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

Jamie N. Pugh[1], Samuel G. Impey[1], Dominic A. Doran[1], Simon C. Fleming[2], James P. Morton[1], Graeme L. Close[1]

[1]Research Institute for Sport and Exercise Sciences

Liverpool John Moores University

United Kingdom

[2]Royal Cornwall Hospital

Truro, UK

Corresponding author:

Professor Graeme Close

Research Institute for Sport and Exercise Sciences

Liverpool John Moores University

Tom Reilly Building

Byrom St Campus

Liverpool

L3 3AF

United Kingdom

Email: G.L.Close@ljmu.ac.uk

Tel: +44 151 904 6266

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:rhamnose ratio (0.051 ± 0.016 vs. 0.031 ± 0.021, $p = 0.0047$; 95% CI $= 0.006 - 0.036$) and sucrose concentrations (0.388 ± 0.217 vs 0.137 ± 0.148 mg.l^{-1}; $p < 0.001$; 95% CI $= 0.152 - 0.350$). In contrast, urinary lactulose:rhamnose (0.032 ± 0.005 vs 0.030 ± 0.005; $p = 0.3$; 95% CI $= -0.012 - 0.009$) or sucrose concentrations ($0.169 \pm 0.168\%$ vs $0.123 \pm 0.120\%$; $p = 0.54$; 95% CI $= -0.199 - 0.108$) 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, we provide data showing that it is possible to detect changes in intestinal permeability that is not observed using urinary sampling over a shorter time-period.

Key Words: HIIT Exercise, I-FABP, intestinal permeability, gastrointestinal discomfort

Abbreviations: ANOVA Analysis of variance

ELISA Enzyme-linked immunosorbent assay

GI Gastrointestinal

HIIT High-intensity interval training

HR Heart rate

I-FABP Intestinal-fatty acid binding protein

L:R Lactulose:rhamnose ratio

NSAID non-steroidal anti-inflammatory drug

RPE Ratings of perceived exertion

TC Thermal comfort

Introduction

Gastrointestinal (GI) discomfort is common among distance runners, with prevalence estimated to range between 30-90%, depending upon the event, sex and the screening methods (de Oliveira et al. 2014). During ultra-endurance running events, as many as 96% of participants have been reported as experiencing GI symptoms (Stuempfle et al. 2015). Symptoms expressed during endurance exercise typically include nausea, cramping, bloating and diarrhoea (Peters et al. 1999; Ter Steege et al. 2008). The severity of such symptoms can range from mild, which does not impair performance, to severe which may not only impact performance but also health (de Oliveira et al. 2014; Riddoch and Trinick 1988). Situated between these ends of the symptomology continuum, acute exercise activity may also impact upon digestion, absorption and importantly, the recovery process (Riddoch et al. 1988; van Wijck et al. 2013a; de Oliveira et al. 2014).

The aetiology of exercise-related GI symptoms is thought to be multifactorial, but decreased splanchnic perfusion has been postulated as a key mechanism (van Wijck et al. 2012). During exercise, there is a redistribution of blood flow, which results in splanchnic hypoperfusion (Otte et al. 2001). Decreased blood flow, and the resulting decrease in oxygenation of the intestinal epithelial cells, can lead to a loss in epithelial integrity, as can increased epithelial temperature, which have been associated with increased gastrointestinal permeability, bacterial and endotoxin translocation, and intestinal inflammation (Dokladny et al. 2006; van Wijck et al. 2012). It has been shown that there is an inverse relationship between splanchnic blood flow and exercise intensity (Ott et al. 2001) with increased exercise intensity resulting in more pronounced intestinal permeability (Pals et al. 1997). Increased intestinal permeability has also been suggested to play a role in exertional heatstroke (Lambert 2004). Some connection has been made between increases in endotoxaemia and GI symptoms in athletes during and following prolonged strenuous exercise (Brock-Utne et al. 1988; Jeukendrup et al. 2000; Altenhoefer et al. 2004) although markers of GI permeability and symptoms are not always connected. Athletes have presented both increases in permeability or elevated endotoxin concentrations without subsequent GI symptoms, or GI symptoms have been reported without increases in GI permeability (Jeukendrup et al. 2000; Moncada-Jiménez et al. 2009). There is also some discrepancy between some field studies showing increases in GI damage and symptoms (Brock-Utne et al. 1988), and laboratory studies showing increases in GI damage but not symptoms (Lambert et al. 2008).

Traditional assessment of GI permeability has relied upon a 5 h urinary collection period whilst the subject remains fasted, which may be impractical with athletic populations. It has been shown that determination of the ratio of saccharide concentrations in serum correlates with the 5 h urine ratio and has been shown to be an acceptable alternative to urine and can reduce the sample collection time to 90-120 minutes (Fleming et al. 1996). Determination of saccharide

concentrations in plasma have also been used to show increases in GI permeability following exercise (Van Wijck et al. 2011; 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). It has though, been shown that more than 50% of orally ingested saccharides in solution reach the colon in just 2 h and the mono- to di-saccharide ratio from urine output after 0-2 h maybe representative of colonic and small intestinal permeability (Camilleri et al. 2010).

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). It has been shown that increases in I-FABP correlate with splanchnic hypoperfusion (Van Wijck et al. 2011). Whilst multiple models of exercise have been shown to alter I-FABP (van Wijck et al. 2012; Barberio et al. 2015), increases in I-FABP have not been conclusively shown to correlate to the onset or severity of GI symptoms (Van Wijck et al. 2011).

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 intervals 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 et al. 2013), to the authors' knowledge the effects of acute HIIT protocols on markers of GI damage and permeability, and symptoms of GI discomfort are not well characterised. Furthermore, given that GI symptoms are reported to be more common during "hard" runs compared to "easy" type training sessions (Riddoch and Trinick 1988), there is a definitive need to better understand the acute effect of HIIT on GI profile.

We therefore conducted the present study 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, we hypothesised that acute HIIT (using a model considered relevant for elite runners) would significantly increase markers of intestinal damage (I-FABP), permeability (primarily lactulose:rhamnose ratio) and symptoms of GI discomfort.

Methods

Eleven trained runners (mean $\pm$ SD $\dot{V}O_{2max}$ 60.0 $\pm$ 3.2 mL$\cdot$ kg^{-1}$\cdot$min^{-1}, body mass 75.1 $\pm$ 5.8 kg, height 179.1 $\pm$ 8.9 cm, age 33.1 $\pm$ 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. Runners were excluded if they had been adherent to medications at any time one month prior to participation, had a history of GI symptomology i.e. IBS or disease, or previous abdominal surgery. All runners were informed about the purpose of the study, the practical details, and the risks associated with the process before giving their written consent. The Ethics Committee of Liverpool John Moores University approved the study.

Experimental design

In a repeated measures counter-balanced design, and after previously completing an assessment of $\dot{V}O_{2max}$, 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 and 10:00. No alcohol consumption, non-steroidal anti-inflammatory drug (NSAID) consumption, fibre-rich or spicy food product consumption, unaccustomed or strenuous exercise was permitted during this period. 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. After obtaining measures of nude body mass, an indwelling cannula (Safety Lock 22G, BD Biosciences, West Sussex UK) was inserted into the anticubital vein in the anterior crease of the forearm and a resting blood sample drawn. After the resting blood sample, the cannula was flushed with ~5 ml of sterile saline to keep the cannula patent. This procedure was repeated after each subsequent blood draw. Standing, posture-controlled venous blood samples were taken pre-exercise, after each set of exercise, and 2 h post baseline. Participants also provided a urine sample pre, and 120 minutes post exercise, and at the same corresponding time points during non-exercise rest trial. At each sample point, urine volume was recorded and 20 mL was directly transferred to pre-chilled containers, and frozen at -80 $^{\circ}$C for later analysis.

Assessment of maximal oxygen uptake

$\dot{V}O_{2max}$ 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 km$\cdot$h^{-1} followed by 2-min stages at 12 km$\cdot$h^{-1}, 14 km$\cdot$h^{-1}, and 16 km$\cdot$h^{-1}. On completion of the 16 km$\cdot$h^{-1} stage, the treadmill was inclined by 1% every 2 minutes thereafter until volitional exhaustion. $\dot{V}O_{2max}$ was stated as being achieved by the following endpoint

criteria: (1) heart rate within 10 beats min^{-1} of age predicted maximum, (2) respiratory exchange ratio >1.1 and (3) plateau of oxygen consumption despite increased workload. Peak $\dot{V}O_2$ was determined from the mean of last 10 s of each 2 minutes of each interval. Based on the results of the incremental test, the running speed corresponding to 100% $\dot{V}O_{2max}$ was estimated for each participant using a linear regression equation. The running speed corresponding to 120% was then calculated.

HIIT protocol

The high intensity interval exercise protocol consisted first of a 5 minute rest period, then a 5 minute warm-up run at a velocity corresponding to 50% $\dot{V}O_{2max}$, 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 $\dot{V}O_{2max}$, corresponding to 120% of their $\dot{V}O_{2max}$. Each interval was followed by running at a velocity associated with 50% $\dot{V}O_{2max}$ 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. Measurements of heart rate (HR) (Polar FT1, Finland), and subjective ratings of perceived exertion (RPE) (Borg 1970), thermal comfort (TC) (Matzarakis et al. 1999) and gastrointestinal discomfort using a modified Likert scale (Nieman et al. 2006) were obtained during each trial. 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. On completion of exercise, participants remained in the laboratory, resting until 2 h post-baseline for further sampling. During the resting protocol, the participants remain seated for 120

minutes. All measurements and sampling took place at times mimicking the sampling points of the HIIT exercise protocol (Fig. 1).

Assessment of intestinal damage

I-FABP concentrations from EDTA plasma were determined using an ELISA (Hycult Biotechnology, Uden, the Netherlands; detection window 47 – 3000 pg/ml) according to the manufacturer's instructions. Plasma samples were taken at baseline, following each of the 3 sets of 6 x 400 m runs, and at 90 and 120 minutes. The coefficient of variance was 8% for between-sample duplicates.

Assessment of intestinal permeability

Permeability was assessed using a previously published protocol (Fleming, Duncan et al. 1996), with the modification of using L-rhamnose instead of mannitol as the monosaccharide probe. Briefly, at baseline, participants drank a standardized sugar solution containing 5 g lactulose, 2 g rhamnose, 1 g sucrose and 0.5 g D-xylose in a total of 50 ml water. Saccharide concentrations were determined from serum samples 120 minutes post baseline. Participants also provided urine samples 120 post baseline for analysis. The various sugars were separated by HPLC and quantitated by use of a pulsed electrochemical detector using a gold working electrode and silver/silver chloride reference electrode. The detection potential was -0.01 V (0-0.5 s), the oxidation potential was +0.75 V (0.51-0.64 s), the reduction potential was -0.75 V (0.65-0.75 s), and the integration period was 0.05 to 0.5 s. The coefficient of variation using this method has been found to be between 1.8 – 8.5%.

Assessment of GI discomfort

During both experimental protocols GI symptoms were assessed using a GI discomfort scale (adapted from Pfeiffer et al. 2009). During exercise, global GI symptoms were scored on a 9-point scale, with a score > 4 being regarded as serious. At the end of exercise participants completed a more detailed questionnaire (adapted from Pfeiffer et al. 2012) in order to assess more specific symptoms of GI discomfort during the session, such as bloating, flatulence and urge to vomit. GI symptoms were scored on a 10-point scale (0 = no symptoms and 10 = extreme symptoms) with a score >4 being regarded as serious. This questionnaire was completed again at 24 h post activity and participants were asked to assess GI symptoms during the last 24 h, since the experimental visit. Using this questionnaire, participants also rated symptoms typically experienced in their habitual training during the following types of training runs; short steady-state runs (< 60 minutes), long steady-state runs (> 60 minutes), and high intensity interval runs, as well as symptoms typically experienced at rest.

Statistical analyses

Statistical analysis was conducted using the Statistical Package for the Social Sciences software programme (SPSS, version 23). 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. Mean values were compared with the use of Wilcoxon Signed Rank tests. Pearson 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 ± standard deviation (SD), data not normally distributed are reported as median and range. $P < 0.05$ was considered statistically significant.

Results

Physiological responses to acute HIIT protocol

Participants ran a total of 7.2 km^{-1} at a velocity of 17.7 ± 1.0 $km.h^{-1}$, while recovery running totalled 2.2 ± 0.1 km at a velocity of 8.9 ± 0.5 $km.h^{-1}$. HR, RPE and thermal comfort increased incrementally throughout interval bouts with peak values of 187 ± 10 bpm (95 ± 6% HR_{max}), 19 ± 1, and 8.5 ± 0.7, respectively.

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 ± 334 pg/mL to mean peak levels of 829 ± 448 pg/mL immediately post exercise and then decreased during subsequent recovery, whilst there was no change during rest (see Figure 2). There was no significant difference in baseline I-FABP concentrations between HIIT (481 ± 334 pg/mL) and rest condition (263 ± 149 pg/mL) ($p = 0.2$).

Gastrointestinal permeability

The lactulose:rhamnose ratio (L:R) in serum was 59% higher following HIIT when compared to the resting condition (0.051 ± 0.016 vs. 0.031 ± 0.021, $p = 0.0047$) (see Figure 3A). However, there was no significant difference in urinary L:R between rest and HIIT trial ($p = 0.37$) (see

Figure 3B). There was no correlation between serum and urinary L:R (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 difference in urinary sucrose recovery following HIIT (0.169 ± 0.168%) compared to rest (0.123 ± 0.120%, $p > 0.05$).

Gastrointestinal discomfort

During habitual training, participants reported uncertain increases in symptoms including "Bloating", "Flatulence", and "Urge to defecate" during both long steady state and high intensity interval runs compared with rest and short steady state runs ($p > 0.05$, data not shown), although symptoms were typically low to mild (≤ 4). During experimental visits, global peak 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 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 L:R (see Table 2).

Discussion

The aim of the present study was to characterise the acute effects of a HIIT running session on markers of small intestinal damage, intestinal permeability and symptoms of GI discomfort. We report for the first time, that in a group of trained male runners, acute HIIT increased

markers of gastrointestinal permeability (both L:R and sucrose) and small intestinal injury (I-FABP). Runners also reported acute symptoms of GI discomfort (bloating, urge to burp, stomach cramps and flatulence) during and in the 24 h post exercise, although these markers of discomfort did not correlate with measures of GI permeability or damage.

During exercise, there is a redistribution of blood away from the splanchnic area, analogous with increased blood flow to heart and contracting skeletal muscle. 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. 2003). As such, plasma I-FABP is considered a sensitive measurement of small intestinal damage (Pelsers et al. 2003; Derikx et al. 2008). Here, we report for the first time that I-FABP is increased during HIIT running session following a similar temporal sequence to that reported by others following steady state exercise. The increase in these plasma parameters could, theoretically, be partly attributed to an exercise-induced reduction in plasma volume. The increases, however, largely exceeded the decreases in plasma volume that have been described in athletes following HIIT exercise of up to 20% (Kargotich et al. 1998; Kavouras 2002; Graham et al. 2016). Also, given that I-FABP is a small (15 kDa), water-soluble protein, changes in plasma volume may have less of an effect, should it permeate through the microvascular system at a proportionate rate to plasma solvent (Michel et al. 1999). Nonetheless, peak values exceed those previously seen following 60 minutes of cycling at 70% peak power output (Van Wijck et al. 2011; van Wijck et al. 2012), 60 minutes of running at 70% $\dot{V}O_{2max}$ in the heat (Sessions et al. 2016) but are lower than I-FABP levels reported following exhaustive running in the heat (Barberio et al. 2015). Differences between studies

may be attributable to analytical procedures, subject characteristics and exercise intensities and durations. However, taken together, such data further suggest that factors affecting splanchnic blood flow (i.e. intensity, exhaustion, heat stress etc.) are potent regulators of intestinal damage. Of note in the present study is the seemingly large day to day variations in I-FABP concentrations within participants, and the large variation in response to exercise. For example, although pre-test conditions were standardised as much as possible, one participant's baseline values were 318 pg/mL and 1218 pg/mL during rest and HIIT conditions respectively. In total, nine of the eleven participants' baseline values were higher during HIIT compared to resting condition. This may be due to poor compliance with pre-test conditions, psychological factors such as anxiety of having to perform strenuous exercise, which has been shown to effect GI symptoms (Haug et al. 2002), or other factors unknown to the authors. As such, further studies may be required to examine day-to-day variations, as well as the effect of other factors on I-FABP.

Intestinal permeability has been clinically defined as the non-mediated diffusion of large, normally prohibited molecules (> 0.15 kDa molecular mass) from the intestinal lumen to the blood (Travis et al. 1992; Zuhl et al. 2012). Due to differences in the dosages of the probes used, time delivered in relation to exercise, and analytical methods, it is difficult to compare L:R values between studies. However, the relative increase in L:R here (59%) is similar to those seen previously following 60 minutes of continuous running of 60-70% $\dot{V}O_{2max}$, (33-78%) (Pals et al. 1997; Lambert et al. 2008). Nonetheless, the methodological approach used here holds promise for future investigations. Traditionally, 5 hour urine collection and percentage recovery of sugar probes has been used to assess intestinal permeability. This method has been shown to be sensitive to changes in permeability caused by exercise of varying modalities, durations and intensities (Pals et al. 1997; Nieuwenhoven 1999; Lambert et al. 2008; 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. Given that both saccharides are assumed to be affected in the same way to pre- and postmucosal confounders such as gastric dilution, gastrointestinal motility, bacterial degradation, renal function or changes in plasma volume (Bischoff et al. 2014), changes in GI permeability may also be detected from plasma or serum. We have shown that by measuring sugar probes in serum, we could quantify exercise induced increase in L:R and sucrose concentration 2 hours after probe ingestion. Conversely, analysis of urine collected at 2 hours did not show any differences, both of which agrees with previous works (van Wijck et al. 2011). It has been suggested that the use of plasma and serum samples increases the detection sensitivity, thus reducing the need for large oral doses (van Wijck et al. 2013b), and reduces the collection time (Fleming et al. 1996). We show here that serum sampling is a more time efficient method of assessing GI permeability and we were able to detect increases in permeability 2 hours after probe ingestion but were not able to using urine sampling over the same time course.

Although we have shown that an acute bout of HIIT increases I-FABP and L:R, these did not correlate to any increases in ratings of GI discomfort, either during or in the following 24 hours after exercise and overall scores were low to mild (all median values < 4). 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 no history of GI disease. This is consistent 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 (Lambert et al. 2008; van Wijck et al. 2011) or have not reported GI symptoms at all (Marchbank et al. 2011; Zuhl et al. 2014). Even 60 minutes of running in the heat, 30 minutes of which was a distance time trial, mean symptoms were rated as very mild to noticeable (Morrison et al. 2014). This apparent discrepancy between symptom expression 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 time via the central nervous system (Brouns et al. 1993). There may also be specific nutritional strategies employed during competition that lead to GI symptoms, that are not used during the training cycle, such as carbohydrate loading and/or carbohydrate ingestion during exercise (de Oliveira et al. 2011). Participants in the current study reported only low to mild symptoms experienced during typical training runs. Further investigations should look to better identify whether there are unique aetiologies relating to different exercise modalities (i.e. long duration, steady state vs. shorter, high intensity) and between training and competition.

Although the present study provides novel measurements of intestinal damage following HIIT running which have not been reported before, there are some limitations to be considered. Firstly, there was no steady-state exercise to act as a control. Whilst we have shown that HIIT running leads to increases in markers of damage, had a workload-matched, steady-state running protocol been included, this may have shown whether HIIT caused additional damage due to the activity profile. For example, it is known that upper GI symptoms may be exacerbated by HIIT due to a reduction in gastric emptying compared to steady-state exercise (Leiper et al. 2001). The current sample also did not include any females despite GI symptoms appearing to be more prevalent in female athletes (de Oliveira et al. 2014), and as such, further studies should examine any separate effects of exercise on markers of intestinal damage in females. Finally, while splanchnic hypoperfusion is considered to be the primary factor in exercise-

induced intestinal damage, this was not directly measured here, due to obvious technical difficulties this would incur during high intensity running.

In summary, we report for the first time that acute high intensity interval running increases gastrointestinal permeability and intestinal cellular damage in trained male runners though such markers do not correlate to symptoms of GI discomfort. Furthermore, by using serum sampling, we also provide novel data by observing that it is possible to detect changes in intestinal permeability over a shorter time-period, which could not be detected via urinary sampling, which may advantageous for any future investigations that involve the participation of elite athletes. Future studies should continue to examine the functional relevance of markers of intestinal damage, particularly during "real-world" training sessions and when there is an absence of symptoms of GI discomfort. Finally, more work is required to accurately report the prevalence of GI symptoms during, and following, different training modalities and further investigations could lead to more understanding of any unique aetiology of symptoms linked with such exercise or the associated training and competition practices.

Conflict of Interest

The authors have no competing interests to declare associated with this manuscript.

References

Altenhoefer, A., Oswald, S., Sonnenborn, U., Enders, C., Schulze, J., Hacker, J., et al. 2004. The probiotic Escherichia coli strain Nissle 1917 interferes with invasion of human intestinal epithelial cells by different enteroinvasive bacterial pathogens. FEMS. Immuno. Med. Mic. **40**: 223-229. doi:10.1016/S0928-8244(03)00368-7

Barberio, M., Elmer, D., Laird, R., Lee, K., Gladden, B., andPascoe, D. 2015. Systemic LPS and Inflammatory Response during Consecutive Days of Exercise in Heat. Int. J. Sports Med. **36**: 262-270. doi:10.1055/s-0034-1389904

Bartlett, J.D., Close, G.L., MacLaren, D.P., Gregson, W., Drust, B., and Morton, J.P. 2011. High-intensity interval running is perceived to be more enjoyable than moderate-intensity continuous exercise: implications for exercise adherence. J. Sports. Sci **29**: 547-553. doi:10.1080/02640414.2010.545427

Billat, L.V. 2001. Interval training for performance: a scientific and empirical practice. Sports. Med. **31**: 13-31.

Bischoff, S.C., Barbara, G., Buurman, W., Ockhuizen, T., Schulzke, J.-D., Serino, M., et al. 2014. Intestinal permeability–a new target for disease prevention and therapy. BMC gastroenterology, **14**: 1. doi:10.1186/s12876-014-0189-7

Borg, G. 1970. Physical training. 3. Perceived exertion in physical work. Lakartidningen, **67**: 4548-57.

Brock-Utne, J., Gaffin, S., Wells, M., Gathiram, P., Sohar, E., James, M., et al. 1988. Endotoxaemia in exhausted runners after a long-distance race. S. Afr. Med. J. **73**: 533-536.

Brouns, F. and Beckers, E. 1993. Is the gut an athletic organ? Sports Medicine, **15**: 242-257.

Camilleri, M., Nadeau, A., Lamsam, J., Linker Nord, S., Ryks, M., Burton, D., et al. 2010. Understanding measurements of intestinal permeability in healthy humans with urine lactulose and mannitol excretion. Neurogastroent. Motil. **22**: e15-e26. doi:10.1111/j.1365-2982.2009.01361.x

Daussin, F.N., Zoll, J., Dufour, S.P., Ponsot, E., Lonsdorfer-Wolf, E., Doutreleau, S., et al. 2008. Effect of interval versus continuous training on cardiorespiratory and mitochondrial functions: relationship to aerobic performance improvements in sedentary subjects. Am. J. Physiol-reg. J. **295**: R264-R272. doi:10.1152/ajpregu.00875.2007

de Oliveira, E.P. and Burini, R.C. 2011. Food-dependent, exercise-induced gastrointestinal distress. J. Int. Soc. Sports Nutr. **8**: 1.

de Oliveira, E.P., Burini, R.C., and Jeukendrup, A. 2014. Gastrointestinal complaints during exercise: prevalence, etiology, and nutritional recommendations. Sports. Med. **44**: 79-85. doi:10.1007/s40279-014-0153-2

Derikx, J.P., Matthijsen, R.A., de Bruïne, A.P., van Bijnen, A.A., Heineman, E., van Dam, R.M., et al. 2008. Rapid reversal of human intestinal ischemia-reperfusion induced damage by shedding of injured enterocytes and reepithelialisation. PloS one, **3**: e3428. doi:10.1371/journal.pone.0003428

Dokladny, K., Moseley, P.L., and Ma, T.Y. 2006. Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. Am. J. Physiol-Gastr. L. Physiology, **290**: G204-G212. doi:10.1152/ajpgi.00401.2005

Fleming, S., Duncan, A., Russell, R., and Laker, M. 1996. Measurement of sugar probes in serum: an alternative to urine measurement in intestinal permeability testing. Clin. Chem. **42**: 445-448.

Gillen, J.B. and Gibala, M.J. 2013. Is high-intensity interval training a time-efficient exercise strategy to improve health and fitness? Appl. Physiol. Nutr. Metab. **39**: 409-412. doi:10.1139/apnm-2013-0187

Graham, M.J., Lucas, S.J., Francois, M.E., Stavrianeas, S., Parr, E.B., Thomas, K.N., et al. 2016. Low-volume intense exercise elicits post-exercise hypotension and subsequent hypervolemia, irrespective of which limbs are exercised. Frontiers in Physiology, **7**. doi:10.3389/fphys.2016.00199

Haug, T.T., Mykletun, A., and Dahl, A. 2002. Are anxiety and depression related to gastrointestinal symptoms in the general population? Scand. J. Gastroentero. **37**: 294-298.

JanssenDuijghuijsen, L.M., Mensink, M., Lenaerts, K., Fiedorowicz, E., van Dartel, D.A., Mes, J.J., et al. 2016. The effect of endurance exercise on intestinal integrity in well-trained healthy men. Physiological Reports, **4**: e12994. doi:10.14814/phy2.12994

Jeukendrup, A., Vet-Joop, K., Sturk, A., Stegen, J., Senden, J., Saris, W., et al. 2000. Relationship between gastro-intestinal complaints and endotoxaemia, cytokine release and the acute-phase reaction during and after a long-distance triathlon in highly trained men. Clin. Sci. **98**: 47-55. doi:10.1042/Cs19990258

Kargotich, S., Goodman, C., Keast, D., and Morton, A.R. 1998. The influence of exercise-induced plasma volume changes on the interpretation of biochemical parameters used for monitoring exercise, training and sport. Sports. Med. **26**: 101-117. doi:10.2165/00007256-199826020-00004

Kavouras, S.A. 2002. Assessing hydration status. Curr. Opin. Clin. Nutr. Metab. Care. **5**: 519-524.

Lambert, G., Lang, J., Bull, A., Pfeifer, P., Eckerson, J., Moore, G., et al. 2008. Fluid restriction during running increases GI permeability. Int. J. Sports Med. **29**: 194-198. doi:10.1055/s-2007-965163

Lambert, G.P. 2004. Role of gastrointestinal permeability in exertional heatstroke. Exercise. Sport. Sci. R. **32**: 185-190.

Leiper, J.B., Broad, N.P., and Maughan, R.J. 2001. Effect of intermittent high-intensity exercise on gastric emptying in man. Med. Sci. Sport. Exer. **33**: 1270-1278.

Marchbank, T., Davison, G., Oakes, J.R., Ghatei, M.A., Patterson, M., Moyer, M.P., et al. 2011. The nutriceutical bovine colostrum truncates the increase in gut permeability caused by heavy exercise in athletes. Am. J. Physiol-Gastr. L. **300**: G477-G484. doi:10.1152/ajpgi.00281.2010

Matzarakis, A., Mayer, H., and Iziomon, M.G. 1999. Applications of a universal thermal index: physiological equivalent temperature. Int. J. Biometeorol. **43**: 76-84.

McAllister, R.M. 1998. Adaptations in control of blood flow with training: splanchnic and renal blood flows. Med. Sci. Sport. Exer. **30**: 375-381.

Michel, C. and Curry, F. 1999. Microvascular permeability. Physiological reviews, **79**: 703-761.

Moncada-Jiménez, J., Plaisance, E.P., Mestek, M.L., Araya-Ramírez, F., Ratcliff, L., Taylor, J.K., et al. 2009. Initial metabolic state and exercise-induced endotoxaemia are unrelated to gastrointestinal symptoms during exercise. J. Sports Sci. Med. **8**: 252.

Morrison, S.A., Cheung, S.S., and Cotter, J.D. 2014. Bovine colostrum, training status, and gastrointestinal permeability during exercise in the heat: a placebo-controlled double-blind study. Appl. Physiol. Nutr. Metab. **39**: 1070-1082. doi:10.1139/apnm-2013-0583

Murray, R. 2006. Training the gut for competition. Curr. Sports. Med. Rep. **5**: 161-164.

Nieman, D.C., Henson, D.A., Dumke, C.L., Oley, K., McAnulty, S.R., Davis, J.M., et al. 2006. Ibuprofen use, endotoxemia, inflammation, and plasma cytokines during ultramarathon competition. Brain. Behav. Immun. **20**: 578-584.

Nieuwenhoven, V. 1999. The effect of physical exercise on parameters of gastrointestinal function. Neurogastroent. Motil. **11**: 431-439.

Otte, J.A., Oostveen, E., Geelkerken, R.H., Groeneveld, A.J., and Kolkman, J.J. 2001. Exercise induces gastric ischemia in healthy volunteers: a tonometry study. J. App. Physiol. **91**: 866-871.

Palma, G., Collins, S.M., Bercik, P., and Verdu, E.F. 2014. The microbiota–gut–brain axis in gastrointestinal disorders: stressed bugs, stressed brain or both? J. Physiol. **592**: 2989-2997.

Pals, K.L., Chang, R.-T., Ryan, A.J., and Gisolfi, C.V. 1997. Effect of running intensity on intestinal permeability. J. App. Physiol. **82**: 571-576.

Pelsers, M.M., Namiot, Z., Kisielewski, W., Namiot, A., Januszkiewicz, M., Hermens, W.T., et al. 2003. Intestinal-type and liver-type fatty acid-binding protein in the intestine. Tissue distribution and clinical utility. Clin. Biochem. **36**: 529-535.

Peters, H., Bos, M., Seebregts, L., Akkermans, L., van Berge Henegouwen, G., Bol, E., et al. 1999. Gastrointestinal symptoms in long-distance runners, cyclists, and triathletes: prevalence, medication, and etiology. Am. J. Gastroenterol. **94**: 1570-1581. doi:10.1111/j.1572-0241.1999.01147.x

Pfeiffer, B., Cotterill, A., Grathwohl, D., Stellingwerff, T., and Jeukendrup, A.E. 2009. The effect of carbohydrate gels on gastrointestinal tolerance during a 16-km run. Int. J. Sport Nutr. **19**: 485.

Pfeiffer, B., Stellingwerff, T., Hodgson, A.B., Randell, R., Pottgen, K., Res, P., et al. 2012. Nutritional intake and gastrointestinal problems during competitive endurance events. Med. Sci. Sports. Exerc. **44**: 344-51. doi:10.1249/MSS.0b013e31822dc809

Riddoch, C. and Trinick, T. 1988. Gastrointestinal disturbances in marathon runners. Br. J. Sports Med. **22**: 71-74.

Seiler, S. 2010. What is best practice for training intensity and duration distribution in endurance athletes. Int. J. Sports. Physiol. Perform. **5**: 276-291.

Sessions, J., Bourbeau, K., Rosinski, M., Szczygiel, T., Nelson, R., Sharma, N., et al. 2016. Carbohydrate gel ingestion during running in the heat on markers of gastrointestinal distress. Eur. J. Sport Sci. **16**: 1-9. doi:10.1080/17461391.2016.1140231

Stuempfle, K.J. and Hoffman, M.D. 2015. Gastrointestinal distress is common during a 161-km ultramarathon. J. Sports Sci. **33**: 1814-1821. doi:10.1080/02640414.2015.1012104

Ter Steege, R.W., Van Der Palen, J., and Kolkman, J.J. 2008. Prevalence of gastrointestinal complaints in runners competing in a long-distance run: an internet-based observational study in 1281 subjects. Scand. J. Gastroenterol., **43**: 1477-1482. doi:10.1080/00365520802321170

Trapp, E., Chisholm, D., Freund, J., and Boutcher, S. 2008. The effects of high-intensity intermittent exercise training on fat loss and fasting insulin levels of young women. Int. J. Obes. **32**: 684-691. doi:10.1038/sj.ijo.0803781

Travis, S. and Menzies, I. 1992. Intestinal permeability: functional assessment and significance. Clin. Sci. **82**: 471-88.

van Wijck, K., Lenaerts, K., Grootjans, J., Wijnands, K.A., Poeze, M., van Loon, L.J., et al. 2012. Physiology and pathophysiology of splanchnic hypoperfusion and intestinal injury during exercise: strategies for evaluation and prevention. Am J. Physiol. Gastrointest. Liver Physiol. **303**: G155-G168. doi:10.1152/ajpgi.00066.2012

Van Wijck, K., Lenaerts, K., Van Loon, L.J., Peters, W.H., Buurman, W.A., andDejong, C.H. 2011. Exercise-induced splanchnic hypoperfusion results in gut dysfunction in healthy men. PloS one, **6**: e22366. doi:10.1371/journal.pone.0022366

van Wijck, K., Pennings, B., van Bijnen, A.A., Senden, J.M., Buurman, W.A., Dejong, C.H., et al. 2013a. Dietary protein digestion and absorption are impaired during acute postexercise recovery in young men. Am. J. Physiol. Regul. Integr. Comp. Physiol. **304**: R356-R361. doi:10.1152/ajpregu.00294.2012

van Wijck, K., Verlinden, T.J., van Eijk, H.M., Dekker, J., Buurman, W.A., Dejong, C.H., et al. 2013b. Novel multi-sugar assay for site-specific gastrointestinal permeability analysis: a randomized controlled crossover trial. Clin. Nutr. **32**: 245-251. doi:10.1016/j.clnu.2012.06.014

Zuhl, M., Schneider, S., Lanphere, K., Conn, C., Dokladny, K., and Moseley, P. 2012. Exercise regulation of intestinal tight junction proteins. Bri. J. Sports Med. **48**: bjsports-2012-091585. doi:10.1136/bjsports-2012-091585

Zuhl, M.N., Lanphere, K.R., Kravitz, L., Mermier, C.M., Schneider, S., Dokladny, K., et al. 2014. Effects of oral glutamine supplementation on exercise-induced gastrointestinal permeability and tight junction protein expression. J. App. Physiol. **116**: 183-191. doi:10.1152/japplphysiol.00646.2013

Table 1. GI symptoms during rest and HIIT conditions and the following 24 hours.

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)	2 (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)	2 (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***
Stomach upsets	0 (0-3)	0 (0-4)	0.139	0 (0-1)	0 (0-3)	0.285

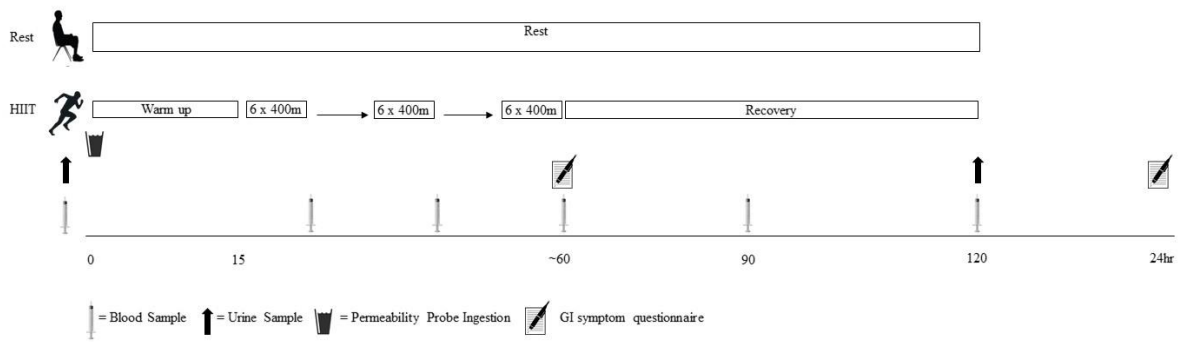Data are median and range. * higher GI symptom scores following HIIT versus rest

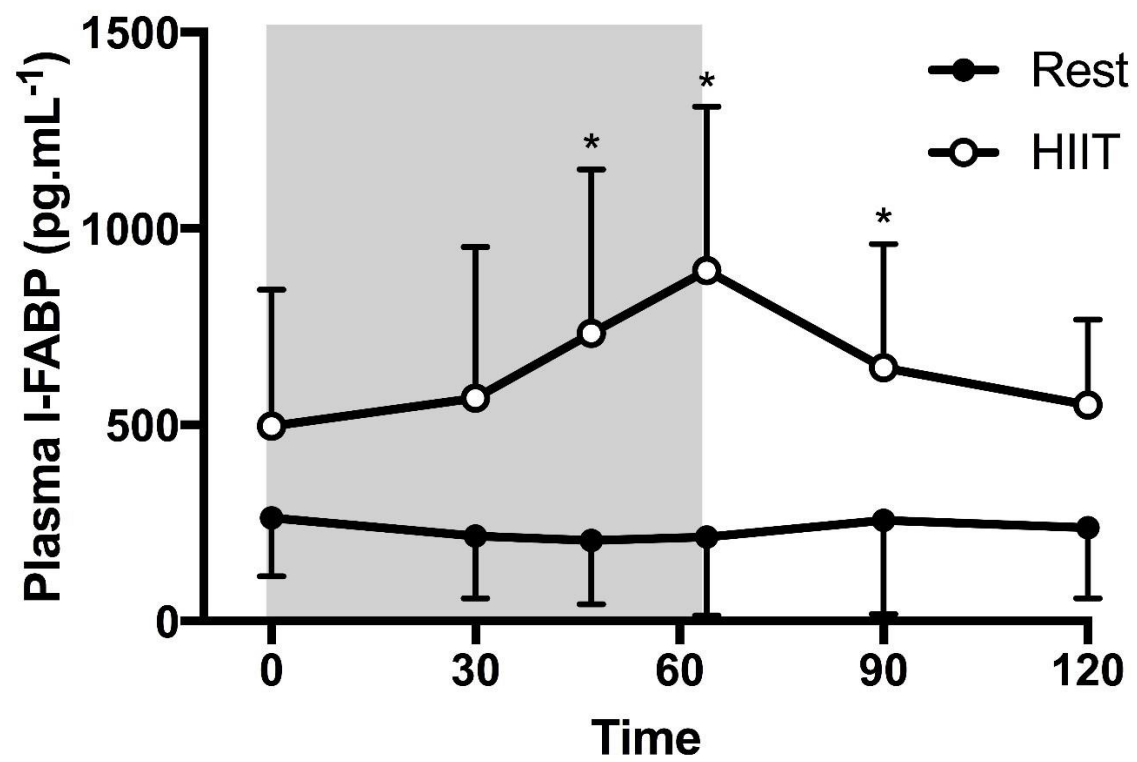Table 2. Correlations between GI symptoms with I-FABP and L:R

Symptom	I-FABP Correlation	p value	L:R Correlation	p value
Exercise				
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
24 hours				
Bloating	-0.066	0.848	-0.172	0.484
Flatulence	0.343	0.302	-0.358	0.384

Fig. 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
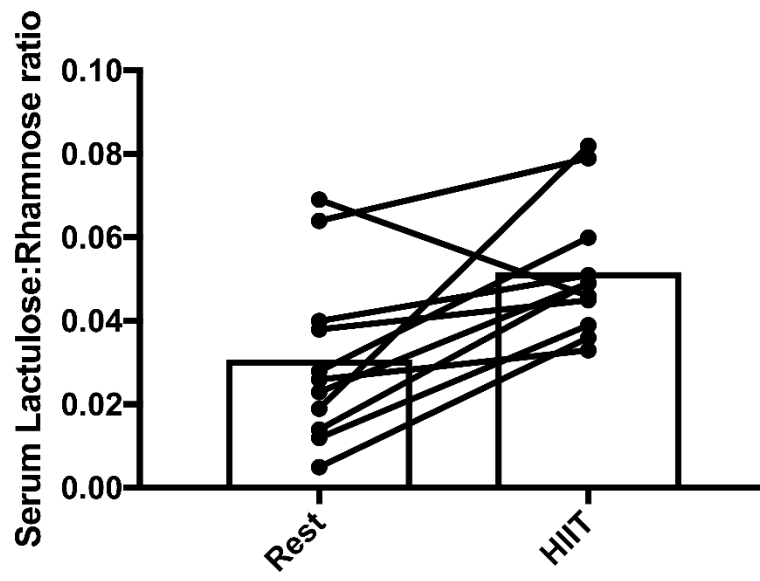
Fig. 2. Mean (±SD) plasma I-FABP concentrations during rest and HIIT conditions. * Significant difference between baseline ($p < 0.05$)

Fig.3 Mean and individual serum (A) and urinary (B) lactulose to rhamnose ratios during rest and HIIT. $p < 0.001$

Rest

Rest

HIIT

| Warm up | 6 x 400m | → | 6 x 400m | → | 6 x 400m | Recovery |

0   15   ~60   90   120   24hr

= Blood Sample   = Urine Sample   = Permeability Probe Ingestion   = GI symptom questionnaire

(A)

(B)