depending on the bacterial strains, dosage, and duration of supplementation. One multi-strain product used in a number of research studies is the LAB4 probiotic, containing *Lactobacillus.acidophilus* CUL-60 and CUL-21, *Bifidobacterium bifidum* CUL-20 and *Bifidobacterium lactis* CUL-34. The LAB4 probiotic has been shown to be beneficial to those undergoing antibiotic treatment (Plummer et al., 2004, Plummer et al., 2005) and in reducing the frequency of URTI and duration of symptoms in schoolchildren (Garaiova et al., 2015). In regards to GI symptoms, a group of participants with IBS supplementing with LAB4 showed greater reductions in GI symptom severity and quality of life scores than those in a placebo group (Williams et al., 2009). Finally, LAB4 has been used specifically in two endurance exercise related studies. Shing et al. (2014) reported increases in running time to fatigue and small, albeit non-significant, decreases in GI symptoms following 4 weeks supplementation with LAB4. In triathletes, supplementation with LAB4 for 12 weeks prior to a long distance triathlon race resulted in an attenuation of increased GI permeability over a training cycle and fewer GI symptoms were reported compared to placebo (Roberts et al., 2016). These data, along with the data from those with IBS, shows that LAB4 may have the potential to be of benefit to endurance athletes in regards to GI symptoms, although more studies are required.

2.5 Summary

In summary, endurance exercise appears to be associated with a high frequency of GI symptoms, particularly during competitive long distance running events. A number of mechanisms have been proposed, but an increase in GI permeability and damage is thought to be a contributing factor, as is CHO consumption during exercise – which appears to exacerbate symptoms. However, there are discrepancies in the measures used to assess GI permeability, and there is a lack of data assessing the relationship between these measures and GI symptoms. There is also a large disparity in the reported frequency and severity of GI symptoms, with a number of methodological approaches used. Research interest in GI symptoms, its measurement, and its causes is growing. There is a need to assess the efficacy of biological markers to assess GI permeability, as well as finding practical strategies to reduce the severity and/or frequency of GI symptoms during exercise.

Chapter 3 - General Methods

General Methods

3.1 Location of testing and ethical approval

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 Moores University. The track marathon took place on an official measured 400m athletics track and pre and post-race samples were collected in the physiology laboratory at Edge Hill University. Ethical approval was granted from the local ethics committee at Liverpool John Moores University. Analysis of L:R and serum cortisol was conducted at the Royal Cornwall Hospital. Analysis of plasma and breath ^{13}C at the University of Surrey. All other analysis was performed at Liverpool John Moores University.

3.2 Subject characteristics

Participant characteristics are described in each study chapter. None of the participants had a history of gastrointestinal disease and none were under pharmacological intervention during any study. Participants were asked to maintain habitual activity levels during each study, and refrain from additional exercise, caffeine and alcohol for at least 24 hours prior to any testing sessions.

3.3 Assessment of cardio-respiratory measures

3.3.1 Assessment of maximal oxygen consumption ($\dot{V}\text{O}_{2\text{max}}$) - running

$\dot{V}\text{O}_{2\text{max}}$ was assessed using an incremental exercise test performed on a motorized treadmill (HP Cosmos, Germany). Oxygen uptake was measured continuously during exercise using an on-line gas analysis system (Oxycon Pro, Carefusion, Germany). The test commenced with a 2-min stage at $10 \text{ km}\cdot\text{h}^{-1}$ followed by 2-min stages at $12 \text{ km}\cdot\text{h}^{-1}$, $14 \text{ km}\cdot\text{h}^{-1}$, and $16 \text{ km}\cdot\text{h}^{-1}$. On completion of the $16 \text{ km}\cdot\text{h}^{-1}$ stage, the treadmill was inclined by 1% every 2 minutes thereafter until volitional exhaustion. $\dot{V}\text{O}_{2\text{max}}$ was stated as being achieved by the following endpoint criteria: (1) heart rate within 10 $\text{beats}\cdot\text{min}^{-1}$ of age predicted maximum, (2) respiratory exchange ratio >1.1 and (3) plateau of oxygen consumption despite increased workload. Based on the results of the incremental test, the running speed corresponding to 100% $\dot{V}\text{O}_{2\text{max}}$ was estimated for each participant using a linear regression equation, allowing for calculation of running speeds of varying intensities.

3.3.2 Assessment of lactate threshold and peak oxygen consumption ($\dot{V}\text{O}_{2\text{peak}}$) – running

Lactate threshold and $\dot{V}\text{O}_{2\text{peak}}$ were determined via an incremental running test as previously described (Jones 2006). Briefly, participants ran a minimum of six stages on a motorized treadmill (HP Cosmos Saturn, Traunstein, Germany). Each stage was 3 minutes in duration, interspersed with 30-second breaks to facilitate fingertip capillary blood sampling and lactate concentration analysis (Lactate Pro™ Analyzer, Arkray, KDK Corporation, Kyoto, Japan). Running speed was increased by $1 \text{ km}\cdot\text{h}^{-1}$ at the end of each stage, until runners reached volitional fatigue. Oxygen uptake was measured continuously

during exercise using an on-line gas analysis system (Oxycon Pro, Carefusion, Germany). $\dot{V}O_{2\text{peak}}$ was determined from the mean of last 10 s of each 3 minute interval.

3.3.3 Assessment of peak oxygen consumption ($\dot{V}O_{2\text{peak}}$) and peak aerobic power output (PPO) - cycling

$\dot{V}O_{2\text{peak}}$ and peak aerobic power output (PPO) were determined on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) during an incremental exhaustive exercise test. Work rate commenced at 95 W for 3 min, followed by incremental steps of 35 W every 3 min until volitional exhaustion. Oxygen uptake was measured continuously during exercise using an on-line gas analysis system (Moxus modular metabolic system, AEI technologies Inc, Pennsylvania, USA). $\dot{V}O_{2\text{peak}}$ was determined from the mean of last 10 s of each 3 minute interval. PPO was calculated from the last completed work rate, plus the fraction of time spent in the final non-completed work rate multiplied by the work rate increment (Jeukendrup et al., 1996).

3.4 Psycho-physiological measures

3.4.1 Ratings of perceived exertion

Participants reported ratings of perceived exertion (see Table 3.1) during exercise according to a 15-point Likert scale devised by Borg (1970).

Table 3.1 Ratings of perceived exertion

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

3.4.2 Rating of global GI symptoms

Global gastrointestinal symptoms were recorded every 15 min during each experimental protocol using a GI discomfort scale adapted from Pfeiffer et al. (2009). Participants rated their symptoms on a 10-point scale, ranging from 0 ('no problem at all') to 10 ('the worst it has ever been'), with a score

> 4 being regarded as 'moderate'. Participants were instructed to consider symptoms of bloating, the urge to defecate, burping, nausea, flatulence, and/or the urge to vomit.

3.4.3 Rating of specific GI symptoms

After exercise, participants were asked to complete a more detailed questionnaire (adapted from Pfeiffer et al., 2012) to assess any specific symptoms of GI discomfort of bloating, flatulence, stitch, belching, nausea, urge to vomit, urge to defecate, and stomach cramps (Appendix 1). GI symptoms were scored on a 10-point scale (0 = no pain and 10 = worst possible pain) with a score > 4 being regarded as moderate. Lower GI symptoms were classified as flatulence, bloating, diarrhoea, and urgent need to defecate and upper GI symptoms as nausea, belching, stitch and urge to vomit. To ensure understanding, specific symptoms were explained and described to participants.

3.4.4 Gastrointestinal symptom rating scale (GSRS)

The Gastrointestinal Symptom Rating Scale (GSRS) (Svedlund et al., 1988b) contains 15 items, each rated on a seven-point Likert scale from no discomfort to very severe discomfort relating to; abdominal pain, hunger pains, nausea, heartburn, acid regurgitation, diarrhea, loose stools, rumbling, abdominal distension, belching, increased flatulence, constipation, hard stools, and feeling of incomplete evacuation. This questionnaire was chosen for use with remote participants (Chapter 4), as it has been validated with a large number of participants when used as an online resource (e.g. Spiegel et al., 2014).

3.5 Collection, storage and analysis of blood samples

Blood samples were drawn from a superficial vein in the antecubital crease of the forearm using either standard venepuncture techniques or via an indwelling cannula. Samples were collected into serum separation tubes (SST), K₂EDTA or lithium heparin vacutainers (BD Biosciences, UK). K₂EDTA and lithium heparin tubes were stored on ice while SST vacutainers were stored at room temperature for 1 h before centrifugation at 1500 g x 15 minutes at 4 °C. Following centrifugation, plasma or serum was separated into aliquots and stored at -80 °C for later analysis.

3.5.1 Assessment of intestinal permeability

Intestinal permeability was assessed by analysing serum samples using a previously published protocol (Fleming et al., 1996b), with the modification of using rhamnose instead of mannitol as the monosaccharide probe. A 50 mL sugar probe solution (5 g lactulose, 2 g rhamnose) was consumed and the ratio of the sugars was measured from serum (Chapters 5, 6, 7) and urine (Chapter 5) samples post exercise. The respective sugars were separated using high-pressure liquid chromatography 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 rhamnose and 6.1 min for lactulose using 120mmol·L⁻¹ NaOH as an isocratic eluent. The coefficient of variation for lactulose and rhamnose combined was 11%. Timings for administration of LR probe solutions, and serum sample collection during each chapter are given in Table 3.2.

Table 3.2 Timing of LR drink solution in each chapter

	LR probe administration	Serum sample collection
Chapter 5	Immediately pre-exercise	Immediately post-exercise (~1hr after administration) 1hr post exercise (~2hr after administration)
Chapter 6	Immediately post-exercise	60 min post exercise (60 min after administration)
Chapter 7	Baseline	60 min after administration
	Immediately post-race	60 min post-race (60 min after administration)

3.5.2 Assessment of intestinal-fatty acid protein (I-FABP)

I-FABP concentrations from EDTA plasma were determined using an enzyme-linked immunosorbent assay (ELISA) (Hycult Biotechnology, Uden, the Netherlands; detection window 47 - 3000 pg·ml⁻¹) according to the manufacturer's instructions. The ELISA is a solid-phase enzyme-linked immunosorbent assay based on the sandwich principle. Samples were diluted 1:2 with a provided sample dilution buffer and plates were read at 450 nm. The coefficient of variance was 8% for between-sample duplicates.

3.5.3 Cytokine analysis

Cytokine concentrations were measured using cytometric bead array (CBA, BD Biosciences, San Diego, USA) for the cytokines (as described in relevant chapters) using the manufacturers instructions with bead populations with distinct fluorescence intensities coated with capture antibodies specific for each protein. Following acquisition of sample data using the flow cytometer, the sample results were generated in graphical and tabular format using the BD CBA Analysis Software. The coefficients of variation were 9.7%.

3.5.4 Circulating metabolite analysis

Plasma glucose, lactate, non-esterified fatty acids, and glycerol and were analysed using a Randox Daytona spectrophotometer and commercially available kits (Randox Laboratories, Ireland). The coefficient of variation for plasma glucose, lactate in our laboratory was ≤ 5 %.

3.5.5 Serum cortisol analysis

Serum cortisol was measured using an ELISA kit according to the manufacturer's instructions (Elecsys Cortisol assay, Cobas-Roche, UK). The range of measures was 0.5-1750 nmol·L⁻¹ with a CV of 2.9%.

3.5.6 Plasma sCD14

Plasma sCD14 was measured with a commercial enzyme-linked immunosorbent assay kit (R&D Systems, Inc., Minneapolis, Minnesota) according to the manufacturer's instructions. The range of measures was 250.0 - 16,000 pg·mL⁻¹ with a CV of 5.9%.

Chapter 4 - Prevalence, Severity and Potential Nutritional Causes of Gastrointestinal Symptoms during a Marathon in Recreational Runners

This study was published in *Nutrients* in 2017 (Appendix 2)

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Prevalence and severity of GI symptoms in recreational marathon runners

4.1 Abstract

Purpose: To investigate the prevalence of gastrointestinal (GI) symptoms amongst recreational runners during a marathon race, and any potential nutritional factors that may contribute.

Methods: Runners (n=96) of the 2017 Liverpool and Dublin marathon were recruited. GI symptoms were retrospectively assessed in relation to the 7 days prior to the marathon and during the marathon using the Gastrointestinal Symptom Rating Scale (Svedlund et al, 1988b), while nutritional intake was recorded using food diaries for the day before the race, morning of the race, and during the race.

Results: 43% of participants reported moderate (≥ 4) GIS in the 7 days prior to the marathon and 27% reported moderate symptoms during the marathon with most common symptoms being flatulence (16%) during training, and nausea (8%) during the marathon race. Correlations between nutritional intake (total Kcal, carbohydrate, fibre, fat, protein, fluid intake) and GIS were not statistically significant ($p > 0.05$). There were significant correlations between total GIS score ($r = 0.510, p < 0.001$), upper GIS score ($r = 0.346, p = 0.001$) and lower GIS score ($r = 0.483, p < 0.001$) in training and during the marathon.

Conclusion: There appears to be a modest prevalence of GIS in recreational runners, in the week prior to a marathon and during marathon running, although there was no association with nutritional intake before or during the race.

4.2 Introduction

Gastrointestinal (GI) symptoms are widely reported in athletes participating in prolonged endurance events including; cyclists, triathletes and marathon runners. However, there is a large estimated range of symptom prevalence reported between studies. In a recent review of endurance events the reported prevalence of GI symptoms was 4–96% of participants (Costa et al., 2017a). Numerous potential factors may explain the large variance in reported symptoms such as the mode, duration or intensity of exercise, environmental conditions, nutritional intake, type of assessment tool, and method used to classify a “symptom”. For example, studies have used 4, 9, 10, and 11-point scales, each with differing vernacular, to quantify GIS (Pfeiffer et al., 2012, Ter Steege et al., 2008, Stuempfle and Hoffman, 2015, Wilson, 2017). Positive responses, of any magnitude, including those that do not affect performance, could be seen to overestimate the prevalence of symptoms, or may lead to erroneous conclusions regarding symptom severity. For example, studies that have reported data for GI symptoms in marathon runners without acknowledging severity have shown prevalence rates of 52% and 71% (Rehrer et al., 1989, Peters et al., 1999). Contrarily, when symptoms were described as ‘moderate’ or ‘serious’ in severity, prevalence has been reported as 4–7% (Pfeiffer et al., 2012, Ter Steege et al., 2008). Furthermore, symptom severity in the scales used is a subjective measure and not further quantified by, for example, duration or impact on performance.

GI symptoms can have a number of aetiologies, including underlying pathology such as inflammatory bowel disease, the physiological changes that occur with exercise such as the reduction of splanchnic blood flow, changes to the physiology of digestion and transit, and the gut–brain axis (Costa et al., 2017a). One potential cause of GI symptoms during marathon running is nutritional intake before and/or during the race. Carbohydrate (CHO) intake in both the days before and during endurance exercise has been shown to be beneficial to performance (Stellingwerff and Cox, 2014, Helge, 2017) yet there appears to be an association between carbohydrate intake during endurance exercise and GI symptoms (Costa et al., 2017b, de Oliveira and Burini, 2014, ten Haaf et al., 2014). The mechanisms through which this may occur include potential malabsorption leading to luminal distension, delayed gastric emptying and gas production (Major et al., 2017, Shin et al., 2013). It has been shown that reducing GI symptoms associated with CHO intake during exercise was associated with improvements in performance (Costa et al., 2017b). However, to date, there has been little research into habitual dietary intake of recreational marathon runners, and their association with GI symptoms during a race. The aim of the present study was to document the dietary intake and prevalence and severity of GI symptoms during a marathon and to investigate any association between them.

4.3 Methods

4.3.1 Marathon runners

Runners registered to participate in the 2017 Rock n Roll Liverpool and SSE Airtricity Dublin marathons were invited to participate in this study via email sent out by the race organisers. Informed consent to participate was provided through registration via an internet-based online data collection tool. A total of 216 runners across both races registered to participate, with 100 of these runners completing the online questionnaire. Of these, four participants did not provide sufficient detail to be included in the dataset. Characteristics of runners from each race are presented in Table 4.1. During the races, mean ambient temperatures were 16 °C and 12 °C and mean relative humidity was 80% and 82% for Liverpool and Dublin respectively. There was 0 mm of precipitation during both races. The race routes had total ascension (vertical distance and maximum elevations of 410 m and 72 m (Liverpool) and 120 m and 58 m (Dublin). Mean time to complete the marathon in each race was 260 (176–361) and 236 (183–278) min, for the Liverpool and Dublin marathon, respectively. Self-reported training data are shown in Table 4.1.

Table 4.1. Subject characteristics of recreational marathon runners (values are mean \pm standard deviation (SD)).

	Liverpool Marathon (<i>n</i> = 66)	Dublin Marathon (<i>n</i> = 30)
Age (years)	43.4 \pm 9.5	42.3 \pm 8.8
Body mass (kg)	70.4 \pm 12.5	66.1 \pm 11.7
Height (cm)	163.7 \pm 36.7	163.7 \pm 34.0
Number of previous marathons	7 \pm 10	15 \pm 29
Quickest marathon in last 2 years (min)	245.7 \pm 42.0	238.2 \pm 29.4
Highest weekly mileage	41.5 \pm 14.3	46.7 \pm 14.6
Longest single training run (miles)	20.4 \pm 4.8	20.4 \pm 3.5

4.3.2 Experimental design

One week before the marathon, participants were sent e-mail instructions in regard to the timing of subsequent communications, what information they would be asked to provide, and the importance of accuracy in all of their responses. Forty-eight hours before the race, a food diary template was sent to participants along with instructions on how to complete it, examples of completed food diaries, and images of different weights of common foods. Participants were required to prospectively record all food and fluid consumed in the 24 h before the race, as well as the morning of the race. For in-race nutrition, participants were informed that this information would be required, and to try to recall all

in-race foods and fluids consumed. The information from food diaries, as well as in-race nutrition, were input into relevant sections of the questionnaire. The dietary information reported was analysed to quantify using Nutritics professional diet analysis software (Nutritics LTD, Dublin, Ireland) by two blinded, independent, and trained nutritionists.

4.3.3 Questionnaire

In the evening following the race, participants received an email link to the online questionnaire, and were asked to complete this as soon as possible. Reminder emails were sent 24 and 48 h later. Participants were required to report their age, gender, weight, height and details regarding their race history and training for the marathon. In order to differentiate between habitual GI symptoms, and race specific GI symptoms, participants completed a modified version of the Gastrointestinal Symptom Rating Scale (GSRS) (Svedlund et al., 1988b), relating to the 7 days prior to the marathon, and then specifically symptoms during the marathon. The GSRS gives explanations of each symptom and was used as it has been shown to be understandable and has good reproducibility for measuring the presence of GI symptoms compared to interviews (Bovenschen et al., 2006). This questionnaire has been previously used in large scale investigations (Tielemans et al., 2013, van Kerkhoven et al., 2008). Symptoms include upper abdominal pain, epigastric pain, heartburn, regurgitation, abdominal rumbling, bloating, nausea, empty feeling in the stomach, early satiety, postprandial fullness, belching, flatulence, haematemesis, dysphagia, and questions on defecation. Subjects were asked to rate the severity of GI symptoms on a seven-point Likert scale (1 = absent; 2 = minor; 3 = mild; 4 = moderate; 5 = moderately severe; 6 = severe and 7 = very severe). In analysis, a score of ≥ 2 was defined as symptom presence and ≥ 4 was defined as moderate symptom presence. Lower GI symptoms were classified as gas/flatus, bloating, diarrhea, and urgent need to defecate and upper GI symptoms as nausea, heartburn, acid reflux, hunger, burping.

4.3.5 Analysis

Descriptive statistics (mean \pm SD) were calculated for all variables. Differences between symptomatic and asymptomatic runners were analysed using unpaired *t*-tests. As GI symptoms were not normally distributed, a non-parametric approach to analysing associations with nutritional intake was used. Spearman's rank correlation was used when GI symptoms were considered. $P < 0.05$ was considered statistically significant. Based on a Mann–Whitney U test, no differences in total GI symptoms (summed score from GSRS) were apparent between males and females ($Z = -1.14$, $p = 0.25$) nor between participants in both races ($Z = -0.068$, $p = 0.946$). Thus, correlations were carried out with all participants combined. A two-sided p -value ≤ 0.05 was used as the threshold for statistical significance.

4.4 Results

In the 24 hr prior to the race participants consumed 2222 ± 758 Kcal, 262 ± 98 g CHO, 93 ± 40 g protein, 85 ± 47 g fat, and 2496 ± 1271 mL water. Nutritional intake of participants for each individual race 24 hr before the marathon are shown in Table 4.2. On the morning of the race, participants consumed 515 ± 301 Kcal, 70 ± 44 g CHO, 17 ± 11 g protein, 6 ± 4 g fat, and 685 ± 506 mL water. There were no significant differences between variables of nutritional intakes between each race either 24 hr before the race or on the morning of the race ($p > 0.05$).

Table 4.2. Nutritional intake on the day before the race and during the race day breakfast

	Day before race		Race day breakfast	
	Liverpool	Dublin	Liverpool	Dublin
Total Energy Intake (Kcal)	2329 ± 775	1987 ± 692	552 ± 316	436 ± 251
CHO (g)	262 ± 93	262 ± 120	76 ± 45	59 ± 41
CHO ($\text{g}\cdot\text{kg}^{-1}$)	3.8 ± 1.4	3.4 ± 2.5	1.1 ± 0.9	0.8 ± 0.8
Sugars (g)	89 ± 57	100 ± 74	39 ± 29	27 ± 25
Starch (g)	168 ± 66	148 ± 63	36 ± 30	34 ± 21
Fibre (g)	22 ± 9	21 ± 8	6 ± 4	6 ± 5
Protein (g)	98 ± 39	85 ± 41	19 ± 12	13 ± 8
Protein ($\text{g}\cdot\text{kg}^{-1}$)	1.4 ± 0.8	1.1 ± 0.8	0.3 ± 0.2	0.2 ± 0.2
Fat (g)	95 ± 52	66 ± 36	17 ± 18	18 ± 36
Saturated fat (g)	34 ± 17	22 ± 14	7 ± 8	4 ± 4
Sodium (mg)	2478 ± 1434	1937 ± 1094	436 ± 402	304 ± 281
Water (mL)	2672 ± 1369	2110 ± 1057	795 ± 598	443 ± 306

During the race, participants consumed 470 ± 282 Kcal, 108 ± 61 g CHO (0.4 ± 0.2 $\text{g}\cdot\text{min}^{-1}$), 2.9 ± 6.9 g protein, 1.9 ± 4.2 g fat, and 1314 ± 745 mL water. Nutritional intake of participants for each individual race are shown in Table 4.3. There were no significant differences between variables of nutritional intakes between each race ($p > 0.05$).

Table 4.3. Nutritional intake during the marathon race

	During the race	
	Liverpool	Dublin
Total Energy Intake (Kcal)	439 ± 277	540 ± 312
CHO (g)	100.9 ± 60.1	126.3 ± 64.1
CHO ($\text{g}\cdot\text{min}^{-1}$)	0.4 ± 0.2	0.5 ± 0.3
Sugars (g)	37.2 ± 31.8	36.6 ± 34.2
Starch (g)	64.2 ± 45.9	72.4 ± 55.8
Fibre (g)	1.0 ± 2.8	2.3 ± 4.6
Protein (g)	2.4 ± 6.5	4.3 ± 7.9
Fat (g)	1.4 ± 3.6	3.3 ± 5.8
Saturated fat (g)	0.4 ± 1.1	0.8 ± 1.5
Sodium (mg)	259 ± 268	286 ± 323
Water (mL)	1390 ± 765	1149 ± 693

From both races, 41% and 47% of participants reported at least one moderate symptom during the previous 7 days, while 30% and 20% reported experiencing moderate symptoms during the race for Liverpool and Dublin marathon respectively. Prevalence of individual GI symptoms reported for each race are shown in Table 4.4. To identify potential associative factors, GI symptom scores were summed to give lower, upper and total scores. Correlations between GI symptoms during the race and all nutritional factors were low and insignificant ($r < 0.20$, $p > 0.05$). There were significant correlations between symptoms in the 7 days prior to the race and during the race for total GI score ($r = 0.510$, $p < 0.001$), upper GI score ($r = 0.346$, $p = 0.001$) and lower GI symptom score ($r = 0.483$, $p < 0.001$).

Table 4.4. Prevalence of individual symptom scores (≥ 2) and moderate (≥ 4) symptoms. Data are percentage of participants in each race.

	Liverpool Marathon ($n = 66$)		Dublin Marathon ($n = 30$)	
	≥ 2	≥ 4	≥ 2	≥ 4
Nausea	32	17	18	2
Heartburn	8	5	2	2
Acid Reflux	9	2	3	0
Hunger	18	3	12	5
Burping	30	8	11	2
Bloated	24	11	5	0
Gas/Flatus	30	9	14	2
Diarrhea	15	8	5	2
Urgent need to defecate	20	9	5	2

4.5 Discussion

The current study assessed the incidence and severity of numerous GI symptoms, using a previously validated questionnaire to document the dietary intake and GIS during training in the week before a marathon and during a marathon in order to explore potential predictive factors of GI symptoms. The data here indicates that there is a significant prevalence of moderate GI symptoms in the week leading to a marathon race, and during the race amongst recreational runners. It is shown that 42% of participants reported moderate GI symptoms in the 7 days prior to the marathon and 27% reported moderate symptoms during the marathon with most common symptoms being flatus (16%) during training, and nausea (8%) during the marathon race. However, it was found that there was no association between nutritional intake and symptoms, neither in the 24 h prior to, during the meal before, nor during the race.

Gastrointestinal symptoms during endurance competitions have been previously reported by 4–96% of participants (Costa et al., 2017a). Differences between studies could be due to a number of factors such as exercise intensity or duration, and environmental temperatures, which have been shown to

exacerbate GI damage and increase symptoms (Pfeiffer et al., 2012, Pals et al., 1997, Peters et al., 1999, Costa et al., 2017a). Variances may also arise from the questionnaires used, the symptoms that are included, and the criteria for classifying a symptom. Studies that have reported data for GI symptoms in marathon runners, without acknowledging severity, have shown prevalence rates of 52% and 71% (Rehrer et al., 1989, Peters et al., 1999), while 'moderate' or 'serious' GIS prevalence has been reported as 4–7% (Pfeiffer et al., 2012, Ter Steege et al., 2008). In the present study, 70% of participants reported having any symptoms, while only 27% had symptoms recorded as moderate or worse, with nausea being the most common (12% of all runners). This highlights the need to differentiate symptom severity within studies, as well as the specific symptom, as these may have different aetiologies, and therefore require different interventions for attenuation. Future studies should ensure pathology is excluded (bloods and faecal calprotectin, endoscopy etc.) and psychological factors, along with validating symptoms against Rome III or IV diagnostic criteria for irritable bowel syndrome.

Gastrointestinal symptoms have been shown to relate to higher CHO intake, higher fat intake, and, in particular, lower fluid consumption during ultra-distance events of longer duration (Stuempfle et al., 2013, Pfeiffer et al., 2012). However, this has not been as clear in marathon running or events of shorter durations (Pfeiffer et al., 2012, van Nieuwenhoven et al., 2005). Here, there was no correlation between total, upper or lower GIS scores and any nutritional factor recorded. This includes dietary intake in the 24 h prior to the race, breakfast on race-day, or in-race nutrition. The difference may be due to the duration of the event. For example, it has been shown that the majority of symptoms during ultra-distance running events did not occur until after 50 km of running (Stuempfle and Hoffman, 2015, Stuempfle et al., 2013). Longer duration events, and therefore greater total CHO intake, increase the likelihood of CHO malabsorption (Peters et al., 1995, Costa et al., 2017b). However, with the recorded CHO intakes during the marathon (mean of $0.4 \text{ g}\cdot\text{min}^{-1}$), this was unlikely to be seen here. While not the primary aim of the present study, it should be noted that the mean values for CHO intake both before and during the race were below those recommended for marathon performance (Jeukendrup, 2011). This nutritional intake data is in close agreement with previous studies in marathon runners (Atkinson et al., 2011, Wilson et al., 2013). Recreational runners' performance could therefore be improved with appropriate CHO intake. As no association was observed here between nutritional intake and GIS, some other factors, not assessed here may contribute to the prevalence of GI symptoms found.

Gastrointestinal symptoms are often more prevalent during marathon running and other endurance events in individuals with a history of symptoms (Peters et al., 1999, Pfeiffer et al., 2012, Stuempfle et al., 2013). The results here showed significant, moderate ($r > 0.3$) correlation between symptom

scores during the 7 days before the race and during the race. This corroborates previous study findings and may be due to a number of factors. These individuals may consistently be those becoming dehydrated, they may have some underlying pathology, or they may experience greater levels of stress and/or anxiety which has been shown to increase gastrointestinal symptomology (Wilson, 2018), although this was not assessed in the present study.

4.6 Conclusion

The current study has identified a high prevalence of GI symptoms in recreational runners, both in training in the week prior to a marathon and during marathon running. The most common symptoms were flatus and nausea during training and marathon running, respectively. In this population of runners, there was no clear association between any nutritional factors and symptoms, although CHO intake was generally low. However, there was a significant correlation between symptoms during training, and symptoms during the marathon. The current study highlights the need to further quantify not only the prevalence, but also the potential aetiology of GI symptoms to develop interventions that may attenuate symptoms. This may then lead to increased athletic performance, quality of life, and health.

Chapter 5 - Acute high-intensity interval running increases markers of gastrointestinal damage and permeability but not gastrointestinal symptoms

This study was published in *Applied Physiology, Nutrition and Metabolism* in 2017 (Appendix 3)
Pugh, J.N., Impey, S.G., Doran, D.A., Fleming, S.C., Morton, J.P. and Close, G.L., 2017. Acute high-intensity interval running increases markers of gastrointestinal damage and permeability but not gastrointestinal symptoms. *Applied Physiology, Nutrition, and Metabolism*. 42(9):941-947.

Acute high-intensity interval running increases markers of gastrointestinal damage and permeability but not gastrointestinal symptoms

5.1 Abstract

Purpose: To investigate the effects of high-intensity interval (HIIT) running on markers of gastrointestinal (GI) damage and permeability alongside subjective symptoms of GI discomfort.

Methods: Eleven male runners completed an acute bout of HIIT (eighteen 400 m runs at 120% $\dot{V}O_{2max}$) where markers of GI permeability, intestinal damage and GI discomfort symptoms were assessed and compared with resting conditions.

Results: Compared to rest, HIIT significantly increased serum lactulose:ramnose ratio (0.051 ± 0.016 vs. 0.031 ± 0.021 , $p = 0.005$) and sucrose concentrations (0.388 ± 0.217 vs 0.137 ± 0.148 $mg \cdot l^{-1}$; $p < 0.001$). In contrast, urinary lactulose:ramnose (0.032 ± 0.005 vs 0.030 ± 0.005 ; $p = 0.3$) or sucrose concentrations (0.169 ± 0.168 vs 0.123 ± 0.120 $mg \cdot l^{-1}$; $p = 0.54$) did not differ between HIIT and resting conditions. Plasma I-FABP was significantly increased ($p < 0.001$) during and in the recovery period from HIIT whereas no changes were observed during rest. Mild-symptoms of GI discomfort, were reported immediately- and 24 h post-HIIT, although these symptoms did not correlate to GI permeability or I-FABP.

Conclusion: Acute HIIT increased GI permeability and intestinal I-FABP release, although these do not correlate with symptoms of GI discomfort. Furthermore, by using serum sampling, data is provided showing that it is possible to detect changes in intestinal permeability that is not observed using urinary sampling over a shorter time-period.

5.2 Introduction

Endurance training in both elite and non-elite athletes typically involves exercise that can vary in frequency, duration and intensity (Seiler, 2010). Descriptive studies of elite runners have shown that while 80% of training sessions appear to be steady state and performed at low intensities (< 2 mM blood lactate), around 20% of training consists of periods of high-intensity exercise (> 4 mM blood lactate), that is usually completed as repetitions at approximately 90% $\dot{V}O_{2max}$ (Seiler 2010). High-intensity interval training (HIIT) is central to athlete training, based on the premise that it improves middle distance running performance, $\dot{V}O_{2max}$ and running economy (Billat, 2001, Daussin et al., 2008), induces fat loss (Trapp et al., 2008) and is perceived to be more enjoyable than moderate intensity steady state exercise (Bartlett et al., 2011). However, despite the growing realisation and understanding as to the physiological benefits of HIIT (Gillen and Gibala, 2013), the effects of acute HIIT protocols on markers of GI damage and permeability, and symptoms of GI discomfort are not well characterised. In Chapter 4 it was shown that 42% of participants reported moderate GI symptoms in the 7 days prior to the marathon. Previous studies have equally shown that GI symptoms are common for athletes during training, as well as competition (de Oliveira et al., 2014). Furthermore, given that GI symptoms are reported to be more common during “hard” runs compared to “easy” type training sessions (Keeffe et al., 1984, Riddoch and Trinick, 1988), there is a definitive need to better understand the acute effect of HIIT on GI profile.

Traditional assessment of GI permeability has relied upon the ingestion of a bolus saccharide probe followed by a 5 h urinary collection period whilst the subject remains fasted, which may be impractical with athletic populations. Determination of the ratio of saccharide concentrations in serum correlates with the 5 h urine ratio and is an acceptable alternative to urine and can reduce the sample collection time to 90-120 minutes (Fleming et al., 1996a). Determination of saccharide concentrations in plasma is able to show increases in GI permeability following exercise (van Wijck et al., 2013b, JanssenDuijghuijsen et al., 2016). Plasma analysis revealed 60 minutes cycling at 70% maximal capacity increased intestinal permeability, measured at 140 minutes, while urine samples at 2 h showed a non-significant increase in permeability (Van Wijck et al., 2011).

More recently, the use of I-FABP, a small (15 kDa) cytosolic protein specifically present in mature enterocytes of the small intestine, has also emerged as an early and sensitive marker of small intestinal injury (Pelsers et al. 2003; Derikx et al. 2008) and increases in I-FABP correlate with exercise-induced splanchnic hypoperfusion (van Wijck et al. 2011). Whilst multiple models of exercise increase plasma I-FABP concentrations (van Wijck et al. 2012; Barberio et al. 2015), these increases have not been conclusively shown to correlate to the onset or severity of GI symptoms (van Wijck et al. 2011).

The present study was therefore conducted to characterise the acute effects of a HIIT running session on markers of small intestinal damage, intestinal permeability and whether these were associated with symptoms of GI discomfort. When compared with resting conditions, it was hypothesised that acute HIIT (using a model considered relevant for elite runners) would significantly increase markers of intestinal damage (I-FABP), permeability (primarily LR ratio) and symptoms of GI discomfort. It was also hypothesised that measurements of LR in serum would provide a more time sensitive method of analysis compared to urine.

5.3 Methods

5.3.1 Participants

Eleven trained male runners (mean \pm SD $\dot{V}O_{2\max}$ 60.0 ± 3.2 mL \cdot kg $^{-1}\cdot$ min $^{-1}$, body mass 75.1 ± 5.8 kg, height 179.1 ± 8.9 cm, age 33.1 ± 10.4 years) completed the study. The criteria used for selection was a minimum 10km race performance of 39 min, and a minimum of 5 training sessions a week. Exclusion criteria are outlined in Section 3.2.

5.3.2 Assessment of maximal oxygen uptake

$\dot{V}O_{2\max}$ was determined as described in 3.3.1. Based on the results of the incremental test, the running speed corresponding to 100% $\dot{V}O_{2\max}$ was estimated for each participant using a linear regression equation. The running speed corresponding to 120% was then calculated.

5.3.3 Experimental design and HIIT protocol

In a repeated measures counter-balanced design, participants reported to the laboratory on 2 occasions, separated by a minimum of 7 days, to complete the HIIT and rest trials. Prior to the first visit, participants completed a 24 h food diary and repeated this diet prior to the second visit. For a given participant, each trial was conducted at the same time of day, beginning between 07:00 h and 10:00 h. No alcohol consumption, non-steroidal anti-inflammatory drug (NSAID) consumption, fibre-rich or spicy food consumption, unaccustomed or strenuous exercise was permitted 24 h prior to experimental visits. On the morning of each trial, participants were informed to eat a small breakfast, typical of that consumed prior to training or competition and that this breakfast should remain the same for each visit. Participants arrived at the laboratory on the morning of the trial. The HIIT exercise protocol consisted first of a 5 minute rest period, then a 5 minute warm-up run at a velocity corresponding to 50% $\dot{V}O_{2\max}$ and finally 5 minutes of active stretching. Participants then performed a total of 18 x 400 m interval efforts, performed on a motorized treadmill (HP Cosmos, Germany). The running pace for the interval runs was based on individual's pre-assessed $\dot{V}O_{2\max}$, corresponding to 120% of their $\dot{V}O_{2\max}$. Each repetition was followed by an interval of running at a velocity associated with 50% $\dot{V}O_{2\max}$ for an amount of time equal to 75% of that taken to run the 400 m. These were

divided into 3 sets of 6 × 400 m runs separated by 3 minutes of complete rest. See Figure 5.1 for a schematic representation of the study overview. Participants were permitted to consume water ad libitum during and after each trial; drinking patterns were not recorded although participants were encouraged to consume fluid during the trials in order to prevent dehydration. Total volume consumed was less than 250 mL for all participants.

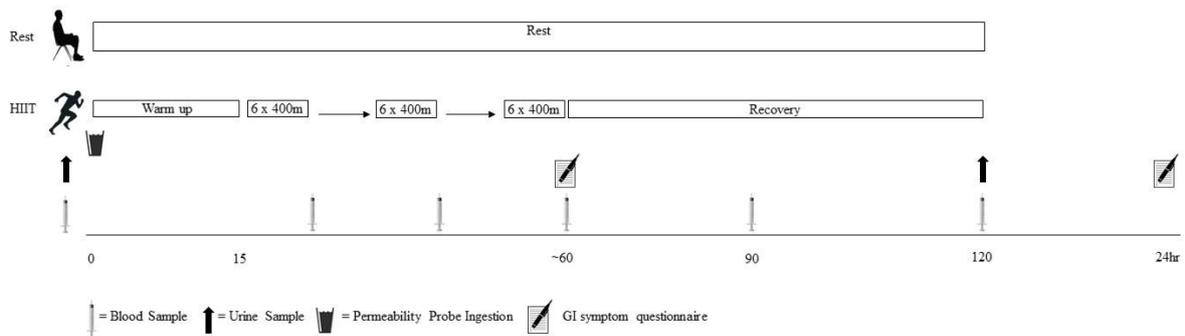


Figure 5.1 Schematic overview of the experimental protocol. Urine and blood samples were taken at the same corresponding time points during rest as they were during HIIT

5.3.4 Blood analysis

Blood samples were collected and analysed for LR and I-FABP as described in Section 3.5.

5.3.5 Assessment of GI discomfort

Global gastrointestinal symptoms were recorded after each set of six 400m runs as described in section 3.4.2. Immediately post-exercise, and 24 hr post, specific GI symptoms were recorded as described in section 3.4.3.

5.3.6 Statistical analyses

Statistical analysis was conducted using the Statistical Package for the Social Sciences software programme (SPSS, version 23, IBM, Armonk, NY). A two-factor repeated measure ANOVA was used to analyse plasma I-FABP with condition (rest, exercise) and various time points (pre, set 1, set 2, post, 90 min, 2 h) as the independent variables. Pairwise t-tests with a Bonferroni corrections were used to compare time points between conditions. Intestinal permeability was assessed using paired t-tests. To evaluate data on GI symptoms, a nonparametric statistical approach was chosen, as scores on GI symptoms were mainly reported on the low end of the scale and not normally distributed. Symptom scores and prevalence were compared with the use of Wilcoxon Signed Rank tests. Spearman-rank order correlation was used to analyse the relationship between significantly increased GI symptoms, with post exercise I-FABP and intestinal permeability measured in serum. All normally distributed data

are presented as mean \pm standard deviation (SD), data not normally distributed are reported as median and range. $P < 0.05$ was considered statistically significant.

5.4 Results

5.4.1 Physiological responses to acute HIIT protocol

Participants ran a total of 7.2 km at a velocity of $17.7 \pm 1.0 \text{ km}\cdot\text{h}^{-1}$ (mean \pm SD), while recovery running totalled $2.2 \pm 0.1 \text{ km}$ at a velocity of $8.9 \pm 0.5 \text{ km}\cdot\text{h}^{-1}$. HR, RPE and thermal comfort increased incrementally throughout interval bouts with peak values of $187 \pm 10 \text{ bpm}$, 19 ± 1 , and 8.5 ± 0.7 , respectively.

5.4.2 I-FABP as a biomarker of intestinal damage

A significant interaction effect between condition and time was found ($p = 0.002$). I-FABP increased 72% during HIIT from baseline concentration of $481 \pm 334 \text{ pg}\cdot\text{mL}^{-1}$ to mean peak levels of $829 \pm 448 \text{ pg}\cdot\text{mL}^{-1}$ immediately post exercise and then decreased during subsequent recovery, whilst there was no change during rest (Fig 5.2). There was no significant difference in baseline I-FABP concentrations between HIIT ($481 \pm 334 \text{ pg}\cdot\text{mL}^{-1}$) and rest condition ($263 \pm 149 \text{ pg}\cdot\text{mL}^{-1}$) ($p = 0.225$).

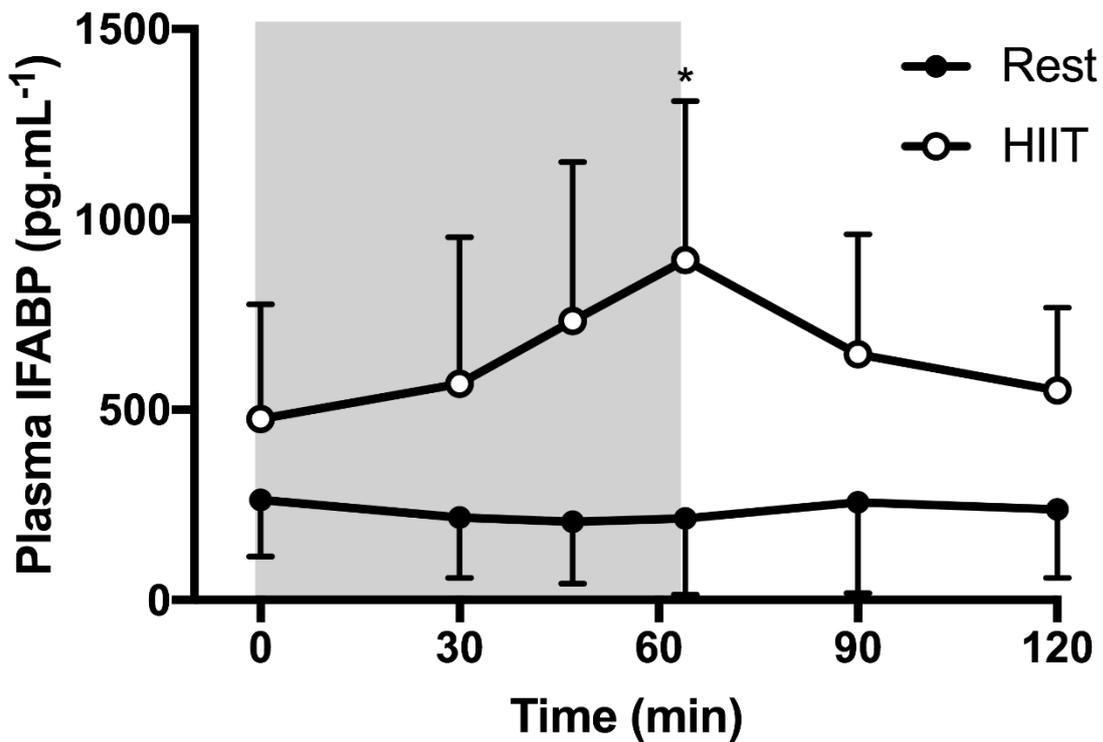


Figure 5.2. Mean (\pm SD) plasma I-FABP concentrations during rest and HIIT condition. * Significant difference to baseline ($p < 0.05$)

5.4.3 Gastrointestinal permeability

LR in serum was 59% higher following HIIT when compared to the resting condition (0.051 ± 0.016 vs. 0.031 ± 0.021 , $p = 0.005$) (Fig 5.3A). However, there was no significant difference in urinary LR between rest and HIIT trial ($p = 0.37$) (Fig 5.3B). There was a weak, negative correlation between serum and urinary LR ($r = -0.179$, $p = 0.21$), Serum sucrose recovery was also significantly higher during the HIIT trial when compared to the resting condition (0.388 ± 0.217 vs. 0.137 ± 0.148 , $p < 0.001$). There was no significant difference in urinary sucrose recovery following HIIT (0.169 ± 0.168) compared to rest (0.123 ± 0.120 , $p > 0.05$).

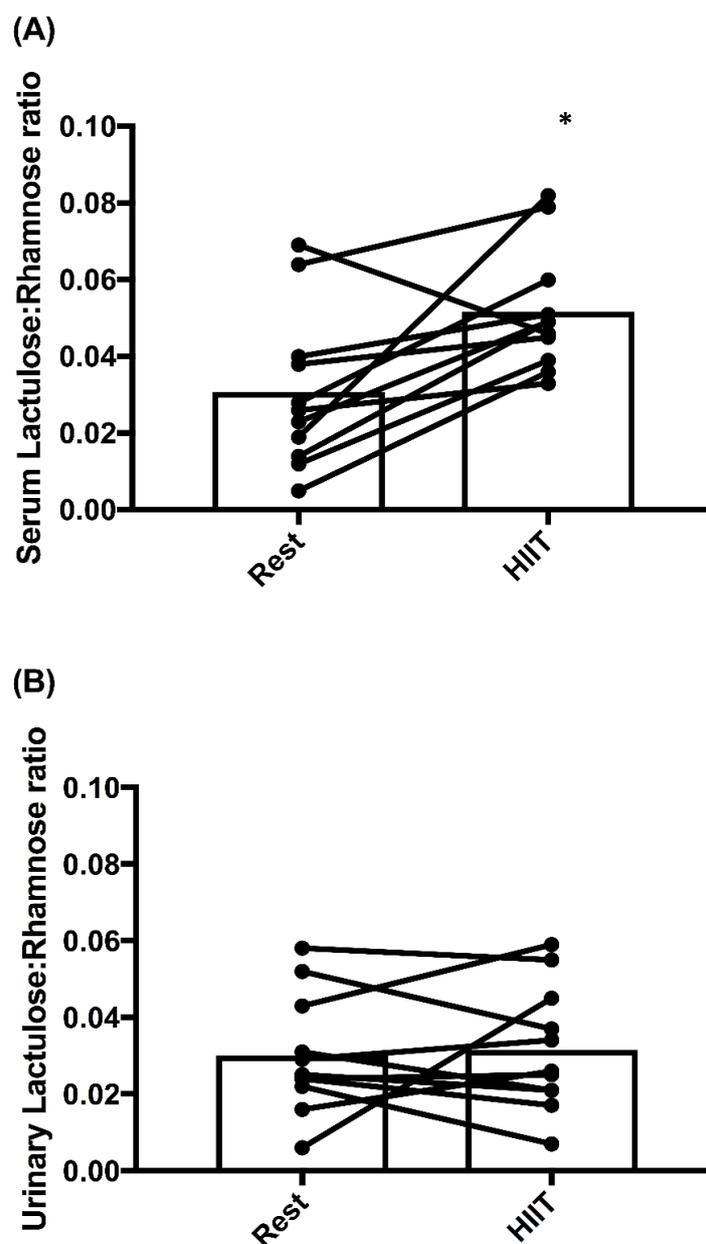


Figure 5.3. Mean serum post (A) and 2hr (B), and urinary (C) lactulose to rhamnose ratios during rest and HIIT conditions. *Significant difference $p < 0.001$

5.4.4 Gastrointestinal discomfort

During experimental visits, peak global GI symptoms were higher during HIIT compared with rest (3.0 ± 2.5 vs. 0.3 ± 0.5 , $p < 0.01$). The results of the specific GI symptoms are displayed in Table 5.1. During both trials, GI symptoms were mostly scored at the low end of the scale (“no problems at all”, “very minor problems”). For symptoms during activity, severity scores for “Bloating”, “Urge to burp” and “Flatulence” were higher, and 24h “Bloating” and “Stomach cramps” were higher in HIIT compared to the rest condition ($p < 0.05$). From these symptoms which were significantly increased, there was no significant correlation with any of these to either post-exercise I-FABP or serum LR (Table 5.2).

Table 5.1. GI symptoms during rest and HIIT conditions assessed at 2 and 24 hours from baseline.

Symptom	Exercise			24 hours		
	Rest	HIIT	Wilcoxon p value	Rest	HIIT	Wilcoxon p value
Side stitch	0 (0)	0 (0-1)	0.317	0 (0)	0 (0)	1.000
Nausea	0 (0-1)	0 (0-5)	0.157	0 (0)	0 (0-1)	0.157
Bloating	0 (0-1)	1 (0-6)	0.027*	1 (0-2)	3 (0-8)	0.034*
Urge to burp	0 (0-2)	1 (0-6)	0.027*	0 (0-3)	0 (0-2)	0.915
Urge to vomit	0 (0)	0 (0-4)	0.109	0 (0)	0 (0-2)	0.317
Urge to defecate	0 (0-1)	0 (0-8)	0.062	2 (0-5)	3 (0-6)	0.150
Diarrhea	0 (0-3)	0 (0-3)	0.0785	0 (0-3)	0 (0-3)	0.785
Flatulence	1 (0-2)	3 (0-6)	0.037*	0 (0-4)	2 (0-5)	0.018*
Stomach Cramps	0 (0-5)	0 (0-6)	0.833	0 (0)	0.9 (0-8)	0.042*

Data are median and range. * Significantly difference between HIIT and rest ($p < 0.05$)

Table 5.2. Correlations between GI symptoms with I-FABP and LR

Symptom	I-FABP		L:R	
	Correlation	p value	Correlation	p value
<i>Exercise</i>				
Bloating	0.0114	0.739	0.084	0.826
Urge to burp	0.071	0.835	-0.324	0.392
Flatulence	0.206	0.544	-0.068	0.861
<i>24 hours</i>				
Bloating	-0.066	0.848	-0.172	0.484
Flatulence	0.343	0.302	-0.358	0.384
Stomach Cramps	0.088	0.891	-0.103	0.564

5.4 Discussion

The purpose of this study was to investigate the effect of a HIIT running session on markers of small intestinal damage, intestinal permeability and subjective symptoms of GI discomfort. The main findings were that HIIT increased markers of intestinal permeability and small intestinal injury but they were not strongly related to increases in subjective measures of GI discomfort.

During exercise, there is a redistribution of blood away from the splanchnic area. The magnitude of such reductions in splanchnic blood flow appears to be related to relative exercise intensity (McAllister, 1998). It has been shown previously that the extent of this exercise-induced splanchnic hypoperfusion significantly correlates with intestinal damage, measured as plasma I-FABP concentration (Van Wijck et al., 2011). I-FABP is a cytosolic water-soluble protein, which appears to be present in mature enterocytes of the small and large intestine (Pelsers et al., 2003a). As such, plasma I-FABP is considered a sensitive measurement of small intestinal damage (Pelsers et al., 2003a, Derikx et al., 2008). Here, I-FABP is increased during HIIT running session, following a similar temporal sequence to that reported by others. Peak values exceed those previously seen following 60 minutes of cycling at 70% wattmax (van Wijck et al., 2012a, Van Wijck et al., 2011), 60 minutes of running at 70% $\dot{V}O_{2max}$ in the heat (Sessions et al., 2016) but are lower than I-FABP concentrations reported following exhaustive running in the heat (Barberio et al., 2015).

Traditionally, 5 h urine collection and percentage recovery of sugar probes has been used to assess intestinal permeability. This method is sensitive to changes in permeability caused by exercise of varying modalities, durations and intensities (Pals et al., 1997, Lambert et al., 2008, van Nieuwenhoven et al., 1999, Marchbank et al., 2011). However, the main disadvantage of this method is the need to collect all urinary output for 5 hours, with participants normally remaining fasted. It is shown here that by measuring sugar probes in serum, it is possible to show an exercise-induced increase in LR and sucrose concentration 1 and 2 hours after probe ingestion. However, analysis of urine collected over 2 hours did not show any differences. It is suggested that the use of plasma and serum samples increases the sensitivity, thus reducing the need for large oral doses (van Wijck et al., 2013b), and reduces the collection time (Fleming et al., 1996a). Due to differences in the dosages of the probes used, time delivered in relation to exercise, and analytical methods, it is difficult to compare LR values between studies. Here though, it has been shown that HIIT increases LR, as detected from serum measurements.

Although HIIT increases I-FABP and LR, these did not correlate with symptoms either during or in the proceeding 24 hours after exercise. The low scores for GI discomfort may be due to the fact that the cohort were healthy, well trained males who were also accustomed to the exercise modality, well

hydrated and had with no history of GI disease. This is in line with much of the recent laboratory-based research into single exercise sessions and markers of GI damage. Many of these have reported measures of increased intestinal damage but reported either low or mild scores of GI discomfort during acute exercise bouts (Van Wijck et al., 2011, Lambert et al., 2008) or have not reported subjective GI symptoms at all (Zuhl et al., 2014, Marchbank et al., 2011). Yet, as described in Section 2.1.1, there is a significant prevalence of GI symptoms reported in competitive endurance events. This apparent discrepancy between symptom frequency between field and laboratory studies may be due to a number of factors. 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. Competitive events could also cause increases in mental stress not seen in laboratory studies which could exacerbate GI symptoms due to further decreases in splanchnic blood flow (Murray, 2006), direct changes to intestinal bacterial composition (Palma et al., 2014) or effects on GI transit via the central nervous system (Brouns and Beckers, 1993). The difference may also lie in the measurement and reporting of symptoms, with some studies documenting any symptom, of any severity, others reporting 'moderate' symptoms, and studies using different assessment scales or questionnaires. Here, symptoms varied between participants and so reporting individual symptoms gave low average and mean scores.

5.6 Conclusion

In summary, it has been shown that high intensity interval running increases gastrointestinal permeability and intestinal cellular damage, without subsequently causing symptoms of GI discomfort. Furthermore, by using serum sampling, novel data is provided that shows it is possible to detect changes in intestinal permeability over a shorter time-period. Such changes could not be detected via urinary sampling. Serum sampling therefore appears to offer a more time sensitive method for any future investigations.

Chapter 6 - Probiotic
supplementation increases
total and exogenous CHO
oxidation in trained male
cyclists: a randomized, double-
blind, placebo-controlled
cross-over trial

Probiotic supplementation improves CHO oxidation in trained male cyclists: a randomized, double-blind, placebo-controlled cross-over trial

6.1 Abstract

Purpose - In vitro and animal studies suggest probiotic supplementation can enhance glucose oxidation. This study aimed to investigate the effects of multi-strain probiotics supplementation on substrate utilization, markers of gastrointestinal damage, permeability, subjective symptoms of discomfort and performance during endurance cycling.

Methods - Nine male cyclists (age 23 ± 4 yrs, $\dot{V}O_{2\max}$ 62.1 ± 4.7 mL·kg⁻¹·min⁻¹) were randomized to two periods of daily supplementation with a probiotics (PRO) capsule (25 billion CFU of *Lactobacillus acidophilus* (CUL60 and CUL21), *Bifidobacterium bifidum* (CUL20), *Bifidobacterium animalis* subsp. *lactis* (CUL34), Proven Probiotics) or placebo (PLC) for four weeks, separated by a 14-day washout period (double-blind cross-over trial). After each supplementation period, cyclists consumed a 10% maltodextrin solution (initial 8 mL·kg⁻¹ bolus at commencement of exercise and 2 mL·kg⁻¹ every subsequent 15 min) while exercising for 120 minutes at 55% W_{\max} followed immediately by a 100 kJ time trial performance test. Markers of GI permeability, damage and GI discomfort were assessed as well as substrate utilization via venous blood and breath samples.

Results - Probiotic supplementation resulted in an increase of total carbohydrate (CHO) oxidation (2.12 ± 0.30 vs 1.81 ± 0.44 g·min⁻¹, $P = 0.019$) and the oxidation of an ingested maltodextrin drink (0.79 ± 0.10 vs 0.75 ± 0.11 g·min⁻¹ of glucose equivalents, $P = 0.024$) during the final hour of exercise. Total fat oxidation was reduced following probiotic supplementation compared to placebo ($P = 0.004$). There were also significant increases in plasma insulin and reductions in NEFA and glycerol in PRO compared to PLC. Differences between markers of GI damage and permeability were not significant, as was time trial performance ($P > 0.05$).

Conclusion - Probiotic supplementation enhances total and ingested CHO oxidation while simultaneously attenuating total fat oxidation during moderate intensity cycling. Probiotic supplementation did not change GI symptoms, time trial performance and markers of intestinal damage and permeability.

6.2 Introduction

While there was a lack of association between consumed CHO and GI symptoms in marathon runners in Chapter 4, total CHO consumed was lower ($\sim 25 \text{ g}\cdot\text{hr}^{-1}$) than amounts typically recommended for endurance competition ($\geq 60 \text{ g}\cdot\text{hr}^{-1}$). These greater amounts of CHO consumed have been linked to both increases in exercise performance and increased risk of GI symptoms. Adequate CHO availability as the main fuel for skeletal muscle and the central nervous system during endurance exercise lasting 1-2 h is a critical component for optimal performance. Liver and muscle glycogen stores are limited and oral ingestion of CHO before and during exercise has been reported to improve performance (Currell and Jeukendrup, 2008) and delay fatigue during cycling and running (Coyle et al., 1983). This performance benefit has since been reported in numerous publications, with exogenous carbohydrate ingestion showing ergogenic effects for endurance performance in most of these studies (Stellingwerff and Cox, 2014, Pochmuller et al., 2016). However, oxidation rates of orally ingested glucose and maltodextrin (glucose polymer) solutions appear to plateau around $1 \text{ g}\cdot\text{min}^{-1}$ (or $60 \text{ g}\cdot\text{h}^{-1}$) (Wagenmakers et al., 1993b), even with ingestion rates as high as $2.6 \text{ g}\cdot\text{min}^{-1}$ (Jeukendrup et al., 1999b). The capacity of the sodium-glucose transporter (SGLT1) in the small intestine is generally regarded as the limiting factor for glucose absorption and the oxidation rate of glucose and maltodextrin ingested during endurance exercise (Jeukendrup, 2014). While there appears to be a maximal rate of exogenous glucose oxidation of $1 \text{ g}\cdot\text{min}^{-1}$, some studies have failed to reach this level, even when glucose is consumed in excess, suggesting some individual variability (Jeukendrup and Jentjens, 2000). Environmental factors can also reduce the maximal oxidation of consumed carbohydrates, with reductions seen at both an increased temperature (Jentjens et al., 2002) and altitude (O'Hara et al., 2017), most likely due to reductions in splanchnic blood flow and compromised intestinal absorption (Rowell et al., 1968). Such malabsorption of CHO could then increase the risk of GI symptoms during endurance exercise (Costa et al., 2017b). Strategies that may then increase the maximal oxidation rate of consumed glucose, either above the previously established $1 \text{ g}\cdot\text{min}^{-1}$, above an individual's own maximal rate, or when environmental factors compromise oxidation, would be of benefit to endurance athletes.

One novel approach that may increase carbohydrate absorption and CHO oxidation is probiotic supplementation. *In vitro* research has shown that co-incubation of Caco-2 cells (enterocyte model) with metabolites from probiotic strains from the *Lactobacilli* species increases glucose uptake through non-genomic means (Rooj et al., 2010a). Probiotics can also modulate luminal short chain fatty acid production (Rios-Covian et al., 2016) which are known to cause a genomic increase in the abundance and activity of SGLT1 (Tappenden et al., 1997). There are then potential mechanisms by which

probiotics could increase absorption and oxidation of consumed glucose and these findings would have practical and relevant implications for athletes if replicable during endurance exercise.

As well as the potential to increase CHO absorption and oxidation, probiotics have also been proposed to be beneficial to performance via their effects on GI permeability and damage (12-15). It has been shown that probiotic supplementation, or inoculation with the metabolites of probiotic bacteria, can prevent epithelial apoptosis (Yan and Polk, 2002), increase mucin secretion (Caballero-Franco et al., 2007), inhibit pathogenic bacteria attachment (Bernet et al., 1994), as well as increase expression of TJ proteins and decrease secretion of pro-inflammatory cytokines (Mennigen and Bruewer, 2009). However, studies of the protective effects of probiotics against exercise-induced GI damage and symptoms and immune response in *in vivo* human studies are limited.

The aim of the current study was to examine the potential benefits of probiotic supplementation on total CHO oxidation and the oxidation of an ingested maltodextrin drink during 2 h of cycling exercise at 55% W_{max} . It is hypothesised that 4 weeks of probiotic supplementation would increase the intestinal digestion and absorption of the maltodextrin drink, the percent contribution of the drink to carbohydrate oxidation rates and total carbohydrate oxidation rates. It is also hypothesised that the ingestion of the probiotic supplement would significantly reduce the LR ratio and intestinal damage (I-FABP) and improve performance during the 2 h of cycling exercise. This hypothesis has been tested using a double blind placebo-controlled cross-over design.

6.3 Methods

6.3.1 Participants

Nine trained cyclists participated in this study (mean \pm SD; age 23 ± 4 yrs, body mass 74.8 ± 6.7 Kg, $\dot{V}O_{2peak}$ 62.1 ± 4.7 mL \cdot kg $^{-1}\cdot$ min $^{-1}$). Total carbohydrate oxidation was measured in all of them. The oxidation rate and appearance of the ingested maltodextrin in the plasma due to financial restrictions was only measured in seven of the cyclists. Exclusion criteria are outlined in Section 3.2.

6.3.2 Pre-testing

At least 7 days prior to the first experimental trial, subjects completed preliminary testing. $\dot{V}O_{2peak}$ and maximal aerobic power output (W_{max}) were determined as described in Section 3.3.3. After a rest period of 30-60 minutes, participants then completed 1 h of cycling exercise at 55% W_{max} with the prescribed drinking protocol and followed by a time trial to familiarise themselves to the real testing procedures described in the following paragraphs.

6.3.3 Treatment allocation

In a randomized, double-blind, placebo-controlled crossover design, each subject completed two 28 day periods of supplementation with a 14-day washout period between them. Subjects also consumed an additional supplement capsule on the morning of each trial, one hour before commencing exercise. Participants were randomized to consume either a capsule of a commercially available probiotic (PRO) or a visually identical placebo daily for 28 days. The PRO supplement contained the active strains *Lactobacillus acidophilus* (CUL60), *Lactobacillus acidophilus* (CUL21), *Bifidobacterium bifidum* (CUL20) and *Bifidobacterium animalis* subsp. *lactis* (CUL34) (Proven Probiotics, Port Talbot, UK). The minimum concentration was 25 billion colony-forming units (CFU). The PLC capsules were visually identical and consisted of starch only (Proven Probiotics, Port Talbot, UK). Subjects were instructed to swallow the capsule daily after their first meal. The randomization code was held by a third party (Cultech Ltd) and unlocked for statistical analyses, by the authors, upon completion of sample analysis. During the supplementation period, participants were informed to avoid consumption of probiotic foods such as fermented foods and yogurts.

6.3.4 Experimental trials

Each subject underwent four experimental trials; one prior to and at the end of each supplementation period. Trials consisted of 120 min of cycling at 55% W_{max} followed by a time trial amounting to 100 kJ of work. Subjects were instructed not to perform any strenuous exercise and to avoid caffeine, alcohol, and any spicy food 24 hr prior to testing. Subjects also recorded their food intake in the 24 hr before the first trial and repeated this for each subsequent visit. All trials were undertaken in ambient laboratory temperatures ($\sim 21^{\circ}\text{C}$).

Subjects reported to the laboratory at the same time ($\sim 7:30\text{am}$) for each trial after an overnight fast of at least 12 hours. A cannula (Safety Lock 22G, BD Biosciences, West Sussex UK) was inserted into the antecubital vein and baseline blood sample was taken. Resting breath samples were collected over a 5 min period (Moxus modular metabolic system, AEI technologies Inc, Pennsylvania, USA) and exetainer tubes were filled directly from the mixing chamber to determine the $^{13}\text{C}/^{12}\text{C}$ ratio in expired CO_2 . Subjects then began cycling at 55% W_{max} for 120 minutes. Immediately following this, simulated cycling time trials were undertaken with the ergometer set in a cadence-dependent power output (linear) mode for subjects to complete 100kJ of work. Power output was therefore a function of cadence and a fixed factor (alpha value) was used, as described in the following equation: Power (W) = $L \times \text{rpm}^2$, in which the rpm is the pedalling rate, and L is a linear factor. This factor was chosen in a way that would evoke a pedalling rate of 90 rpm at 100% PPO.

6.3.5 CHO drink

During exercise subjects consumed a 10% CHO drink enriched with the stable isotope [U-¹³C]glucose consisting of 176.4g maltodextrin (Myprotein®Inc, Northwich, UK) and 3.6 g [U-¹³C]glucose, dissolved in water to a total volume of 1800ml. For the two participants in which the oxidation rate of the ingested maltodextrin was not measured, the drink consisted of 10% maltodextrin only. Total drink volume was prescribed according to participant weight with an 8 mL·kg⁻¹·bw bolus in the first 3 minutes of exercise followed by 2 mL·kg⁻¹·bw each subsequent 15 min during 120 min cycling exercise (Jeukendrup, Mensink et al. 1997, van Loon, Greenhaff et al. 2001). Total fluid volume and carbohydrate intakes prescribed were 1790 ± 152 mL and 179 ± 15.2 g respectively. An elemental analyser isotope ratio mass spectrometer (EA-IRMS; Europa Scientific 20–20, Iso-Analytical Ltd, Crewe, UK) was used to accurately measure the ¹³C-enrichment of freeze-dried samples of the maltodextrin/[U-¹³C]glucose drinks and the natural ¹³C-background enrichment of the maltodextrin powder expressed as δ¹³C ‰ vs PDB. The drinks contained 35 mmol·L⁻¹ of sodium chloride as sodium in the 30-50mmol·L⁻¹ range leads to better fluid delivery and retention in endurance trained individuals (Maughan et al., 2018).

6.3.6 ¹²C/¹³C analysis

Breath samples were analysed using an isotope ratio mass spectrometer (Delta XP, coupled with a Gas Bench II and GC Pal autosampler (ThermoElectron, Bremen, Germany). The breath tubes were held in a heated sample tray at 26°C. The breath sample was continuously transferred through a valco sampling port in a flow of helium. Carbon dioxide was separated from the presence of other gases by using a capillary column (PoraPLOTQ; Agilent JW columns) with dimensions of 27.5 m X 0.32 mm X 10 µm. The oven temperature was kept constant at 68°C. Nafion water traps removed H₂O from the sample. Multiple analysis of each sample was achieved by switching the contents of the sample loop to the GC column every 50 seconds. Each switch corresponded to starting the GC separation of the sample coming from the loop. Ions m/z 44 and 45 were monitored for CO₂ and ¹³CO₂ respectively. The ¹³C enrichment results from breath samples were expressed as δ¹³C ‰ vs PDB and were converted to the tracer-to-tracee ratio (TTR) by using the following equation:

$$\text{TTR } (^{13}\text{C}:^{12}\text{C}) = [(\delta^{13}\text{C}\text{‰}/1000) + 1] \times 0.0112372 \text{ (Donmoyer et al., 2001)}$$

Plasma glucose isotope enrichment was measured by gas-chromatography mass spectrometry using a trimethyl silyl-O-methyloxime derivative according to methods previously described (Shojaee-Moradie et al., 1996). The peak areas of the ions m/z 319.2 and m/z 323.2, for glucose and [U-¹³C]glucose respectively, were measured by GC-MS on a Agilent 5975C Inert XL EC/CI MSD (Agilent Technologies, Wokingham, Berks, UK).

6.3.7 Indirect calorimetry and calculations

Respiratory gas exchange variables were measured using a breath-by-breath mode (Moxus modular metabolic system, AEI technologies Inc, Pennsylvania, USA) with oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), and respiratory exchange ratio (RER) continuously obtained at set 15 min intervals during exercise. Following consumption of glucose drink, breath samples were collected in duplicate directly from the mixing chamber of the MOXUS system into sealed vacutainer collection tubes. Total carbohydrate and fat oxidation rates were calculated from indirect calorimetry data assuming negligible protein oxidation (Jeukendrup and Wallis 2005):

$$\text{Glucose oxidation} = 4.55 \times \dot{V}CO_2 - 3.21 \times \dot{V}O_2$$

$$\text{Fat oxidation} = 1.67 \times \dot{V}O_2 - 1.67 \times \dot{V}CO_2$$

Exogenous glucose oxidation was calculated using the formula:

$$\dot{V}CO_2 \times (\delta_{Exp} - \delta_{Expbkg} / \delta_{Ing} - \delta_{Expbkg}) / k$$

in which δ_{Exp} is the ^{13}C enrichment of expired air during exercise at different time points, δ_{Ing} is the ^{13}C enrichment of the ingested drink, δ_{Expbkg} is the ^{13}C enrichment of expired air before exercise (background), and k is the amount of CO_2 (in liters) produced by the oxidation of 1 g of glucose ($k = 0.7467 \text{ l } CO_2/g \text{ glucose}$). Plasma glucose oxidation was calculated using the formula:

$$\dot{V}CO_2 \times (\delta_{Exp} - \delta_{Expbkg} \delta_{PG} - \delta_{PGbkg}) / k$$

in which, δ_{PG} is the plasma glucose ^{13}C enrichment and δ_{PGbkg} is the plasma glucose ^{13}C enrichment before exercise (background). As glycogen stores are also ^{13}C enriched, shifts in substrate utilization may result in a change in background enrichment (Wagenmakers et al., 1993c). It has shown that limiting ^{13}C in the diet, as is the case in European subjects, is effective in reducing the background shift from endogenous substrate stores (Wagenmakers et al., 1993b, Wagenmakers et al., 1993c). Furthermore, the ^{13}C enrichment of the CHO ingested was artificially increased by adding [U- ^{13}C]glucose to the CHO beverage. It is therefore not necessary to correct for the relatively small shift in the background ^{13}C enrichment

Because plasma glucose oxidation represents the oxidation of both glucose coming from the gut (exogenous glucose) and the contribution of the liver (glycogenolysis and gluconeogenesis), liver-derived glucose oxidation and muscle glycogen oxidation could be calculated by the following formulas:

Liver-derived glucose oxidation = plasma glucose oxidation – exogenous glucose oxidation

Muscle glycogen oxidation = total CHO oxidation – plasma glucose oxidation

A methodological consideration when using $^{13}\text{CO}_2$ in expired air to calculate exogenous substrate oxidation is the trapping of $^{13}\text{CO}_2$ in the bicarbonate pool, in which an amount of CO_2 arising from decarboxylation of energy substrates is temporarily trapped. However, during exercise, the CO_2 production increases several fold so that a physiological steady-state condition will occur relatively rapidly, and $^{13}\text{CO}_2$ in the expired air will be equilibrated with the $^{13}\text{CO}_2/\text{H}^{13}\text{CO}_3^-$ pool. Recovery of $^{13}\text{CO}_2$ from [U- ^{13}C]glucose oxidation will approach 100% after 60 min of exercise when dilution in the bicarbonate pool becomes negligible (Jeukendrup et al., 1999a). Therefore, data from the initial 60 min were not used for calculation of exogenous glucose oxidation. As a consequence of this, all calculations on substrate oxidation were performed over last 60 min of exercise (60–120 min).

6.3.8 Blood parameter analysis

Following consumption of glucose solution, blood samples were collected at set 15 minute intervals. Analysis for LR, I-FABP, inflammatory cytokines, and circulating metabolites were performed as described in section 3.5. Post exercise and 1hr post exercise sample concentrations were corrected for plasma volume changes as described by Dill and Costill (1974).

6.3.9 Assessment of gastrointestinal symptoms

Specific GI symptoms were recorded every 30 min during exercise, as described in section 3.4.3

6.3.10 Statistical analysis

ANOVA for repeated measures was used to compare differences in substrate utilization and in blood related parameters over time between the trials. A Tukey's post hoc test was applied in the event of a significant F-ratio. Where appropriate, the comparison of variables between the two conditions was conducted by using a Student's t-test for paired samples. All values are expressed as means \pm SD. Statistical significance was set at $P < 0.05$.

6.4 Results

6.4.1 Physiological response to exercise

Participants cycled for 2 hours at 175 ± 20 W (mean \pm SD) across trials corresponding to 55% of their W_{max} . There were no significant differences between mean heart rate (149 ± 18 vs 146 ± 16 b \cdot min $^{-1}$), $\dot{V}\text{O}_2$ (33.9 ± 3.9 vs 33.5 ± 3.4 mL \cdot kg $^{-1}\cdot$ min $^{-1}$), or RPE (12 ± 1 vs 12 ± 1) for placebo and probiotic conditions respectively. CHO and fat metabolism during each hour are presented in Table 6.1. CHO oxidation was lower during the second hour in both trials. CHO oxidation was higher ($P = 0.019$) in the second hour after probiotic supplementation compared to placebo. Fat oxidation was lower in the probiotic condition during both the first ($P = 0.026$) and the second ($P = 0.004$) hours compared to placebo.

Energy expenditure did not differ between placebo (6292 ± 644 kJ) and probiotic (6232 ± 493 kJ) ($p = 0.662$).

Table 6.1. CHO and fat metabolism during 0-60 and 60-120 min ($n = 9$). Data are mean ± SD. *Significantly different from placebo ($P < 0.05$)

	0-60 min			60-120 min		
	Placebo	Probiotic	<i>P</i> value	Placebo	Probiotic	<i>P</i> value
RER	0.89 ± 0.03	0.92 ± 0.03*	0.037	0.86 ± 0.03	0.90 ± 0.02*	0.005
CHO oxidation (g·min ⁻¹)	2.08 ± 0.45	2.31 ± 0.35	0.052	1.81 ± 0.44	2.12 ± 0.30*	0.019
Fat oxidation (g·min ⁻¹)	0.47 ± 0.15	0.35 ± 0.10*	0.026	0.59 ± 0.12	0.43 ± 0.11*	0.004

6.4.2 Substrate utilisation

The ¹³C-enrichment of the consumed drinks was 1681 ‰ vs PDB. Plasma ¹³C/¹²C glucose ratios increased as a result of maltodextrin/[U-¹³C]glucose drink and was stable during the 60-120 min period (Fig 6.1A). Baseline ¹³C-enrichments from resting breath samples were comparable between placebo (-25.2 ± 3.6 ‰ vs PDB) and probiotic (-25.0 ± 1.8 ‰ vs PDB) ($p > 0.05$). Changes in enrichment after ingestion of the drink at the start of 2 h of endurance exercise at 55% W_{max} are shown in Fig 6.1B. ¹³CO₂ enrichments levelled off from 45 min during both trials and there were no significant differences at any time point between probiotic and placebo.

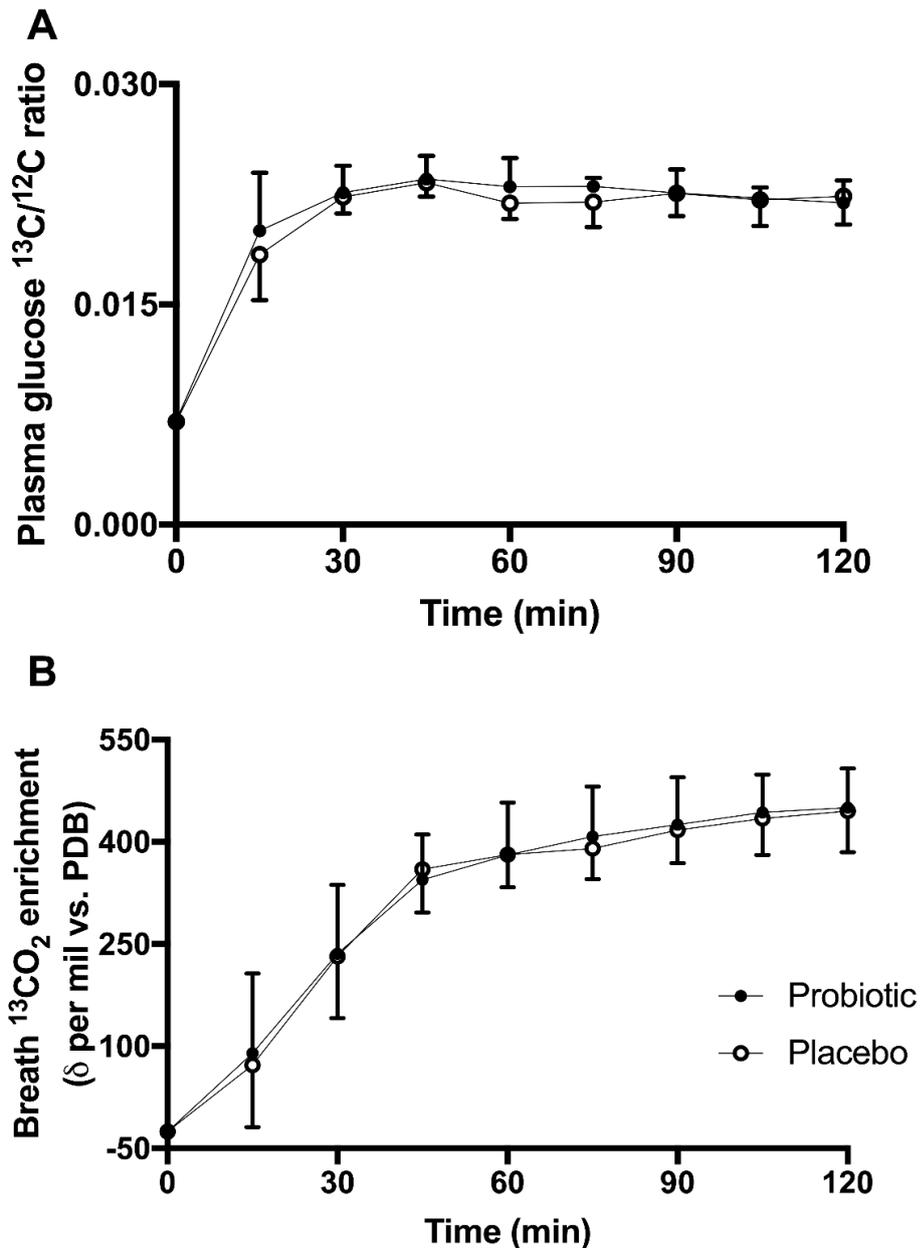


Figure 6.1. (A) Plasma glucose ¹³C/¹²C ratio and (B) Breath ¹³CO₂ enrichment during exercise. Values are means ± SD; n = 7. PDB, Pee Dee Belleminitella

CHO substrate oxidation during 60-120 min is summarised in Table 6.2. Mean oxidation of the ingested maltodextrin/[U-¹³C]glucose drink was higher under probiotic compared to placebo conditions, as was the maximal oxidation observed (0.84 ± 0.10 vs 0.77 ± 0.09 g·min⁻¹, $p = 0.016$) which was achieved at 120 min during both trials. There was no difference in mean liver-derived glucose oxidation and muscle glycogen oxidation tended to be higher under probiotic conditions but did not reach statistical significance.

Table 6.2. CHO utilisation calculated during 60-120 min. Data are presented as $g \cdot min^{-1}$ and are mean \pm SD. $n = 7$. *Significantly different from placebo ($P < 0.05$)

	Placebo	Probiotic	<i>P</i> -value
Ingested maltodextrin	0.75 \pm 0.09	0.79 \pm 0.10*	0.024
Liver-derived glucose	0.21 \pm 0.08	0.19 \pm 0.04	0.323
Muscle glycogen	0.99 \pm 0.41	1.24 \pm 0.28	0.087

Blood metabolites

At 0 min, plasma glucose, lactate, NEFA and glycerol concentrations were all similar in both trials (Fig 6.2). Plasma glucose increased during the first 30 min of exercise before decreasing at 45 min and remained stable for the rest of the exercise bout, with no differences between probiotic and placebo (Fig 6.2A). Plasma lactate increased in response to exercise to $\sim 2 \text{ mmol} \cdot \text{L}^{-1}$ and then gradually declined during the course of the exercise bout (Fig 6.2B). Insulin concentrations were higher at 30, 45 and 75 min in probiotic compared to placebo ($p < 0.05$). NEFA concentrations reduced at the onset of exercise and subsequently increased from 60 min in both trials, with a significant difference between trials by 120 min ($P = 0.043$) (Fig 6.2D). Plasma glycerol increased during exercise in both trials, with significantly lower concentrations at each time point from 90 min during the probiotic trial compared to placebo ($P > 0.001$) (Fig 6.2E).

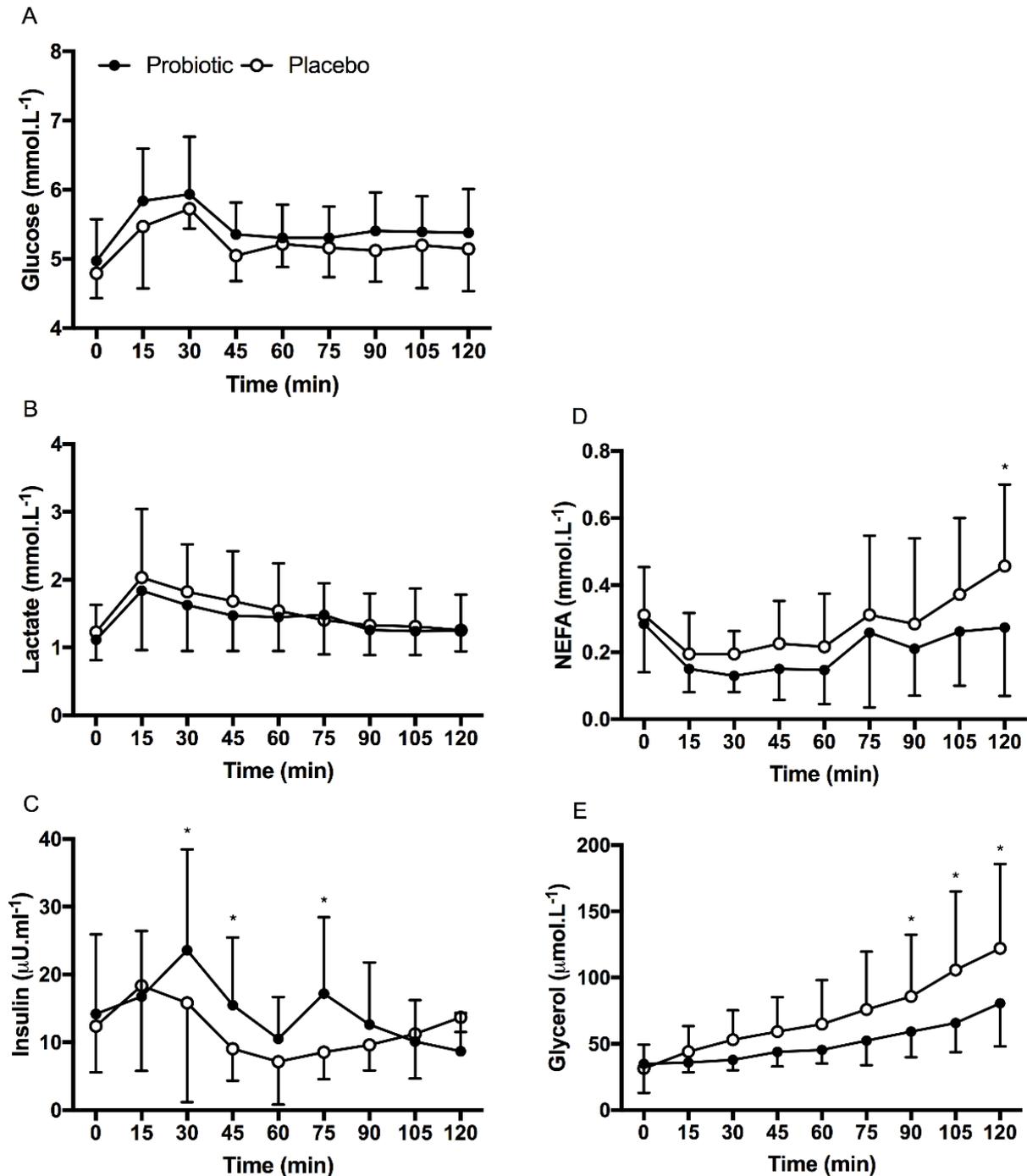


Figure 6.2. Plasma (A) glucose, (B) lactate, (C) insulin, (D) NEFA, and (E) glycerol. n = 9 *significant difference between PRO and PLC at the corresponding time point (p<0.05).

Markers of GI permeability, damage and cytokines

Individual data points for LR during probiotic and placebo are presented in Fig 6.3A. There was no significant difference in LR between probiotic (0.045 ± 0.02) and placebo trials (0.047 ± 0.03) ($p = 0.436$). For I-FABP, there was no significant difference between probiotic and placebo at pre ($p = 0.364$), post ($p = 0.374$) or 1hr post exercise ($p = 0.393$) for PRO and PLC, while there was also no effect of exercise (Fig 6.3B).

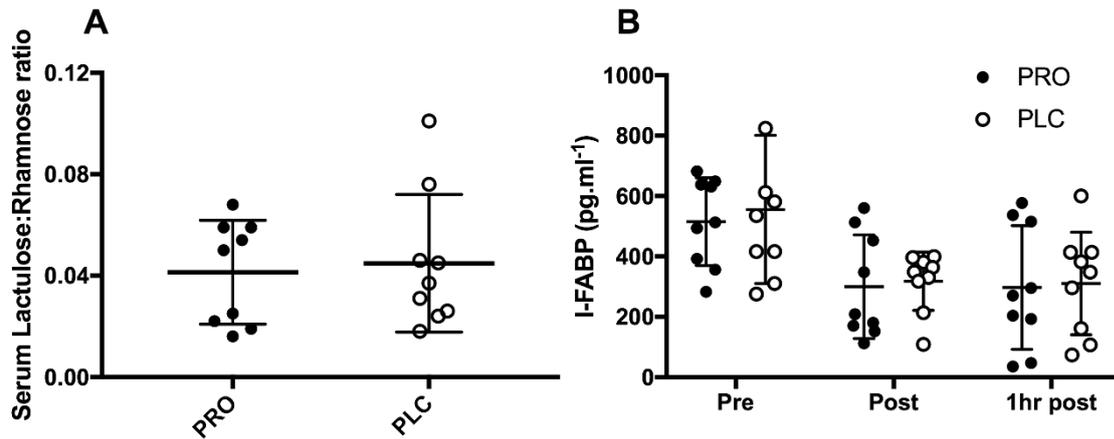


Figure 6.3. A) Serum lactulose:rhamnose ratio for probiotic and placebo. B) Intestinal-fatty acid binding protein (I-FABP) pre, post and 1hr post exercise for probiotic and placebo

Plasma cytokine concentrations for pre and post-exercise are presented in Table 6.4. For pre-exercise measures, IL-1a and IL-6 concentrations were lower during the probiotic trial, while IL-6 was also lower post-exercise. There was a significant correlation between LR and post-exercise concentrations of IL-6 ($r = 0.527$, $p = 0.035$) and IL-10 ($r = 0.554$, $p = 0.026$). Correlations with I-FABP were insignificant for all cytokines.

Table 6.3. Pre and post-exercise cytokine concentrations for placebo and probiotic trials. Data are mean \pm SD. (N = 9)

	Pre-exercise			120 min		
	PLC	PRO	p value	PLC	PRO	p value
IL-1a (pg·mL ⁻¹)	1.35 \pm 0.84	0.61 \pm 0.57	0.0138*	1.59 \pm 1.17	1.52 \pm 1.33	0.4169
IL-6 (pg·mL ⁻¹)	2.13 \pm 1.13	1.24 \pm 0.82	0.0321*	3.33 \pm 1.92	2.13 \pm 1.07	0.0389*
IL-8 (pg·mL ⁻¹)	2.52 \pm 1.06	2.43 \pm 1.24	0.4409	4.49 \pm 1.57	4.15 \pm 2.54	0.3206
IL-10 (pg·mL ⁻¹)	1.81 \pm 1.22	1.14 \pm 0.81	0.0794	2.73 \pm 1.95	2.75 \pm 2.86	0.4853

GI symptoms and time trial performance

During exercise trials, individual GI symptoms assessed were low (≤ 2 on scale of 0-10) even when using maximum values from each trial (Table 6.5). During the 100kJ time trial there was no significant difference in the time to complete between placebo (308 \pm 69s) and probiotic (301 \pm 74s) ($P = 0.714$).

Table 6.4. GI symptoms during PLC and PRO conditions. Data are median and range

Symptom	PLC	PRO	Wilcoxon p value
Side stitch	0 (0)	0 (0-1)	0.817
Nausea	0 (0-1)	0 (0-0)	0.757
Bloating	0 (0-1)	0 (0-1)	0.927

Urge to burp	0 (0-2)	0 (0-1)	0.682
Urge to vomit	0 (0)	0 (0-1)	0.759
Urge to defecate	0 (0-1)	0 (0-1)	0.962
Diarrhea	0 (0)	0 (0)	0.785
Flatulence	1 (0-2)	0 (0-1)	0.653
Stomach Cramps	0 (0-1)	0 (0-2)	0.833

6.5 Discussion

The present study evaluated the influence of probiotic supplementation on carbohydrate oxidation during endurance exercise in human participants. The findings demonstrate that probiotic supplementation enhances both total carbohydrate oxidation and the oxidation of the ingested maltodextrin drink, whilst attenuating fat oxidation during moderate intensity cycling. Additionally, there were no measurable increases in GI damage, permeability or GI symptoms following cycling exercise, so any effect of supplementation could not be seen here. The increases in CHO oxidation did not coincide with a change in subsequent time trial performance. The small but significant effects on oxidation of ingested carbohydrate suggests probiotics can influence exercise metabolism, and these benefits warrant further investigation.

It has long been known that CHO ingestion during exercise can prevent hypoglycemia, maintain high rates of carbohydrate oxidation, and increase endurance capacity compared with placebo ingestion (Jeukendrup, 2014). Numerous factors appear to limit the maximal rate of glucose oxidation including: exercise intensity, muscle glycogen stores, exercise-induced muscle damage, environmental temperature, and training status (Jentjens et al., 2002, Jeukendrup, 2014, Jeukendrup and Jentjens, 2000). However, the ultimate rate-limiting factor appears to be intestinal absorption, potentially due to a saturation of the glucose transporter SGLT1 (Jeukendrup and Jentjens, 2000, Hawley et al., 1994). In exercise models, probiotics have previously been shown to modify CHO oxidation (Iwasa et al., 2013a, Art et al., 1994a) and this may potentially be due to an increase in insulin response to CHO feeding (Simon et al., 2015) and/or increased expression of SGLT1 glucose transporters (Barrenetxe et al., 2006, Rooj et al., 2010b). In the present trial, either of these may have contributed to the increase in total CHO oxidation and small increase in exogenous CHO oxidation. Plasma insulin was shown to be increased during portions of the exercise bout following probiotic supplementation, with subsequent reductions in both fat oxidation and plasma NEFA and glycerol, a relationship that has been acknowledged (Costill et al., 1977). The effects of plasma insulin levels on exogenous oxidation rates are not though well understood, with suggestions that they may (Jeukendrup et al., 1996) or

may not (Massicotte et al., 1990) have an effect. Peak rates of exogenous CHO oxidation may then have been higher following probiotic supplementation due to an increase in the absorption rate of ingested CHO although this was not measured here. While the difference shown in exogenous CHO oxidation might be small, further studies may be warranted to investigate any effect when exercising at higher environmental temperatures or at altitude, both of which have been shown to compromise exogenous oxidation due to reductions in intestinal absorption (Jentjens et al., 2002, O'Hara et al., 2017).

The data presented here showed that there were no increases in measures for I-FABP or GI symptoms during 120 min of cycling, while GI permeability was not different between trials and many values were similar to resting values previously reported in our laboratory (Pugh et al., 2017a, Pugh et al., 2017b). GI permeability has previously been reported to only significantly increase compared to resting values at exercise intensity of $\geq 80\% \dot{V}O_{2max}$ at ambient temperatures (Pals et al., 1997), while in the present trial it was $\sim 55\% \dot{V}O_{2max}$. There was also no increase in plasma I-FABP following exercise, data that corroborates previous data showing no increase at this exercise intensity, particularly when subjects consume CHO, during moderate intensity exercise (Trommelen et al., 2017). Maintaining euhydration and CHO intake in liquid solutions have both been found to ameliorate GI permeability and epithelial injury (Lambert et al., 2008, Snipe et al., 2017) potentially due to attenuation of the decrease in splanchnic blood flow during exercise (Perko et al., 1998, ter Steege and Kolkman, 2012). In regards to GI symptoms, these were generally low, again most likely do the exercise modality, duration, and intensity which do not appear to have led to a sustained functional challenge to the GI system. To better investigate the effects of probiotics on exercise induced GI permeability, damage and symptoms, particularly in the presence of carbohydrate ingestion, exercise of a greater intensity and duration should be considered. Equally, it should be investigated to find whether probiotics are beneficial for those exercising in warmer temperatures where there is an increased risk of GI damage and CHO malabsorption.

When undertaking exercise metabolism studies utilising stable isotope tracers, a number of methodological considerations are needed. It has previously been shown that the natural [U-¹³C]-enrichment of the glycogen stores in humans depends on whether the CHO-sources routinely ingested by the volunteers originate from C₃ or C₄ plants (Wagenmakers et al., 1993c). This can lead to measurable increases in breath ¹³CO₂ enrichment during exercise in American volunteers (primarily ingesting CHO sources from C₄ plants such as maize and sugar cane) only ingesting water. Under these conditions the increases in Europeans, primarily ingesting CHO sources from C₃ plants such as potato and sugar beet, were minimal though (Wagenmakers et al., 1993c). In the current study, the maltodextrin source was enriched with [U-¹³C]glucose to a value of 1681 ‰ vs PDB, while the increases

in breath $^{13}\text{CO}_2$ during exercise in European volunteers were maximally 1 ‰ vs PDB when only water was ingested during exercise (Wagenmakers et al., 1993c). It is generally assumed that maltodextrins are readily digestible (Peters et al., 2011, Cisse et al., 2017) and maximal oxidation rates during exercise have been shown to be as high values observed when consuming glucose (Wagenmakers et al., 1993a). In the present study a [^{13}C]glucose tracer has been added to a maltodextrin source. Similar increases in the profile of the breath $^{13}\text{CO}_2$ enrichment curve over the 2 h of exercise were shown as that when using naturally enriched maltodextrins (Wagenmakers et al., 1993c). There also was an increase and plateauing of plasma $^{13}\text{C}/^{13}\text{C}$ glucose ratio. Together, these indicate that the glucose tracer was not absorbed faster than the maltodextrin source, but instead has mixed with glucose originating from digestion of maltodextrin before being absorbed by the intestine and oxidised by skeletal muscle.

In summary, the present data demonstrate that both total and exogenous CHO oxidation are increased following 4-weeks of probiotic supplementation. Furthermore, this was reflected with reductions in fat oxidation and plasma NEFA and glycerol concentrations. However, as GI symptoms of discomfort were low throughout the trial, the efficacy of probiotics could not be elucidated. This was likely due to the intensity and mode of exercise, as there was also no demonstrated effect on markers of GI damage or permeability following the exercise bout.

Chapter 7 - Probiotic
supplementation reduces GI
symptoms in recreational
marathon runners, but not
markers of GI permeability or
damage

Twenty eight days of probiotic supplementation reduces GI symptoms during a marathon race in recreational runners, which appears related to the maintenance of running speed

7.1 Abstract

Purpose: To evaluate the effects of probiotic supplementation on gastrointestinal (GI) symptoms, circulatory markers of GI permeability, damage and markers of immune response during a marathon race.

Methods: In a match-pairs design, twenty-four recreational runners were randomly assigned to either a group supplementing with a probiotic capsule (25 billion CFU *Lactobacillus acidophilus* (CUL60 and CUL21), *Bifidobacterium bifidum* (CUL20), *Bifidobacterium animalis* subs p. *Lactis* (CUL34)) (PRO) or placebo (PLC) for 28 days prior to a simulated marathon race. GI symptoms were recorded in a diary during the supplement period and every 20 minutes during the race. Lactulose:rhamnose ratio, intestinal-fatty acid binding protein, sCD14 and cytokines were measured pre- and post-race.

Results: Prevalence of moderate GI symptoms were lower during the third and fourth weeks of the supplement period compared to the first and second weeks in PRO ($p < 0.05$) but not PLC ($p > 0.05$). During the marathon, the severity of GI symptoms during the final third of the race was significantly lower in PRO compared to PLC ($p = 0.010$). Although there was no difference in final finish time between groups ($p > 0.05$), there was a significant difference in the reduction of average speed from the first to the last third of the race between PLC ($-14.2 \pm 5.8\%$) and PRO ($-7.9 \pm 7.5\%$), ($p = 0.04$). There was also a significant, correlation between severity of GI symptoms and the reduction in running speed during the final third of the race ($r = 0.562$, $p = 0.01$). The lactulose:rhamnose ratio, intestinal-fatty acid binding protein, plasma cytokines and sCD14 increased pre to post race in both PRO and PLC, with no difference between conditions ($p > 0.05$).

Conclusion: Probiotics reduce the incidence and severity of GI symptoms in marathon runners although the exact mechanisms are yet to be elucidated. Reducing GI symptoms during a marathon race may help maintain running pace during the latter stages of the event.

7.2 Introduction

As outlined in Chapter 4, 27% of recreational runners reported moderate or even more severe GI symptoms during a marathon race. The pathogenesis of such symptomology is still poorly understood although it is likely multifactorial in nature. The exercise-induced reduction in splanchnic blood flow is well characterised (McAllister, 1998, Otte et al., 2001) and results in dysregulation of the intestinal barrier. This likely leads to endotoxaemia and an immunological response, which has been associated with GI symptoms during ultra-endurance events (Jeukendrup et al., 2000, Stuempfle et al., 2016). Carbohydrate (CHO) intake during exercise is also suggested to be a potential causative and or aggravating factor, due to malabsorption when consumed in excess (de Oliveira and Burini, 2014), although tolerance levels may differ for individuals (Costa et al., 2017b), and data in Chapter 6 showed that large amounts of CHO can be consumed during cycling exercise with low prevalence and severity of GI symptoms reported. There may also be a predisposition to symptoms, as individuals with a history of recurrent exercise-associated GI symptoms, appear to suffer greater prevalence and severity of symptoms during exercise (Ter Steege et al., 2008). However, due to the range of symptom types (each with their own unique aetiology) coupled with differences in study methodologies to assess GI symptoms; studies to date have yet to find a single mechanism. Nonetheless, GI symptoms during marathon running remain detrimental to both enjoyment of exercise and performance in recreational and elite runners hence potential strategies to reduce such remains an attractive area of research.

One such strategy that may reduce GI symptoms during endurance exercise is probiotic supplementation. While probiotics can relieve lower GI symptoms in irritable bowel syndrome IBS (Hungin et al., 2018), there is less consensus with regard to their efficacy in modulating exercise-associated GI symptoms. A reduction in the duration of GI symptoms was noted in a group of recreational runners two weeks post-marathon race following probiotic supplementation, although only severe symptoms (diarrhea, vomiting, stomach ache) were recorded and no differences were found during the period of supplementation (Kekkonen et al., 2007). The severity of GI symptoms during training in novice triathletes was also reduced when supplementing with a multi-strain probiotic, with subsequent reductions in GI permeability following an Ironman man distance triathlon race compared to placebo (Roberts et al., 2016); however, permeability was assessed six days post-race and in-race GI symptoms were not assessed. Potential mechanisms by which symptoms could be ameliorated include modulation of CHO absorption and oxidation (Roos et al., 2010b), and attenuation of exercise-induced GI damage or permeability and modulation of tight junctional proteins within the epithelial barrier (Anderson et al., 2010, Karczewski et al., 2010, Lamprecht et al., 2012).

There are many circulatory markers of GI dysfunction that have been assessed post-exercise. Indirect measures of GI permeability utilise dual sugar probes, such as lactulose and rhamnose (van Wijck et al., 2013b), while intestinal-fatty acid binding protein (I-FABP) has often been used as a sensitive marker to intestinal mucosal damage (Van Wijck et al., 2011). Both LR and I-FABP have been assessed in post-exercise in Chapters 5 and 6. However, to date, the relationship between such markers and exercise-induced GI symptoms is unclear. Downstream circulatory markers of immune activation and inflammatory response have been assessed following competitive endurance exercise (Moore et al., 1995, Nieman et al., 2006), although to date, only one marker has been associated with the occurrence of GI symptoms. sCD14 is a phosphatidylinositol-linked membrane glycoprotein on polymorphonuclear leucocytes that serves as a receptor for endotoxin and has been suggested to provide a more stable marker for increased exposure to LPS (Liu et al., 1998). Plasma sCD14 concentrations were increased to a greater extent in ultramarathon runners reporting symptoms of nausea relative to those without (Stuempfle et al., 2016), and therefore warrants further investigation. Given the prevalence of GI issues in endurance athletes and the effects of this on performance and enjoyment, the purpose of this study was to evaluate the effects of probiotic supplementation on GI symptoms during marathon training and racing. It was hypothesised that GI symptoms during training and a marathon race would be less frequent and less severe with probiotic supplementation. In addition, it was hypothesised that probiotic supplementation would attenuate circulatory markers of exercise-induced GI permeability, damage and immunological response.

7.3 Methods

7.3.1 Subjects

A total of 24 runners (20 male, 4 female) participated in the study. The recruitment process as well as study dropouts are shown in Figure 7.1. All participants were required to have run a marathon race quicker than 5 hr within the previous two years. Participant characteristics are presented in Table 7.1. Exclusion criteria are outlined in Section 3.2.

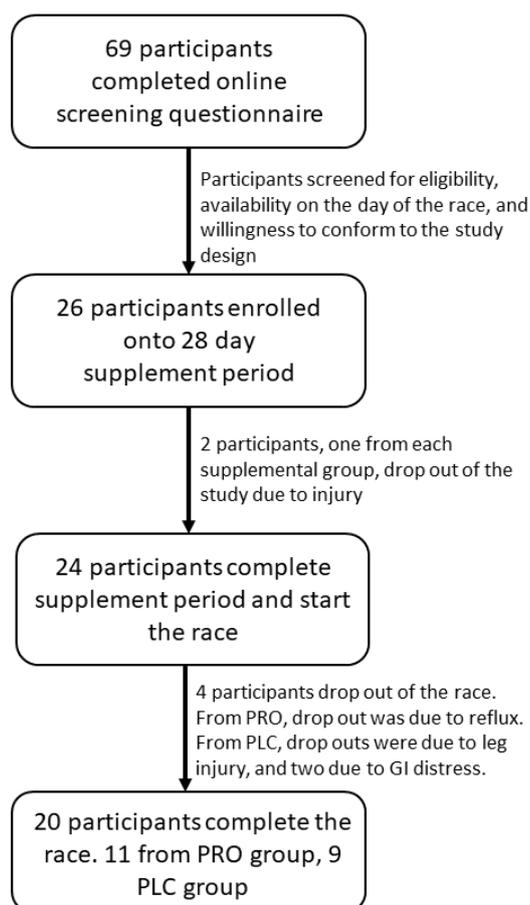


Figure 7.1. Schematic of participant recruitment, and dropouts at various stages of the study.

7.3.2 Baseline testing

At least 4 weeks before the marathon, and before the supplement period, participants completed an incremental running test to determine lactate threshold (LT) and peak oxygen uptake ($\dot{V}O_{2peak}$) as previously described in Section 3.3.2. During this laboratory visit, participants also completed the Gastrointestinal Symptom Rating Scale to assess baseline GI symptoms (Svedlund et al., 1988b).

Table 7.1. Subject characteristics. Values are means \pm SD. Differences between groups for all measures were not significant ($P < 0.05$).

	PLC		PRO	
	Mean \pm SD	Range	Mean \pm SD	Range
Age (yr)	36.1 \pm 7.5	29 – 50	34.8 \pm 6.9	22 - 43
Height (cm)	175.4 \pm 11.1	152 – 186	179.0 \pm 6.3	168 – 190
Weight (kg)	73.5 \pm 11.3	48 – 95	76.5 \pm 9.4	61 – 92
$\dot{V}O_{2peak}$ (mL·kg ⁻¹ ·min ⁻¹)	56.4 \pm 8.6	47.2 – 70.0	57.6 \pm 8.0	48.1 – 66.7
LT (km·h ⁻¹)	11.9 \pm 1.9	9 – 16.0	12.3 \pm 1.9	10 – 15.5
Most recent marathon time (min)	220 \pm 40	150 - 283	222 \pm 46	152 - 315

7.3.3 Supplement Period

After baseline testing, in a double-blind, randomised, matched-pairs design, participants underwent a 28 day period of supplementation consuming either a commercially available probiotic (PRO) or a visually identical placebo (PLC). Participants also consumed an additional supplement capsule on the morning of the race, two hours before the start. Participants were matched according to their most recent marathon performance (PRO: 222 ± 46 min; PLC 220 ± 40 min) and body mass (Table 7.1). The probiotic supplement capsules contained the active strains *Lactobacillus acidophilus* CUL60, *Lactobacillus acidophilus* CUL21, *Bifidobacterium bifidum* CUL20 and *Bifidobacterium animalis* subs p. *Lactis* CUL34 (Proven Probiotics Ltd, Port Talbot, Wales). The placebo capsules consisted of cornstarch only. The minimum concentration was 25 billion colony forming units (CFU) per gram. Participants were instructed to swallow the capsule daily after their first meal. The randomization code was held by a third party, unlocked for analyses upon sample analysis completion. During the supplementation period, participants were instructed to refrain from all probiotic foods (i.e., fermented yogurts). For the full 28 day supplement period, participants were required to complete a daily training and GI symptom diary.

7.3.4 Marathon Race

During the 24 h before the race, participants consumed a standardized high CHO, low fibre diet (per kg body mass: 8.0g CHO [0.28g fibre]; 2.0 g protein; 1.0 g fat). Compliance to the diet was confirmed with food diaries and the Remote Food Photography Method (Martin et al., 2009). After an overnight fast, participants reported to the laboratory at ~07:00h and resting venous blood samples were taken. Participants were then provided with a standardized breakfast (572 kcal; 128 g CHO [4.4g fibre], 7 g protein, 3.5 g fat, and a minimum of 500 mL water) before a pre-race venous blood sample was collected. Participants performed self-selected warm-ups before a race briefing to reiterate in-race nutrition and subjective measures. The race started at 12:00pm. Runners ran the 42195 m race on a synthetic 400 m outdoor track (105.48 laps) which was in close proximity to the laboratory. Weather conditions throughout the race were; temperature: 16-17 °C; wind speed: 8-16 km·h⁻¹; precipitation: 0mm. During the race, heart rate was monitored throughout (Firstbeat Sports®, Jyväskylä, Finland) and subjective ratings of perceived exertion (RPE) were recorded every 15 minutes. Each 400m lap time was recorded using electronic chips and a timing mat (BibTag System, MYLAPS, USA). Global GI discomfort was assessed every 15 minutes using a modified Likert scale (Nieman et al., 2006). Muscle biopsies also taken from 7 participants from PRO and 6 from PLA pre and post-race although data from these analyses is not reported.

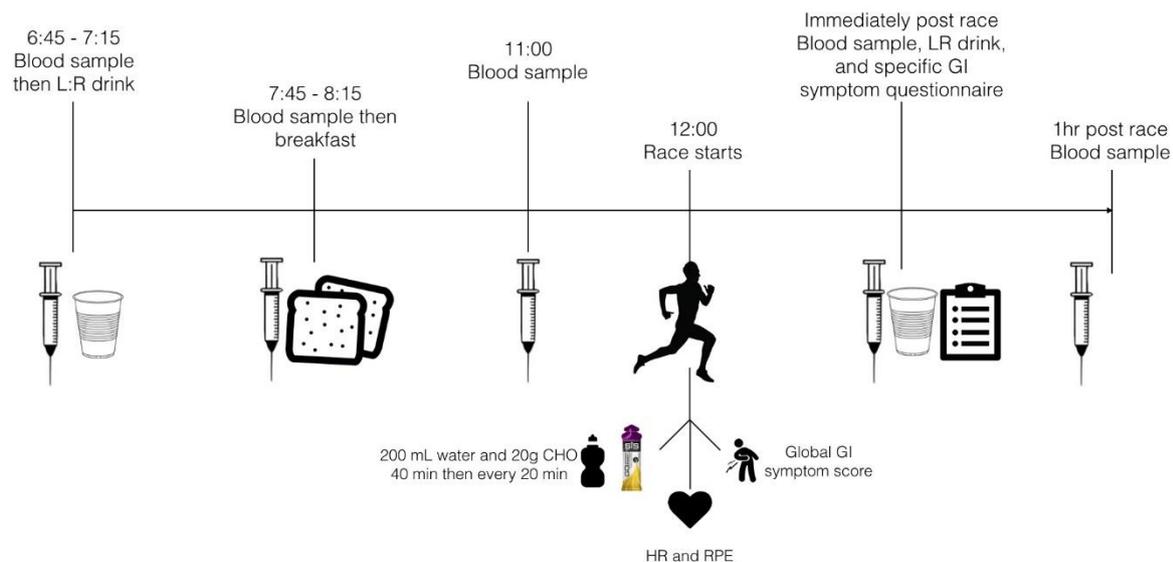


Figure 7.2. Schematic overview of the marathon race day and timing of measurements.

7.3.5 In-race Nutrition

Participants consumed one CHO gel (SIS Isotonic Gel, Blackburn, UK) and 200 mL of water 10 to 15 min before the start of the race and one energy gel with 200 mL of water 40 min after the start of the race and subsequently every 20 min for the remainder of the race. Gels consisted of 22 g maltodextrin, 0.01 g sodium. This provided an average of $66 \text{ g}\cdot\text{hr}^{-1}$ CHO and $600 \text{ mL}\cdot\text{hr}^{-1}$ during the race, a strategy that has been shown to improve performance in non-elite runners relative to a self-selected strategy (Hansen et al., 2014). In order to familiarize with this nutritional strategy, participants were informed of the strategy and provided with identical gels to practice this during their two longest training runs during the four-week supplementation period. This was diarized in the GI symptom and training diary.

7.3.6 Post-race

Blood samples were collected immediately post-race for later analysis. Participants were then asked to complete a more detailed questionnaire (adapted from Pfeiffer et al. 2012) to assess any specific symptoms of GI discomfort, including; bloating, flatulence, stitch, belching, nausea, urge to vomit, urge to defecate, and stomach cramps. These were scored on a 10-point scale (0 = no pain and 10 = worst possible pain) with a score > 4 being regarded as serious. To ensure understanding, specific symptoms were explained and described to participants. This same scale was used in the daily GI symptom diary used during the supplemental period.

7.3.7 Blood analysis

Samples were collected pre-race, as well as immediately and 1 hr post-race. Samples were analysed for LR, I-FABP, sCD14, glucose and inflammatory cytokines as described in section 3.5. Post exercise and 1hr post exercise sample concentrations were corrected for plasma volume changes as described by Dill and Costill (1974).

7.3.7 Statistical analysis

Descriptive statistics were produced for all data sets to check for normal distribution indicated by KolmogorovSmirnov (accepted if $p > 0.05$). A two-factor repeated measure ANOVA was used to LR, I-FABP, sCD14, cytokines and cortisol with condition (PRO and PLC) and various time points as the independent variables. Where significant main effect and interactions were present, pairwise comparisons were analysed according to Bonferoni post-hoc tests in order to locate specific differences. To evaluate data on GI symptoms, a nonparametric statistical approach was chosen, as scores on GI symptoms were mainly reported on the low end of the scale and not normally distributed. Symptom diary variables were compared with the use of Wilcoxon Signed Rank tests. Symptom scores during the race were compared with the use of Mann-Whitney nonparametric U test for independent data. Spearman rank-order correlation was used to analyse the relationship between GI symptoms, with post exercise I-FABP, inflammatory cytokines and sCD14 concentrations. All normally distributed data are presented as mean \pm standard deviation (SD). Data not normally distributed are reported as median and range. $P < 0.05$ was considered statistically significant. Statistical analysis was conducted using the Statistical Package for the Social Sciences software programme (SPSS, version 23).

7.4 Results

7.4.1 GI symptoms during supplementary period

Baseline assessment scores from the GSRS showed no differences in GI symptoms between PRO and PLC for any lower GI (8 (0-20) vs 10 (0-18)) (median and range), upper GI (7 (0-15) vs 6 (0-13)), or total GI symptom scores (15 (0-35) vs 17 (0-30)). From GI symptom diaries, daily average scores for specific symptoms were low (<2) in both PRO and PLC. However, there was a large range of scores for individual symptoms, with 17 participants reporting symptoms with moderate severity or worse during at least one day. Table 7.2 shows the median number of total symptoms scored as moderate (≥ 4) for upper and lower GI symptoms, as well as the number of days with one or more moderate symptom. Data shown represents the first 14 days and the second 14 days. For the probiotic group, there were significant reductions in the number of moderate symptoms reported as well as the

number of days in which moderate symptoms were reported in the second 2 week period of supplementation compared to the first (Table 7.2). There were no differences in the placebo group.

Table 7.2. GI symptoms reported during days 1-14 and 15-28 days during supplementation. Data are presented as median and range.

	PRO (n = 12)			PLC (n = 12)		
	Days 1-14	Days 15-28	Wilcoxon <i>p</i> value	Days 1-14	Days 15-28	Wilcoxon <i>p</i> value
Total number of GI scores \geq 4	4 (0-25)	2 (0-16)	0.007*	5 (0-41)	11 (0-39)	0.336
Total number of upper GI scores \geq 4	0 (0-10)	0 (0-10)	0.317	3 (0-8)	3 (0-6)	0.514
Total number of lower GI scores \geq 4	3 (0-16)	1 (0-8)	0.007*	4 (0-11)	5 (0-12)	0.317
Number of days with one or more symptom scored \geq 4	3 (0-12)	1 (0-6)	0.011*	5 (0-12)	5 (0-10)	0.579

*significantly difference between days 1-14 and 15-28 ($p < 0.05$) (Mann-Whitney *U*-test)

7.4.2 Race completion

A total of 20 runners completed the marathon race. There was one drop-out from PRO and three from PLC. Participants were asked to self-describe their reason for drop out. In PLC participants withdrew from the race due to musculoskeletal injury (22.5 km of the race completed) and two due to severe GI discomfort (12.2 and 30.2 km). In the PRO group, withdrawal was due to reflux (13.4 km). Those runners who completed at least 50% of the total were included for analysis for all blood analysis and GI symptoms, and all participants were included for GI symptom scores during training. Blood samples could not be obtained at any time point from one participant who completed the race. All athletes were 100% compliant to the in-race water prescription. Adherence to the gel consumption nutritional plan was high with only one gel missed by three participants (2 PLA and 1 PRO).

7.4.3 Physiological response to the marathon race

For all physiological measures, there were no significant differences between PLC and PRO ($p > 0.05$) (Table 7.3). For HR and RPE, data are shown for each third of the race distance. For all participants, RPE increased from the first to the final third ($p < 0.001$) of the race. Post-race blood glucose concentrations increased in both groups relative to pre-race samples ($p < 0.001$).

Table 7.3. Physiological responses to the marathon. Data are mean \pm SD

	Distance	PLC (n=9)	PRO (n=11)
Finish time (min)		247 \pm 47	234 \pm 38
Running speed (%LT)		91.3 \pm 8.7	90.2 \pm 9.1
Heart rate (bpm)	1/3	162 \pm 9	156 \pm 13
	2/3	161 \pm 15	160 \pm 9
	3/3	155 \pm 19	161 \pm 8
RPE	1/3	12.6 \pm 0.7	12.5 \pm 1.0
	2/3	14.5 \pm 0.9	14.8 \pm 1.8
	3/3	16.5 \pm 1.7 ^a	16.3 \pm 2.5 ^a
Blood glucose (mmol·L ⁻¹)	Pre	5.3 \pm 0.6	5.2 \pm 1.1
	Post	7.5 \pm 1.1 ^b	8.4 \pm 1.6 ^b
Haemoglobin (g·dL ⁻¹)	Pre	14.2 \pm 0.9	13.8 \pm 1.6
	Post	13.8 \pm 1.9	13.9 \pm 1.2
Haematocrit (%)	Pre	42.5 \pm 3.2	41.5 \pm 3.7
	Post	42.9 \pm 4.2	42.6 \pm 3.1
PV change (%)		2.1 \pm 8.4	-1.4 \pm 6.8

^a = significantly different to 1/3 race distance ($p < 0.001$), ^b = significantly different to pre-race ($p < 0.001$)

7.4.4 Marathon performance and GI symptomology

Marathon completion times ranged between 152-302 minutes. Mean running speed during each lap for PLC and PRO is shown in Fig 7.3A. As two of the three drop outs from PLC were those with the second and third fastest previous personal bests, it was difficult to compare absolute finish times and running speeds between groups. Therefore, average running speeds were calculated for both groups during each third of the race and relative comparisons were made between each third (Fig 7.3B). During the second third of the race, relative reductions in speed were 3.6 \pm 3.5% for PRO and 6.9 \pm 6.9% for PLC, although this was not significant ($p = 0.165$). During the final third, the reduction in average relative speed was greater in PLC (14.2 \pm 5.8%) compared to PRO (7.9 \pm 7.5%), ($p = 0.04$).

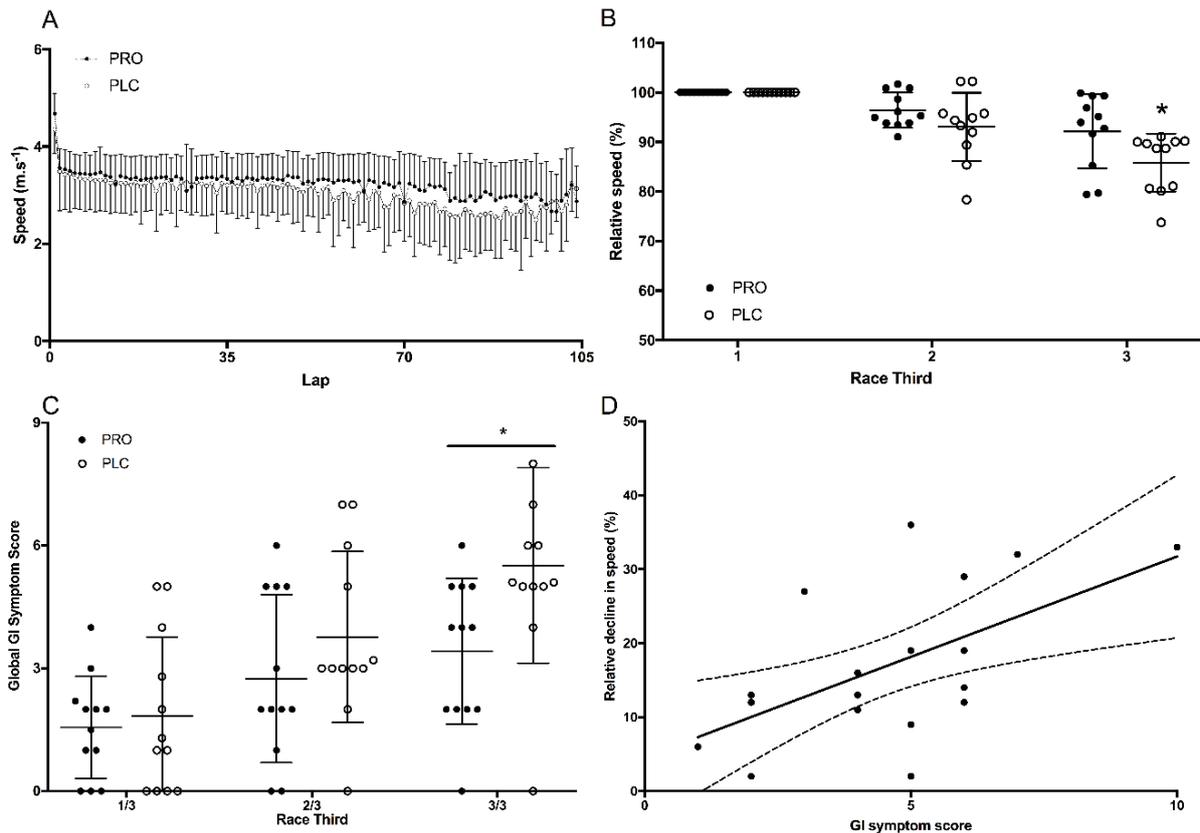


Figure 7.3A: Running speed during each lap of the race. B: Average running speed during each third of the race, relative to first third. * = significant difference between PLC and PRO ($p < 0.05$) C: Global GI symptom scores during each third of the race. * = significant increase compared to PRO. D: Correlation between relative decline in speed and average global GI symptom scores during the final third of the race. $N = 20$

During the race, global GI scores were averaged across each third (Fig 7.3C). There were significant effects of both time and condition. During the second and final third of the race, there were increases in GI symptom severity scores. While differences between groups were not significant for the first ($p = 0.722$) and second ($p = 0.205$) third of the race, GI symptoms were significantly lower in PRO compared with PLC during the final third of the race ($p = 0.010$) (Fig 7.3C). For both groups combined, there was a significant, moderate correlation between average global GI score in the final third, and a reduction in average pace during this final third relative to the first third ($r = 0.562$, $p = 0.010$) (Fig 7.3D).

7.4.5 Circulatory markers of immune activation and GI dysregulation

Differences in sCD14 between PRO and PLC were not significant either pre or post-race (Fig 7.4). For both groups sCD14 was significantly increased post-race (PLC = $8.7 \pm 5.1 \mu\text{g}\cdot\text{mL}^{-1}$, PRO = $9.6 \pm 5.7 \mu\text{g}\cdot\text{mL}^{-1}$) compared to pre-race (PLC $3.3 \pm 1.7 \mu\text{g}\cdot\text{mL}^{-1}$, $p = 0.006$; PRO $3.7 \pm 1.6 \mu\text{g}\cdot\text{mL}^{-1}$, $p = 0.022$). Post-race sCD14 concentrations were significantly correlated with a global GI symptoms reported during the final third of the race ($r = 0.551$, $p = 0.015$).

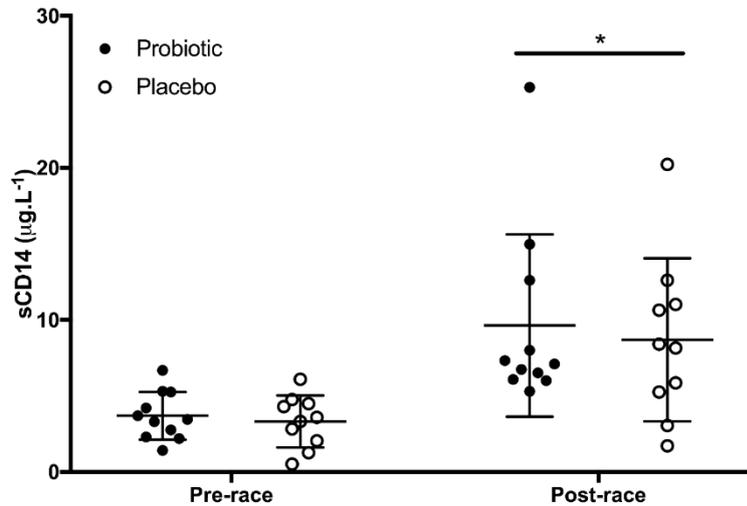


Figure 7.4. Plasma sCD14 concentrations pre and post-race for PRO (n=11) and PLC (n=10) groups. * = significant difference from pre-race ($p < 0.05$).

Differences in LR (Fig 7.4A) and I-FABP (Fig 7.5) were not significant at any time points between PRO and PLC. There was a significant effect of time for LR with a significant difference between pre (PRO = 0.057 ± 0.022 ; PLC = 0.061 ± 0.042) and post-race values (PRO = 0.099 ± 0.062 ; PLC = 0.081 ± 0.036) ($p = 0.040$). Differences in LR pre to post race were due to significant increases in serum lactulose concentration 1h post-race compared to post LR (Fig 7.4B) while there was no significant difference in serum rhamnose concentrations at these time points (Fig 7.4C). For both groups I-FABP was significantly increased post-race (PRO = $1814 \pm 1708 \text{ pg}\cdot\text{mL}^{-1}$; PLC = $1392 \pm 867 \text{ pg}\cdot\text{mL}^{-1}$) compared to pre-race (PRO $455 \pm 190 \text{ pg}\cdot\text{mL}^{-1}$; PLC $460 \pm 221 \text{ pg}\cdot\text{mL}^{-1}$) ($p = 0.0004$). The difference was not significant at 1 hr post-race ($p = 0.925$). Post-race values for LR ($r = -0.250$, $p = 0.289$) and I-FABP ($r = -0.394$, $p = 0.095$) did not significantly correlate with global GI symptom scores.

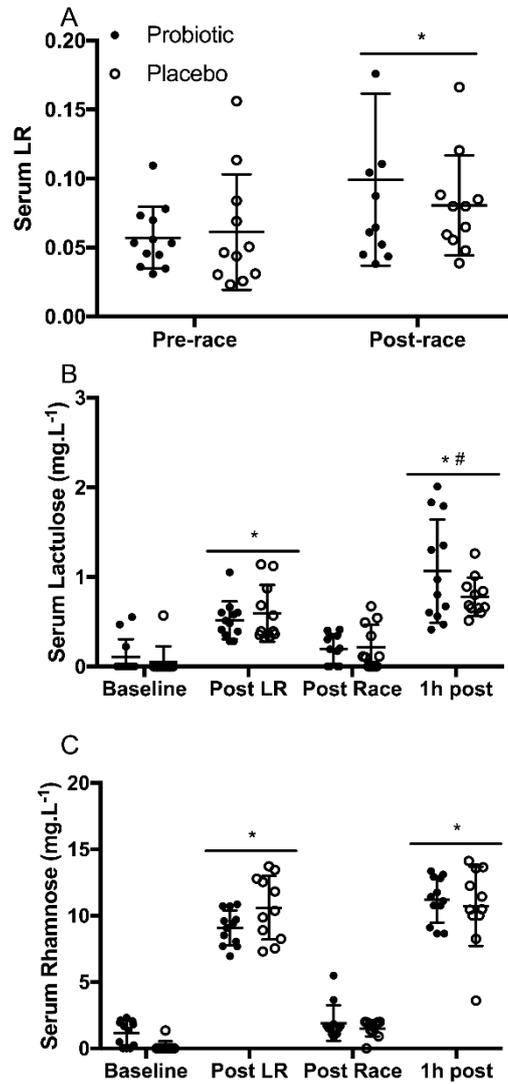


Figure 7.5 Serum lactulose:rhamnose ratio (A), lactulose (B) and rhamnose concentrations (C) at each sampling point race for PRO (n=11) and PLC (n=10) groups. *Significant difference from pre-race ($p < 0.05$), # Significant difference to Post LR ($p < 0.05$).

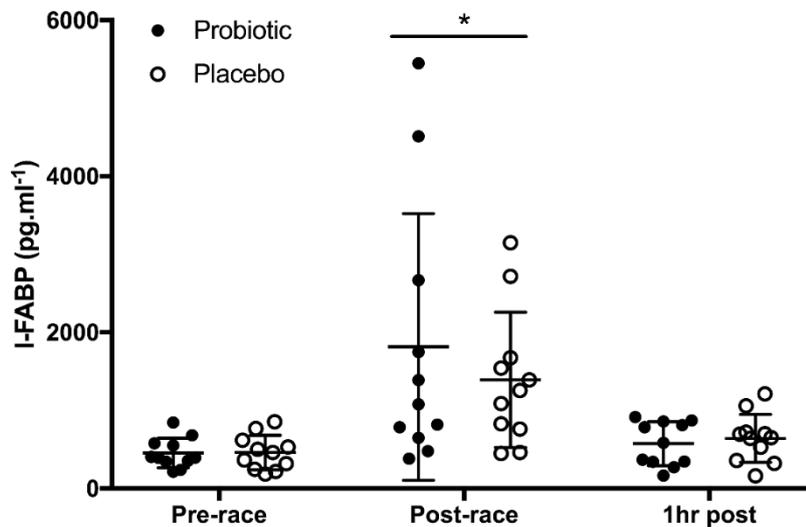


Figure 7.6 Plasma intestinal-fatty acid binding protein (I-FABP) pre, post, and 1hr post-race for PRO (n=11) and PLC (n=10) groups. *Significant difference from pre-race ($p < 0.05$).

The concentrations of IL-6, IL-8, IL-10 and cortisol are presented in Table 7.4. There were no significant differences for either pre- or post-race concentrations between PLC and PRO ($p > 0.05$). For both groups, all measures increased from pre to post-race ($p < 0.001$). Post-race values did not correlate with global GI symptom scores ($p > 0.05$).

Table 7.4. Pre and post-exercise cytokine and cortisol concentrations for PRO (n=11) and PLC (n=10) groups. Data are mean \pm SD.

	PLC		PRO	
	Pre-race	Post-race	Pre-race	Post-race
IL-6 (pg·mL ⁻¹)	0.82 \pm 0.74	13.58 \pm 12.9 ^a	1.01 \pm 0.66	10.96 \pm 7.86 ^a
IL-8 (pg·mL ⁻¹)	2.13 \pm 2.03	12.11 \pm 6.33 ^a	2.24 \pm 0.92	13.45 \pm 11.18 ^a
IL-10 (pg·mL ⁻¹)	0.73 \pm 0.64	5.78 \pm 3.26 ^a	0.91 \pm 0.55	5.27 \pm 3.91 ^a
Cortisol (nmol·L ⁻¹)	614 \pm 119	1083 \pm 196 ^a	653 \pm 155	1148 \pm 242 ^a

^a = significant difference to pre-race ($p > 0.001$)

7.4.6 GI symptoms assessed post-race

Mean scores for specific symptoms immediately post-race and 24 hrs post were all low for both PRO and PLC (≤ 2) with no significant differences between conditions for all symptoms. From these, total, upper, and lower GI symptom scores were calculated, with no significant differences between PRO and PLC (Table 7.5). All participants reported at least one symptom during the race and 24hr post-race (score ≥ 2), while 50% reported at least one moderate symptom (score ≥ 4). Correlations between measures of either any of the individual specific GI measures, or pooled totals (total score, lower GI score, upper GI score) assessed post-race and global GI symptoms were all not significant ($p > 0.05$).

Table 7.5. GI symptoms measured immediately and 24 hrs post-race. Data are presented as median and range

	PLC (n = 11)	PRO (n =10)
Immediately post-race		
Total GI symptom score	15 (3-51)	13 (0-37)
Upper GI symptom score	5 (0-30)	6 (0-16)
Lower GI symptom score	7 (0-21)	10 (0-31)
24hr post-race		
Total GI symptom score	12 (0-43)	16 (0-36)
Upper GI symptom score	4 (0-46)	6 (0-18)
Lower GI symptom score	5 (0-25)	7 (0-28)

7.5 Discussion

The current study is the first to assess the efficacy of probiotic supplementation in reducing GI symptoms in runners during training and in a marathon race. All runners reported GI symptoms in the

present study. However, a range of specific symptoms were reported, as well as a range in severity, with two athletes abandoning the race due to severe GI discomfort. GI symptoms increased in severity during the race and severity of these symptoms appeared to be associated with reductions in running speed. The data presented showed that symptom severity did not correlate with markers of GI damage or permeability but there was an association with sCD14. Finally, runners supplementing with probiotics reported fewer and less severe GI symptoms both in training and during a marathon race. This may have performance benefits, compared to a placebo, although the exact mechanism was not found.

Studies have previously reported that 4-7% of runners report 'moderate' or 'serious' GI symptoms during a marathon race (Pfeiffer et al., 2012, Ter Steege et al., 2008) while the reported incidence was 27% in Chapter 4. In the current study, 50% of participants reported experiencing one or more moderate symptoms during the race. While warmer ambient temperatures are known to increase the prevalence of GI symptoms (Costa et al., 2017a), the temperature during the race in the current study was comparable to those previous (Pfeiffer et al., 2012, Ter Steege et al., 2008). The higher incidence may be due to increased CHO consumption ($66 \text{ g}\cdot\text{hr}^{-1}$) than *ab libitum* intakes reported previously ($35 \pm 26 \text{ g}\cdot\text{hr}^{-1}$) (Pfeiffer et al., 2012) and noted in Chapter 4 ($24 \pm 12 \text{ g}\cdot\text{hr}^{-1}$). Equally, GI symptoms during marathon races are more prevalent when runners ingest food or fluids, but are not accustomed to doing so during training (Ter Steege et al., 2008). While participants were provided with CHO gels to use in two training runs before the marathon race, more opportunities through 'gut-training' runs may have been required. Indeed, two weeks of CHO ingestion during running was shown to reduce lower GI symptoms, and CHO malabsorption, although daily training was utilised (Costa et al., 2017b). It should also be noted a predisposition to GI symptoms and a history of GI symptoms during training is associated with symptoms during running races (Pfeiffer et al., 2012, Ter Steege et al., 2008). A high proportion of runners reported moderate symptoms in the 28 days prior to the race (79%) which may be another factor explaining the higher prevalence of moderate or worse symptoms.

While there was a high prevalence of GI symptoms during the race, there was no association between symptoms and LR or I-FABP concentrations. As there was no significant increase in post-race LR compared to pre-race, the lack of association is unsurprising. Previous data is equivocal in regards to GI permeability measures post-marathon with evidence showing both increases in permeability post-race (Buchman et al., 1999) and no differences compared to resting controls (Smetanka et al., 1999). Post-marathon I-FABP concentrations were significantly elevated compared to pre-race, with some of the highest values reported to date following exercise ($382\text{-}5447 \text{ pg}\cdot\text{ml}^{-1}$). I-FABP has been quantified post-exercise in a number of studies but its association with GI symptoms has often not been considered (Barberio et al., 2015, JanssenDuijghuijsen et al., 2016, Van Wijck et al., 2011). This may

be due to the low frequency of GI symptoms, particularly severe symptoms, reported in studies, as seen in Chapters 5 and 6, even when 'symptomatic' endurance athletes are recruited (Karhu et al., 2017). In the current study, there was no association with I-FABP and any of the measures of GI symptoms. Given the short half-life (Pelsers et al., 2003a, Gollin et al., 1993) and rapid changes in I-FABP concentrations during exercise, as seen in Chapter 5, post-exercise I-FABP concentrations may be affected by changes in running speed, and therefore exercise intensity, at the end of the race, and so not be entirely reflective of mean splanchnic hypoperfusion experienced by runners throughout the race.

While there was no association with GI symptoms and LR or I-FABP, there were significant correlations between a number of the measured GI symptoms and post-race sCD14 concentrations. sCD14 concentrations were elevated post-race, supporting an exercise-associated endotoxaemia shown following extreme endurance exercise (Brock-Utne et al., 1988, Camus et al., 1997, Gill et al., 2015a, Gill et al., 2015b). However, many previous studies have measured plasma LPS concentrations and have found inconsistent associations with GI symptoms. sCD14 is a phosphatidylinositol-linked membrane glycoprotein on polymorphonuclear leucocytes that serves as a receptor for endotoxin and has been suggested to provide a more stable marker for increased exposure to LPS (Liu et al., 1998). In the only other study to investigate the association between sCD14 and exercise-induced GI symptoms, ultramarathon runners reporting symptoms of nausea were found to have significantly elevated plasma sCD14 concentrations post-race (Stuempfle et al., 2016). Interestingly, while sCD14 correlated with a number of symptoms in the present study, including average global symptoms measured during the race, total lower GI symptoms, and vomiting, there was no association between sCD14 and nausea. Perhaps due to differences in participant interpretation of symptoms or due to differences in measurement of symptoms. However, both studies show the potential relationship between GI symptoms during endurance events and sCD14. In studies reporting exercise-induced endotoxaemia, many also show increase cytokine concentrations, believed to contribute to severe exercise-associated GI symptoms (Stuempfle and Hoffman, 2015, Brock-Utne et al., 1988, Camus et al., 1994). It is difficult to show a direct link between the two during exercise given that strenuous exercise is a strong stimulus for cytokine production, independent of GI damage and GI translocation (Pedersen and Hoffman-Goetz, 2000). Furthermore, sCD14 concentrations were not significantly different between groups, despite differences in GI symptoms. Therefore, the effect of probiotics is most likely due to a mechanism other than attenuation of exercise-induced endoxemia.

Evidence of the deleterious effect of GI symptoms on exercise performance has been equivocal. For example, GI symptoms have appeared to negatively affect performance in ultramarathon events (Stuempfle and Hoffman, 2015, Hoffman and Fogard, 2011). However, despite increases in GI

symptoms, which were associated with higher CHO intake, there were no associations with GI symptoms and race performance in a number of endurance races. (Pfeiffer et al., 2012). In part, this may be due to the severity of GI symptoms, and the need to differentiate mild or moderate symptoms, and those that affect performance. In the present study, reductions in running speed during the final third of the marathon were significantly correlated with GI symptoms reported. Although this does not directly imply causation. Reductions in running speed during the final stages of a marathon are known to affect a number of psychological factors and lead to a number of negative emotions (Buman et al., 2008). Given the subjective nature of GI symptoms and their measurement here, GI symptom scores may have been higher or exaggerated in those runners whom were unable to maintain their previous pace, as opposed to GI symptoms being a causative factor in reductions in running speed. However, a comprehensive study has shown that a 'gut-training' protocol that was able to reduce GI symptoms associated with CHO malabsorption during exercise, also resulted in an increase in endurance performance (Miall et al., 2018). This may be a potential mechanism in the present study by which runners undergoing probiotic supplementation reported less severe GI symptoms and were better able to maintain running pace during the final third of the race.

In conclusion, the data presented here shows that during a marathon race, a range in the severity and types of GI symptoms are reported. The severity of these symptoms reported here were associated with reductions in running speed during the final third of the race. Those runners who supplemented with probiotics during the 28 days prior to the race reported significantly fewer GI symptoms during training, and during the marathon and this may have resulted in the maintenance of exercise performance during the latter stage of the race. Finally, sCD14 concentrations correlated with a number of measured GI symptoms while LR and I-FABP did not. There was no difference between supplement groups for any markers of GI permeability, damage or endotoxaemia. Therefore, probiotics offer a promising strategy to reduce the incidence and severity of GI symptoms in endurance runners. While the mechanism could not be elucidated, it does not appear to be due to attenuation of GI damage and endotoxaemia.

Chapter 8 - Synthesis of Findings

8.1 Realisation of Aims

8.1.1 Aim 1 - Investigate the prevalence and severity of GI symptoms during exercise

This aim was addressed in Chapter 4 and Chapter 7. In Chapter 4, 70% of runners reported experiencing symptoms during a marathon race, while only 27% were scored as “moderate” or worse (≥ 4 out of 7). In Chapter 7, 75% of participants reported moderate or worse symptoms during the experimental marathon race (≥ 4 out of 10). This difference may be due to a number of factors. As this is the first-time symptoms have been reported in real-time, during a race, this may be a reflection of a discourse between symptoms experienced during the race, and under-reporting post-race. It may also be due to the difference in *ab libitum* water and CHO intake between recreational runners in Chapter 4 ($\sim 350 \text{ mL}\cdot\text{hr}^{-1}$ and $\sim 25 \text{ g}\cdot\text{hr}^{-1}$, respectively) and that which participants consumed in Chapter 7 ($\sim 750 \text{ mL}\cdot\text{hr}^{-1}$ and $\sim 66 \text{ g}\cdot\text{hr}^{-1}$). It may also be due to differences in the scale used (GSRS in Chapter 4 and a visual analogue scale in Chapter 7). Therefore, in recreational marathon runners, 27-75% of participants have reported moderate to severe GI symptoms. This highlights the need to investigate the aetiology of GI symptoms, and look for potential strategies to alleviate or temper them.

8.1.2 Aim 2 - Explore the role of different circulatory markers to measure GI permeability and damage, and their relation to GI symptoms

This aim has been addressed in Chapters 5, 6, and 7. In Chapters 5 and 6 symptoms were generally low, and there was no correlation between I-FABP or LR and any GI symptom (or accumulation of symptoms). While symptoms were more frequent and more severe in Chapter 7, there was still no association between symptoms and LR or I-FABP. There are a number of reasons for the lack of evidence for a relationship between markers of exercise-induced GI permeability and damage and symptoms. There may be no causal link. However, there is evidence to show links between increases in GI permeability and symptoms in Irritable Bowel Syndrome patients (Shulman et al., 2014, Zhou et al., 2009). The aetiology of GI symptoms, particularly during exercise, is varied, and so may not always be due to increases in permeability. Whilst increases in GI permeability or damage might be a causative factor, this relationship may be difficult to elucidate when other factors (e.g. nutrition) might also be contributing to GI symptoms in the absence of GI permeability or damage.

In Chapter 7, it was observed that plasma sCD14 concentrations, a non-specific marker of monocyte activation, post-race were associated with a number of measured GI symptoms. Specifically, post-race sCD14 concentrations were significantly correlated with mean GI scores during the final third of the marathon race, accumulated total and lower GI scores assessed immediately post-race and total GI symptom score 24hr post-race. Taken together results from this specific objective of the thesis have

suggested that there is a relationship between this marker of immune activation and the severity of GI symptoms, but not LR or I-FABP. Strategies to attenuate the rise in sCD14 concentrations during exercise warrants future studies as a method to reduce the prevalence and severity of GI symptoms.

8.1.3 Investigate the efficacy of probiotics in reducing GI symptoms during prolonged exercise

In Chapter 6, both total and exogenous CHO oxidation were increased following 4 weeks of probiotic supplementation. Furthermore, this was reflected with reductions in fat oxidation and plasma NEFA and glycerol concentrations. GI symptoms of discomfort were low throughout all trials though and, as such, the efficacy of probiotics could not be elucidated. However, the increase in exogenous CHO with probiotics shows a potential mechanism that could lead to reductions in GI symptoms during endurance exercise in which CHO is ingested.

In Chapter 7, moderate or worse GI symptoms were reported by 15 of the 20 finishers during the marathon. Furthermore, those participants in the probiotic supplemental group reported lower symptoms during the final third of the race. During this time in the race, participants had consumed between ~120-300 g CHO. The use of probiotics therefore represents a potential strategy to reduce the prevalence and severity of GI symptoms. That they did not have a significant effect on sCD14 concentrations suggests that their effects were not via alterations in immune response. Future studies are required to identify the mechanism by which this beneficial effect is mediated.

8.1.4 Investigate the efficacy of probiotics to reduce GI permeability and damage during exercise

In Chapter 6, participants cycled for 120 minutes at 55% W_{max} (~53% $\dot{V}O_{2max}$). Following this, there were no increases in markers of GI permeability or damage, and GI symptoms were low during all trials. Therefore, the efficacy of probiotics to attenuate circulatory measures of GI permeability and GI damage could not be established. In Chapter 7, probiotic supplementation was associated with fewer moderate and severe GI symptoms during both 28 days of training, and during a competitive marathon race in recreational runners. However, there was no significant difference between placebo and probiotics on markers of GI permeability (LR) or damage (I-FABP), nor circulating cytokines or sCD14. All of these measures were increased pre to post-race, with some of the highest post-exercise I-FABP concentrations recorded. Therefore, the multi-strain probiotic used did not appear effective at attenuating exercise-induced GI permeability or damage although it did appear effective in reducing symptoms and indirectly increasing performance. Probiotic supplementation may therefore be an appropriate therapeutic strategy for use with athletes, particularly endurance athletes.

8.2 General Discussion

8.2.1 The prevalence and aetiology of gastrointestinal symptoms during endurance running

GI symptoms during sport can range from those which are mild that do not appear to effect exercise performance (Peters et al., 1999) to more severe symptoms which may impact performance or lead to withdrawal from competition (Hoffman and Fogard, 2011) and even requiring medical observation and treatment (Sanchez et al., 2006). GI symptoms typically assessed include those considered to be of the upper GI tract (e.g. heartburn, nausea, belching, vomiting) and the lower GI tract (e.g. bloating, flatulence, increased urge to defecate, abdominal cramps and diarrhoea). This wide range of symptoms, each with their own potential causative factors, make it difficult to identify, isolate or attribute a single aetiopathology. GI Symptoms have been best characterised in endurance athletes, with little data for other sports. Those sports reporting the highest incidence of GI symptoms are typically those involving running exercise, as opposed to cycling (Pfeiffer et al., 2012), and of greater durations. For example, the greatest prevalence of symptoms have been reported in those athletes competing in ultra-endurance running events such as 60 km and 120 km ultramarathon (82% of participants) (Wardenaar et al., 2015), 161 km ultramarathon (96%) (Stuempfle and Hoffman, 2015), and 24 hr continuous running (73%) (Costa et al., 2016). In comparison, the prevalence of symptoms in those running a 42.2 km marathon generally appears to be lower, although there is a large variation between studies with reported incidence rates of between 4-71% (Pfeiffer et al., 2012, Peters et al., 1999, Rehrer et al., 1989). In Chapter 4, 27% of recreational runners reported as experiencing symptoms during a marathon race described as moderate or worse, a figure similar to that reported in a small group of elite marathon runners (Holmich et al., 1988). Variations between studies could be due to a number of factors such as differences in participant characteristics and/or environmental temperatures, which have been shown to exacerbate gastrointestinal damage and increase symptoms (Lambert, 2008), and variance in the questionnaires used, the symptoms that are included, and the criteria for classifying a symptom. As well as during competitive racing, runners also appear to have a significant prevalence of GI symptoms during training. In Chapter 4, 42% of runners reported moderate or worse symptoms in the 7 days prior to a marathon race. In Chapter 7, 79% of participants reported moderate or worse GI symptoms in the 28 days prior to the marathon race. These values corroborate previous data that have shown GI symptoms to occur at rest or during exercise in 20-70% of runners (Peters et al., 1999, Keeffe et al., 1984).

8.2.2 Relationship between gastrointestinal symptoms and circulatory markers

The reported prevalence rates of GI symptoms warrants the further investigation with regard to their aetiology and subsequent development of interventions that may attenuate symptoms. This may then lead to increased athletic performance, quality of life, and health. Due to the number of mechanisms

that can lead to GI symptoms, it can be difficult to investigate them in isolation. There are also individual variations and tolerance levels within each of these factors, which may explain the lack of absolute relationships found to date. Furthermore, there are methodological considerations for many of the circulatory markers used to assess GI permeability and damage, and endotoxaemia that impact their efficacy.

The comparison of the ratio between a larger molecule, such as lactulose, and a smaller molecule, such as rhamnose has long been used to give an index of intestinal permeability (Camilleri et al., 2010), while elevated I-FABP concentrations is suggested to indicate intestinal epithelial cell damage (Bischoff et al., 2014). In this thesis, both LR and I-FABP have been analysed and shown to increase following HIIT exercise (Chapter 5) and a marathon race (Chapter 7) but with no significant association to symptomology. This finding reflects that observed in other field-based studies where intestinal permeability was not significantly related to symptoms during both a half and full marathon (Smetanka et al., 1999, Oktedalen et al., 1992). Furthermore, an inverse correlation between exercise-induced GI permeability and symptoms has also been reported (Costa et al., 2017b) where those with the highest GI permeability reported the lowest symptoms, a finding that has also been found with I-FABP (Costa et al., 2017b). The mechanism by which increased permeability is purported to lead to GI symptoms is via the increased translocation of endotoxin, antigens, leading to a localised immune and inflammatory response (Costa et al., 2017a, van Wijck et al., 2012a). However, it has been suggested that small, inert soluble markers used in these studies do not trace larger macromolecular permeability and so may not reflect antigen handling by the gut (Menard et al., 2010). An intestine leaky to these small molecules (<350 Da) can be tight to macromolecules (>10000 Da) which are most likely to challenge the immune system (Perrier and Cortesy, 2011). This may then explain some of the association between LR and GI symptoms reported by participants in this thesis. However, while increases in GI permeability and damage leading to antigen translocation and potential immune activation is one mechanism of GI symptoms during endurance exercise, it is not the only one. This is perhaps best exemplified by data showing that protein ingestion during exercise reduces markers of GI permeability yet increases subjective GI symptoms (Snipe et al., 2017), suggesting a different mechanism for symptoms.

As well as GI permeability (LR) and damage (I-FABP), many studies have reported increased cytokine concentrations following endurance exercise (Stuempfle and Hoffman, 2015, Brock-Utne et al., 1988, Camus et al., 1994). It is difficult to interpret the relationship of such measures with GI symptoms though given that strenuous exercise is a strong stimulus for cytokine production, independent of GI damage and GI translocation (Pedersen and Hoffman-Goetz, 2000). This may explain the lack of any significant correlations between plasma cytokine and GI symptoms in Chapter 7. Findings in this study

show the potential relationship between GI symptoms during endurance events and immune activation, as measured by sCD14. The association found between sCD14 and exercise-induced GI symptoms is in some agreement to previous research in ultramarathon (Stuempfle et al., 2016). However, there is a paucity of further data for sCD14 concentrations post-exercise. Data in Chapter 7 provides rationale for future study for this as a predictive marker for GI symptoms, as well as investigation of those signalling pathways associated with increased sCD14 concentrations in order to better explain the exact aetiology of exercise-induced GI symptoms.

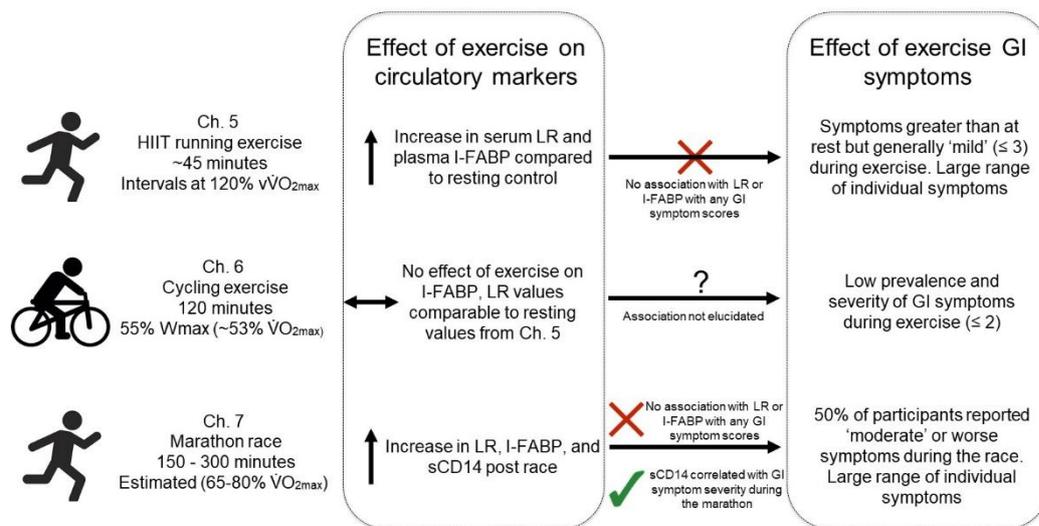


Figure 8.2. Schematic representation of the main findings in this thesis in regards to the relationship between exercise-induced GI symptoms and circulatory measures of GI permeability and damage.

8.2.3 The efficacy of probiotics to reduce GI symptoms during endurance exercise

In this thesis, probiotic supplementation has been shown to be associated with fewer and less severe GI symptoms in weeks prior to and during a marathon race. There was no difference between probiotic and placebo groups for circulatory markers of GI permeability or damage though. This is not surprising given the lack of association between GI symptoms and these circulatory markers, as previously discussed. While there was a relationship between sCD14 and GI symptoms, there was no effect of probiotic supplementation on this marker in Chapter 7. This suggests that either sCD14 was not sensitive enough to detect any beneficial effects of probiotics on GI-induced immune activation, or that the attenuation of GI symptoms was due to another mechanism.

In Chapter 6, data was presented showing that probiotic supplementation altered exercise metabolism when CHO feeding was implemented. Specifically, probiotic supplementation increased oxidation of the ingested CHO. While not directly measured, this increase in exogenous oxidation may be due to alterations in glucose absorption in the duodenum, thought to be the main rate-limiting

step in exogenous CHO oxidation during exercise (Jeukendrup and Jentjens, 2000). It is well established that malabsorption of consumed CHO is a contributing factor to GI symptoms during exercise (Costa et al., 2017b), and that there is a deleterious effect of exercise-induced GI damage on CHO absorption (Zuhl et al., 2012). Therefore, while it may not be possible to assess this in field studies, this is a likely factor for the high prevalence of GI symptoms in endurance races where intensity and duration are sufficient to lead to increased GI permeability and damage, and CHO malabsorption. Probiotic supplementation could therefore offer a strategy to reduce GI symptoms associated with CHO malabsorption.

Participants in Chapter 7 reported that they did not typically consume CHO during training runs, and so the effects of probiotic supplementation on GI symptoms during the second half of the supplement period is most likely not due to any effects on CHO absorption or reductions in malabsorption. As no further measures were taken during this time-period, the exact mechanism for the lower incidence and severity of GI symptoms with probiotics can only be postulated. Probiotics have been shown to mediate both local and systemic pathways that may affect GI symptom prevalence and severity. For example, animal models show probiotics can affect enteric neuron excitability and up-regulate cannabinoid receptors, leading to visceral analgesia, and alter brain neurotransmitters involved in pain modulation (Roman et al., 2018). The use of probiotics to attenuate daily GI symptoms, especially amongst symptomatic athletes, requires further study, given that there is likely separate aetiological factors for exercise-induced GI symptoms and habitual, daily GI symptoms.

Finally, a consideration with probiotic bacteria is that the effects of supplementation are likely to have specific effects at both the species and strain level (Hill et al., 2014). It should also be considered that the colonisation of probiotic bacteria within the GI tract appears to be highly individualised (Zmora et al., 2018) and so is likely a factor for individual response to supplementation. These factors make practical recommendations difficult. Where possible, probiotic supplements should be used based upon the available literature, even if limited. Probiotic species, strains and supplemental dose should be chosen depending on the primary aim of supplementation (e.g. reduce GI symptoms or increase athlete immunity). The probiotic supplements used in this thesis appear to be effective at reducing exercise-related GI symptoms based upon findings here and elsewhere (Roberts et al., 2016). The efficacy of these probiotics for other outcome measures (e.g. traveller's diarrhea, stress and anxiety) is unknown however.

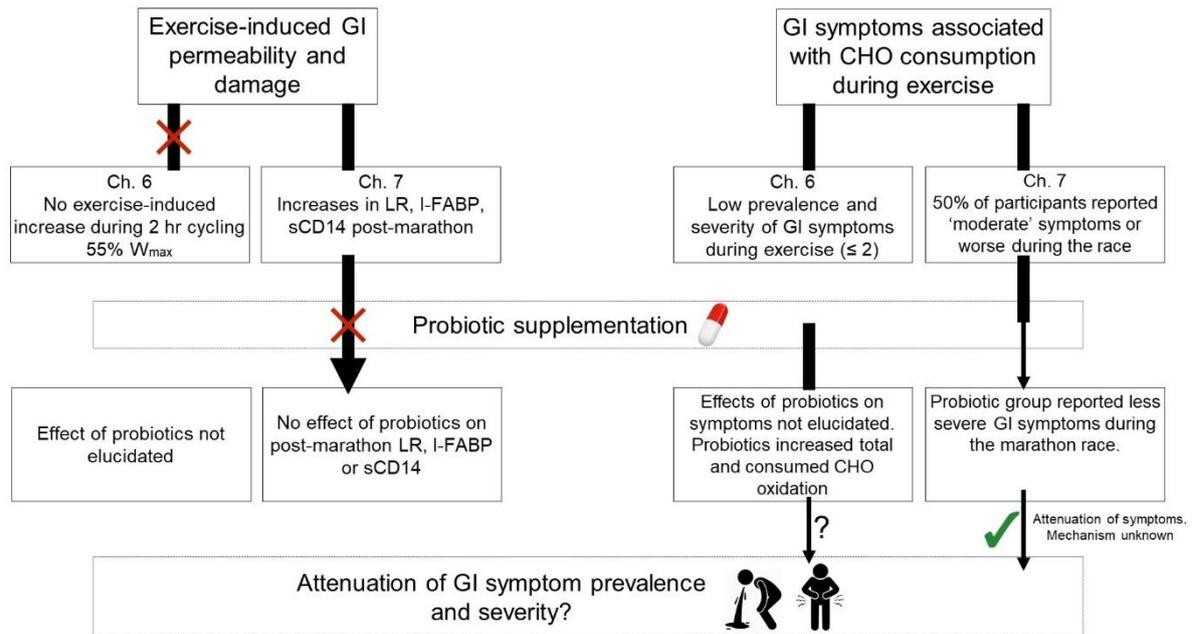


Figure 8.3 Schematic representation of the main findings in this thesis in regards to the efficacy of probiotics to reduce GI symptoms during endurance exercise

8.3 Thesis Limitations

8.3.1 Chapter 4

A limitation in Chapter 4 was the use of food diaries to analyse nutritional habits, with previous research showing a potential under-reporting effect of up to 20% (Burke and Deakin, 2006). It is possible that macronutrient, fibre, and fluid intakes reported were under-estimated. However, total calories and CHO intake reported were in close agreement with previous studies in recreational marathon runners (Atkinson et al., 2011, Wilson et al., 2013). As well, a reduction in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) intake has been shown to alleviate GI symptoms in endurance athletes (Lis et al., 2018). However, FODMAP content was not quantified, and this may have been a factor associated with symptoms seen. Additionally, both marathon races included had over 20,000 participants combined, yet only 216 runners registered to participate, with 96 of these runners completing the online questionnaires to sufficient detail to be included in the dataset. Therefore, the results could be liable to non-response bias (Sedgwick, 2013) raising the possibility that the data are exaggerating the prevalence of GI symptoms, particularly if those who experience symptoms had more interest in the research project, and therefore were more likely to participate in a related study. Finally, as with any study of an observational nature, there are other confounding variables, otherwise not accounted for, whilst it is also difficult to draw any conclusions of cause-and-effect.

8.3.2 Chapter 5

The major limitation in Chapter 5 was that there was no steady-state exercise to act as a control. Had a workload-matched, steady state running protocol been included, this may have shown whether HIIT caused additional damage due to the activity profile. Additionally, the participant cohort did not include any females, as also in Chapter 6. Given that GI symptoms appear to be more prevalent in female athletes (de Oliveira et al., 2014), studies should look to examine any separate effects of exercise in females where aetiology of symptoms may differ. Additionally, while splanchnic hypoperfusion is considered the primary factor in exercise-induced intestinal damage, it was not directly measured, due to obvious technical difficulties this would incur during high intensity running. This is a limitation in Chapters 6 and 7 also. However, I-FABP has been shown to closely correlate to splanchnic hypoperfusion as assessed by gastric tonometry (Van Wijck et al., 2011). Finally, exercise-induced hyperthermia has been suggested to be associated with increases in GI permeability and damage (Pires et al., 2016). A limitation here, and in Chapters 6 and 7 then, is that there were no measures of core temperature. This may have accounted for individual variance in the magnitude of change in some of the markers of damage and permeability, although this has been disputed (Kitic, 2018).

8.3.3 Chapter 6

In Chapter 6, cycling exercise modality was chosen due to the technical issues of regular blood sampling during running (Achten et al., 2003). This is more than likely a factor in the low frequency and severity of GI symptoms that were found. There is typically a greater incidence and severity of GI symptoms during running compared to other exercise modes such as cycling (Costa et al., 2017a). Had running been used as the exercise modality, there would have more than likely been a greater incidence of GI symptoms, which would have better helped answer the research question.

It has been suggested that when ^{14}C glucose was added to insoluble starch, the exogenous oxidation rates calculated may have been overestimated due to differences in absorption of the different CHO sources (Hawley et al., 1991). For the quantification of exogenous glucose oxidation in Chapter 6, a drink containing maltodextrin was further enriched using ^{13}C glucose. However, oxidation rates of glucose and maltodextrin have previously been compared and have been shown to be similar (Rehrer et al., 1992b).

As participants were required to complete the exercise in a fasted state, this will have had an effect on exercise metabolism (Edinburgh et al., 2018). Athletes are generally recommended to begin exercise performance in a fed state (Burke et al., 2011) with the majority of studies showing that pre-exercise feeding enhances prolonged aerobic performance (Aird et al., 2018). Therefore, the

differences observed in exercise metabolism in Chapter 6 may not be applicable to the real-world athlete completing in a fed state. However, the proof of concept observed warrants future research to investigate the effects of probiotic supplementation on exercise metabolism when athletes are in the fed state.

8.3.4 Chapter 7

While the study holds methodological rigour in standardising many of the variables that can affect the incidence of exercise-induced symptoms such as pre-race nutrition, the use of standard, absolute CHO and water intake may not have been the optimum in-race nutrition strategy for each individual participant. It is known that many components of digestion can vary between individuals such as gastric emptying (Costill and Saltin, 1974, Leiper et al., 2001, Rehrer et al., 1992b), and that exogenous oxidation rates can also vary (Rehrer et al., 1992b, Wagenmakers et al., 1993b). Individualised strategies for each participant would have required prior testing, some of which may have been invasive, and so was not possible in the current study. While participants were instructed to consume all of the contents of each gel, this was not assessed systematically and so the possibility remains that some participants consumed less and therefore would not have reached the prescribed CHO intake. Sweat rates can also vary between athletes (Baker et al., 2016), and so runners have experienced differing levels of dehydration, another factor that has been shown to affect GI symptoms (Glance et al., 2002, Lambert et al., 2008, Rehrer et al., 1989).

8.4 Future Directions

Circulatory markers of GI permeability and damage have been shown to have clinical relevance. However, the lack of relationship with GI symptoms seen in this thesis, and in previous studies, reinforces both the complexities and difficulties in assessing the deleterious effects of exercise on the GI tract, its ability to retain functionality during endurance exercise, and the mechanisms of individual GI symptoms. Laboratory based studies have often failed to replicate the demands placed upon the GI tract during endurance races. Therefore, while many of these studies report increases in circulatory markers, many have not reported GI symptoms or have only observed infrequent, mostly mild symptoms. This is likely due to two key factors. First, these studies have often utilised exercise modalities of shortened duration (< 2hr) or lower intensity ($\leq 70\% \dot{V}O_{2max}$) than those races in which GI symptoms are most prevalent. The majority of these have also utilised nutritional practices not commonly utilised by athletes such as undertaking exercise in a fasted state, or without feeding carbohydrate at the rate recommended to enhance performance. These factors most likely explain the low incidence of GI symptoms reported. These factors should be considered for future research

design when for studies to assess the efficacy of interventions to reduce GI permeability or to further examine the aetiology of specific GI symptoms. Conversely, field-based studies have reported a significant prevalence of GI symptoms during a range of endurance race modalities and distances but have found unclear and inconsistent associations with circulatory markers (e.g. Jeukendrup et al., 2000; Smetanka et al., 1999). Future field-based studies, must therefore consider an array of circulatory and subjective measures, although more invasive measures could be needed to fully understand the aetiology of symptoms. The association between sCD14 and GI symptoms during the marathon race does though pose new questions:

1. What signalling pathways mediate the onset and severity of GI symptoms during endurance exercise?
2. Where within this signalling pathway does the increase in sCD14 reside, and can this pathway be utilised to identify new, novel markers associated with GI symptoms?
3. What is the contribution of the GI tract in the increase in sCD14 concentrations during endurance exercise?
4. Given that the increased concentrations were associated with GI symptom severity, would strategies to reduce this immune activation marker result in the attenuation of GI symptoms?

It has also been shown in the current investigations that exogenous CHO oxidation during endurance exercise is increased following 28 days supplementation of probiotics compared to 4 weeks of placebo, as described in Chapter 6. Given that it has been shown that exogenous CHO oxidation is impaired during exercise in the heat relative to more temperate environmental conditions (Jentjens et al., 2002), probiotics may attenuate such reductions, thus maintaining blood glucose and sparing muscle glycogen, possibly providing an ergogenic effect. Equally, it has regularly been shown that, in order to oxidise the purported maximum of $1 \text{ g}\cdot\text{min}^{-1}$ of consumed glucose, the ingestion of $1.2\text{-}1.5 \text{ g}\cdot\text{min}^{-1}$ is required (Jeukendrup and Jentjens, 2000). Taken with the data from Chapter 6, this poses the following questions:

1. Can probiotics increase the oxidation of exogenous CHO during exercise in extreme environments, in which it is usually compromised?
2. Would the increase in efficiency of consumed CHO lead to reductions in malabsorption of CHO and subsequently attenuate GI symptoms?
3. What are the most effective probiotic species and strains, as well as dose, for this benefit in CHO oxidation?
4. Does the baseline GI microbiota of individuals impact this effect of probiotics?

If studies were performed it would also be of greater ecological validity if undertaken by athletes in a fed state. Finally, by selecting running as the exercise modality as opposed to cycling, the efficacy of probiotic to reduce GI symptoms would also be more likely to be elucidated given that symptoms are more regularly reported in these athletes, as shown by data in this thesis.

8.4 Conclusions and implications

As described in the opening chapter of this thesis, and as demonstrated by the data presented, GI symptoms are frequently reported amongst endurance athletes. This is both during training and daily activities, as well as during competition. Data presented in this thesis also shows that such symptoms can be related to decrements in performance (Chapter 7). Investigations to understand the mechanisms of such symptoms, and potential strategies to alleviate them are therefore warranted. It has been shown that circulatory markers of GI permeability and damage are not associated with reported GI symptoms during endurance exercise models used here. While the attenuation of these markers may have implications in clinical investigations, their attenuation is not necessary in regards to supporting athletes with chronic GI symptomology. This is further exemplified by the efficacy of probiotic supplementation to temper the rise increase in GI symptom severity during a marathon race shown, without subsequent effects on a number of circulatory markers. This thesis has shown that probiotics are a simple strategy to reduce GI symptom prevalence and severity in athletes (chapter 7). Finally, the initial work showing the effects of probiotic supplementation on exercise-metabolism is the first of its kind. This data shows the benefits of probiotics to increase the efficiency of oxidation of consumed carbohydrate; an effect with potential performance implications. Probiotic supplementation therefore appears to be a safe and simple strategy for athletes whom regularly suffer from GI discomfort, or those who consume large amounts of carbohydrate during exercise to consider.

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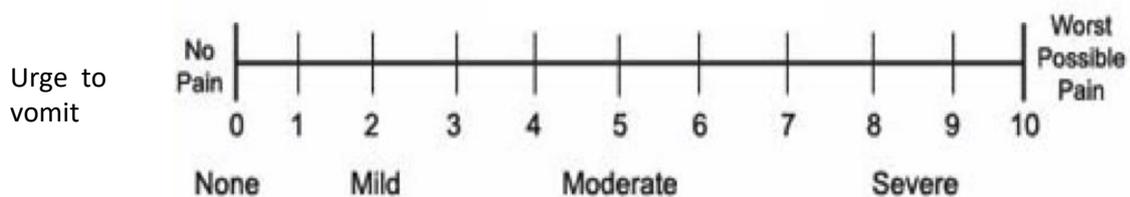
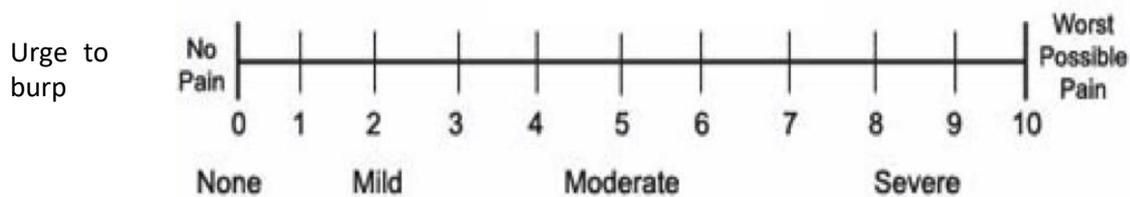
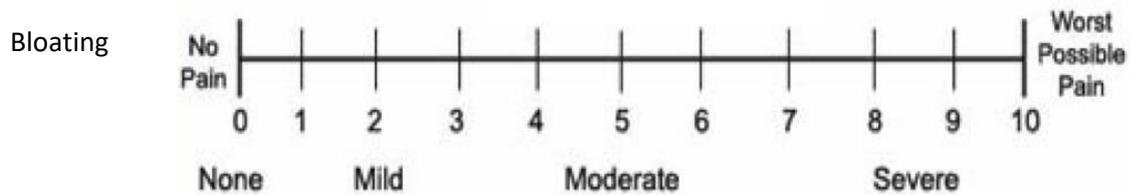
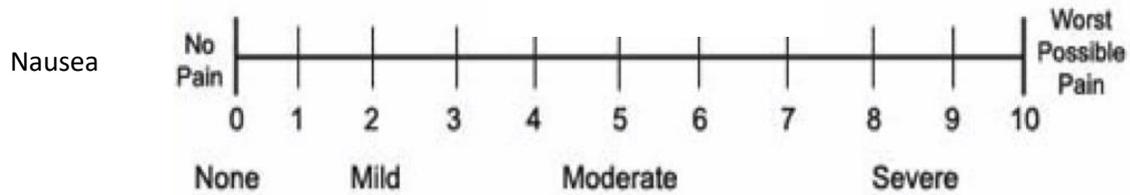
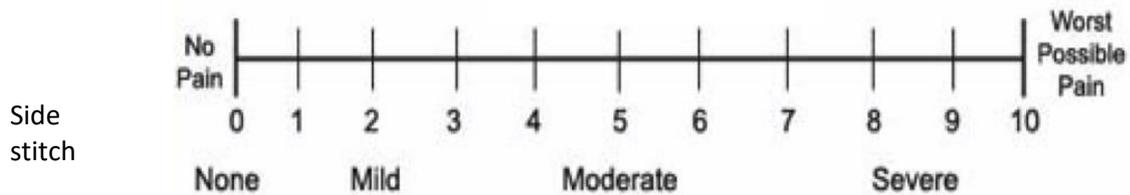
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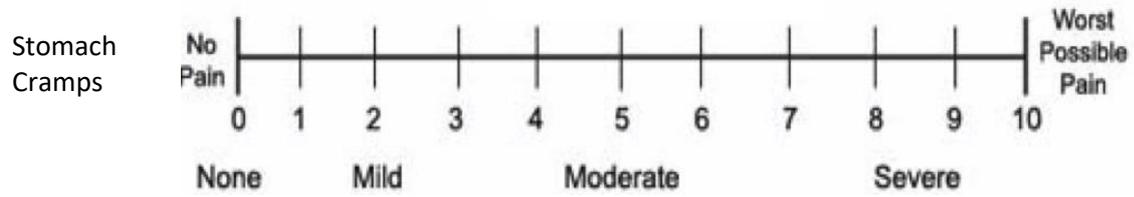
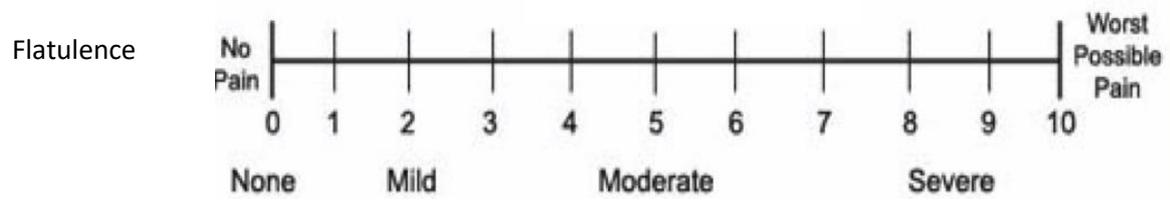
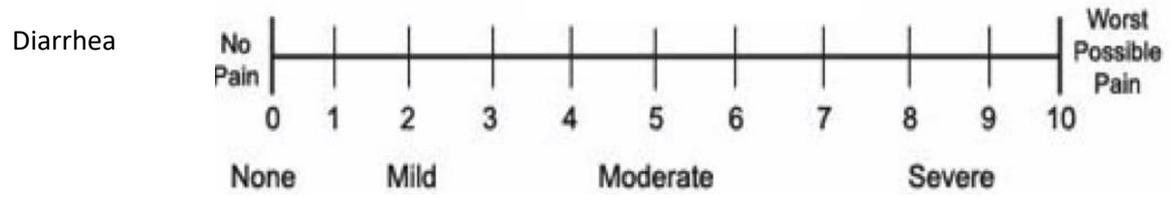
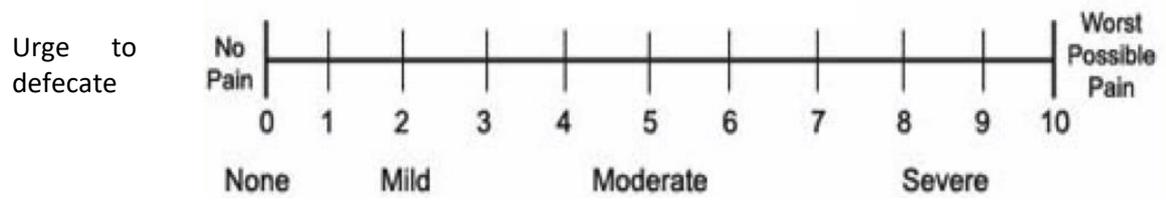
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Appendix 1 – Adapted symptom questionnaire

Below are a series of scales 0-10 regarding symptoms of gastrointestinal symptoms. No symptoms is 0 and 10 is extreme symptoms.





Appendix 2 – Gastrointestinal Symptom Rating Scale

The GASTROINTESTINAL SYMPTOM RATING SCALE (Svedlund et al., 1988a)

This survey contains questions about how you have been feeling and what it has been like DURING THE PAST WEEK. Mark the choice that best applies to you and your situation with an X in the box.

	No discomfort at all	Minor discomfort	Mild discomfort	Moderate discomfort	Moderately severe discomfort	Severe discomfort	Very severe discomfort
Have you been bothered by PAIN OR DISCOMFORT IN YOUR UPPER ABDOMEN OR THE PIT OF YOUR STOMACH during the past week?							
Have you been bothered by HEARTBURN during the past week? <i>By heartburn we mean an unpleasant stinging or burning sensation in the chest</i>							
Have you been bothered by ACID REFLUX during the past week? <i>By acid reflux we mean the sensation of regurgitating small quantities of acid or flow of sour or bitter fluid from the stomach up to the throat</i>							
Have you been bothered by HUNGER PAINS in the stomach during the past week? <i>A hollow feeling in the stomach associated with the need to eat between meals</i>							
Have you been bothered by NAUSEA during the past week? <i>By nausea we mean a feeling of wanting to throw up or vomit</i>							
Have you been bothered by RUMBLING in your stomach during the past week? <i>Rumbling refers to vibrations or noise in the stomach</i>							
Has your stomach felt BLOATED during the past week? <i>Feeling bloated refers to swelling often associated with a sensation of gas or air in the stomach.</i>							

<p>Have you been bothered by BURPING during the past week?</p> <p><i>Burping refers to bringing up air or gas from the stomach via the mouth, often associated with easing a bloated feeling</i></p>							
<p>Have you been bothered by PASSING GAS OR FLATUS during the past week?</p> <p><i>Passing gas or flatus refers to the need to release air or gas from the bowel, often associated with easing a bloated feeling</i></p>							
<p>Have you been bothered by CONSTIPATION during the past week?</p> <p><i>Constipation refers to a reduced ability to empty the bowels</i></p>							
<p>Have you been bothered by DIARRHOEA during the past week?</p> <p><i>Diarrhoea refers to a too frequent emptying of the bowels</i></p>							
<p>Have you been bothered by LOOSE STOOLS during the past week?</p> <p><i>If your stools (motions) have been alternately hard and loose, this question only refers to the extent you have been bothered by the stools being loose</i></p>							
<p>Have you been bothered by HARD STOOLS during the past week?</p> <p><i>If your stools (motions) have been alternately hard and loose, this question only refers to the extent you have been bothered by the stools being hard</i></p>							
<p>Have you been bothered by an URGENT NEED TO HAVE A BOWEL MOVEMENT during the past week?</p> <p><i>An urgent need to go to the toilet is often associated with a feeling that you are not in full control</i></p>							

<p>When going to the toilet during the past week, have you had the SENSATION OF NOT COMPLETELY EMPTYING THE BOWELS?</p> <p><i>A feeling of incomplete emptying means that you still feel a need to pass more stool despite having exerted yourself to do so</i></p>							
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