Intestinal Damage Following Short Duration Exercise at the Same Relative Intensity is Similar in Temperate and Hot Environments.

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Abstract

Increasing temperature and exercise disrupt tight junctions of the gastrointestinal tract although the contribution of environmental temperature to intestinal damage when exercising is unknown. This study investigated the effect of two different environmental temperatures on intestinal damage when exercising at the same relative intensity. Participants (n=12M, mean±SD:81.98±7.95kg, 182.6±7.4cm) completed randomised cycling trials (45min, 70%$\dot{V}O_{2max}$) in 30°C/40%RH and 20°C/40%RH. A subset of participants (n=5) also completed a seated passive trial (30°C/40%RH). Rectal temperature and thermal sensation (TSS) were recorded during each trial and venous blood samples collected pre- and post-trial for the analysis of intestinal fatty acid-binding protein (I-FABP) level as a marker of intestinal damage. $\dot{V}O2$ was similar between 30°C and 20°C exercise trials, as intended (p=0.94). I-FABP increased post-exercise in 30°C (pre-exercise:585±188pg·mL^{-1}, post-exercise:954±411pg·mL-1) and 20°C (pre-exercise:571±175 pg·mL^{-1}, post-exercise:852±317pg·mL^{-1}) (p<0.0001) but the magnitude of damage was similar between temperatures (p=0.58). There was no significant increase in I-FABP concentration following passive heat exposure (p=0.59). Rectal temperature increased during exercise trials (p<0.001), but not the passive trial (p=0.084). TSS increased more when exercising in 30°C compared to 20°C (p<0.001). There was an increase in TSS during the passive heat trial (p=0.03). Intestinal damage, as measured by I-FABP, following exercise in the heat was similar to when exercising in a cooler environment at the same relative intensity. Passive heat exposure did not increase I-FABP. It is suggested that when exercising in conditions of compensable heat stress, the increase in intestinal damage is predominantly attributable to the exercise component, rather than environmental conditions.

Keywords: gastrointestinal; heat stress; Intestinal Fatty Acid-Binding Protein (I-FABP); exercise, intestinal damage

Introduction

The gastrointestinal barrier is the primary intestinal defense layer, composed of epithelial cell membranes held together by tight junction proteins, creating a dynamic and semi-permeable barrier between the intestinal content and the host (Groschwitz and Hogan 2009). During homeostasis an intact epithelial barrier supports nutrient absorption and immunoregulatory functions while efficiently preventing the passage of luminal contents and microorganisms to the bloodstream. During exercise the integrity of this barrier may be compromised, attributable to a reduction in splanchnic blood flow (Qamar and Read 1987; Peters et al. 2001) and ensuing oxidative stress and inflammation that disrupt epithelial tight junctions, leading to intestinal damage. Reperfusion of intestinal tissue following exercise may also lead to further damage (Mallick et al. 2004). Disruptions to the intestinal barrier may have potential negative consequences for health and exercise performance (Costa et al. 2017). These consequences may include increased gastrointestinal symptoms (de Oliveira and Burini 2009), elevated cytokines (eg. IL-1β) associated with central fatigue (Enos et al. 2013) and lowering of postprandial amino acids available for muscle repair, ultimately affecting recovery and subsequent exercise performance (van Wijck et al. 2013). Intestinal damage may also increase bacterial translocation from the lumen to systemic circulation, increasing susceptibility to pathogens and risk of endotoxaemia. Maintaining intestinal integrity during exercise holds promise as a strategy to enhance exercise performance and recovery (Shing et al. 2014; Szymanski et al. 2018) and immune surveillance.

Exercise has been shown to disrupt the intestinal barrier, and it is postulated that greater exercise intensity is associated with greater disruption of the gastrointestinal tract (Pals et al. 1997; van Wijck et al. 2012). Currently, the primary hypothesis to explain this exercise-induced damage is the redistribution of blood flow from the splanchnic area to the active skeletal muscles and skin to dissipate heat as a mechanism of thermoregulation (Rowell et al. 1965), contributing to intestinal ischemia and the consequential oxidative stress and inflammation that have been shown to disrupt intestinal integrity, as reviewed by Doklandy et al. (2016). Just 10 min of cycling at 70% $\dot{V}O_{2max}$ significantly reduces splanchnic perfusion (determined via gastric-arterialized pCO_2) (van Wijck et al. 2011) and this reduction in blood flow is significantly correlated with an increase in circulating marker of intestinal damage, intestinal fatty-acid binding protein (van Wijck et al. 2011).

Intestinal fatty-acid binding protein (I-FABP) is an intra-cellular protein localised to mature enterocytes of both the small and large intestines, but particularly the jejunum (Pelsers et al. 2003). This protein has been found to be released immediately into the circulation upon the loss of enterocyte integrity (Pelsers et al. 2003) making it a sensitive and specific plasma marker for enterocyte damage (Kanda et al. 1996). Indeed, circulating I-FABP level may be used to diagnose acute intestinal ischemia (Sun et al. 2016). Post-exercise increases of I-FABP of 35% have been shown to reduce nutrient uptake (van Wijck et al. 2013), while limiting the post-exercise increase in I-FABP by 29% with an intervention strategy to improve intestinal integrity has been shown to improve thermotolerance, as indicated by a reduction in physiological strain index (Szymanski et al. 2018). It is possible that small increases or small reductions in the magnitude of intestinal damage might have implications for performance and recovery.

Understanding the contribution of physiological and environmental components to intestinal damage in exercising humans is essential to inform strategies to prevent or reduce exercise associated intestinal damage. It has been difficult to determine the influence of environmental heat on intestinal damage as studies often combine increasing temperature with increasing physiological demand (Maw et al. 1993), making it difficult to isolate the effects of the environment. Snipe et al. (Snipe et al. 2016; Snipe et al. 2018a) have reported a dose response in post-exercise intestinal damage, as measured by I-FABP, with increasing environmental temperature from 22°C to 30°C and 22°C to 35°C. While the damage may in part be attributed to increasing ambient temperature, participants were running at the same absolute treadmill speed during each trial and as such exercise intensity may have differed in the hot versus temperate conditions given the possible increased physiological strain and metabolic cost of exercising in the heat (No and Kwak 2016; Collins et al. 2017). In the presence of cardiovascular drift when exercising in the heat at a set workload, heart rate remains a reliable indicator of relative exercise intensity (Wingo 2015) and Snipe et al. (Snipe et al. 2018a; Snipe et al. 2018b) reported significantly higher heart rates (and ratings of perceived exertion) when exercising at the same absolute intensity in 30°C and 35°C compared to 22°C, indicating greater physiological strain when exercising it the heat . It is unknown if the greater increase in intestinal damage reported when exercising in the heat compared to a temperate environment can be attributed to the higher

environmental temperature, as suggested by Pires et al. (2017), or to differences in exercise intensity that would differently affect endogenous heat production and splanchnic blood flow. Given the potential consequences of intestinal damage, using an exercise model associated with intestinal damage this study aimed to determine the effect of two different ambient temperatures on intestinal damage when exercise intensity was matched (70% $\dot{V}O_{2max}$) between environmental conditions.

Materials and Methods

A randomised, cross-over design was employed for this study with participants required to complete two exercise trials. To ensure the allocation of exercise was counterbalanced and randomised, block randomisation (2 groups of 6) was carried out using research randomiser (Urbaniak 2007) (i.e. 6 individuals completed an exercise trial in 30°C, 40%RH first and 6 individuals completed an exercise trial in 20°C, 40%RH first). Participants visited the University of Tasmania Human Performance Laboratory on three separate occasions to complete: 1) an incremental maximal exercise test, 2) a 45min exercise trial at 70% $\dot{V}O_{2max}$in either 30°C, 40%RH or 20°C, 40%RH and 3) a 45min exercise session at 70% $\dot{V}O_{2max}$completed in the alternative environmental condition than the first exercise trial. Trials were separated by two to seven days. A subset of participants (n=5) also completed a fourth trial that entailed all the same procedures as the exercise trials but participants were seated quietly for 45min in 30°C, 40%RH (ie. did not exercise). The experimental protocol was approved by the Human Research Ethics committee at the University of Tasmania and was therefore carried-out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Participants

Twelve physically active males (mean and SD: 81.98 ± 7.95 kg, 182.6 ± 7.4 cm, 22 ± 3 years of age) volunteered to participate in the study. All participants were required to be 18-40 years of age and exercising ≥ 6 hours per week. Exclusion criteria included: history of gastrointestinal (GI) dysfunction, current musculoskeletal injuries, pre-existing medical conditions that could be exacerbated via maximal exercise and/or exercising in high temperature conditions. For 24 hours prior to all sessions, participants were required to abstain from consuming non-steroidal anti-inflammatory drugs, caffeine and alcohol and to avoid strenuous exercise. Participants recorded their dietary intake for 24 hours prior to the first testing session and were required to replicate this prior to each subsequent testing

session, thereby eliminating the effect of different foods on intestinal damage. Additionally, no participant was previously acclimated to the heat. All participants completed a pre-exercise screening questionnaire (Exercise & Sports Science Australia 2012) and were informed of the study procedures prior to providing written informed consent.

Incremental Maximal Exercise Test

Participants completed an incremental maximal exercise test in environmental conditions of 20°C, 40% RH to determine their maximal oxygen uptake ($\dot{V}O_{2max}$). All incremental exercise tests were performed on a Lode Cycle Ergometer (Lode, Groningen, Netherlands) with the initial workload (W) equal to twice the participant's body weight, with an increase of 20W per minute until volitional fatigue. Expired air was collected at 30 s intervals using a metabolic cart (Parvo TrueOne; Parvomedics, UT, USA). Heart rate (Polar Electro Oy, Kempele, Finland), power output and rating of perceived exertion (6-20 Borg scale) (Borg 1982) were recorded at the end of every stage. The metabolic cart was calibrated prior to each exercise session using medical grade gases of known concentrations and a 3.0 L syringe, according to manufacturer's requirements. Data from the $\dot{V}O_{2max}$test was used to determine the workload of the subsequent exercise trials.

Exercise Trials

At least 48 hours after the $\dot{V}O_{2max}$test participants completed their first exercise trial, followed by a two to seven-day interval prior to completing their second exercise trial. All trials took place at the same time of day (± 1 hour). Upon arrival at the laboratory participants rested in a supine position for five min prior to venous blood collection. Participants were then provided with a 9FR rectal temperature probe (Phillips Medical Systems, MA, USA) for insertion 10 cm beyond the anal sphincter after which their body mass was measured. Exercise trials consisted of a standardised warm up and 45min of cycling on a Wattbike ergometer (Wattbike Ltd. WPC Model 2, Nottingham, England) at 70% VO_{2max} in a climate chamber at environmental conditions of either 30°C, 40%RH (hot) or 20°C, 40%RH (temperate). The warm up consisted of two min cycling at 30% peak power output (PPO), two min at 50% PPO and one min building up to an intensity of 70% $\dot{V}O_{2max}$determined using indirect calorimetry. Following the warm up participants cycled at 70% $\dot{V}O_{2max}$for 45min. During each exercise trial rectal temperature was recorded every second (EL-USB-TP-LCD temperature data logger, Lascar. PA,

USA) and averaged over each five min interval, while tympanic temperature (Covidien, Genius 2, MA, USA) was recorded every five min. Heart rate was recorded at 30 s intervals. To ensure participants maintained an intensity of 70% $\dot{V}O_{2max}$throughout the session, expired air was collected and analysed using the metabolic cart at five min intervals. If $\dot{V}O_2$ varied from the prescribed exercise intensity (± 2 $mL \cdot kg \cdot^{-1} min^{-1}$) power output was adjusted accordingly (increased or decreased) and expired air measured for a period until two steady state readings at the target intensity were achieved. Rating of perceived exertion (6-20 Borg scale) (Borg 1982), thermal sensation [rating from -4 (unbearably cold) through to 4 (unbearably hot) in 0.5 increments] (ANSI 2010) and gastrointestinal (GI) symptoms (rating on a scale of 0-4 of the following symptoms: belching, nausea, side stitch, abdominal cramps and urge to defecate) (Hall et al. 2013) were recorded every five min. As fluid restriction during exercise may increase intestinal damage (Lambert et al. 2008), 1.25 $mL \cdot kg^{-1}$body mass of water was administered to the participants every five min to counteract potential effects of dehydration. Following completion of each exercise session, participant body mass was immediately recorded, followed by the collection of a post-exercise blood sample. A post exercise cool-down was conducted following blood collection at a self-directed intensity for approximately five min.

Passive Heat Trial

The passive trial completed by a subset of randomly selected participants (n=5) was conducted in the same manner as the exercise trials, however, participants remained quietly seated in 30°C and 40%RH for 45 min rather than exercising. Blood was collected pre- and post- passive heat exposure while rectal temperature, HR, tympanic temperature, RPE, thermal sensation and GI symptoms were also recorded at the same time points as during the exercise trials.

Blood Collection and Analysis

Venous blood samples were drawn without stasis by venipuncture from the antecubital fossa immediately pre- and post-exercise, or pre- and post-passive rest, from participants in a supine position. Blood samples were collected into 4mL Lithium Heparin Vacutainers® (Becton Dickinson, New Jersey, USA). Haemoglobin concentration was determined in duplicate using a HemoCue® Hb 20 (HemoCue®, Angelholm, Switzerland) while haematocrit was determined in duplicate using the capillary centrifugation method. Capillary tubes were spun at 20,000 rcf for five min. Blood samples

were centrifuged at 4°C for 15 min at 1000 g and plasma aliquoted and stored at -80°C until analysis. Plasma concentrations of I-FABP were adjusted for changes in plasma volume (Dill and Costill 1974).

Intestinal Damage

The concentration of plasma I-FABP, a proven acute and sensitive marker to detect changes in intestinal damage (Timmermans et al. 2015), was determined using a commercial enzyme-linked immunosorbent assay (Hycult Biotechnology, Uden, The Netherlands) according to manufacturer's instructions. Samples were analysed in duplicate with each participants' samples run on the same plate. The intra-assay CVs were 2.5-4.7%. The minimum detectable concentration was 20 $pg \cdot mL^{-1}$ and all samples were within the assay standards range.

Statistical Analysis

A Shapiro-Wilk test was used to determine normality of distribution of all data. I-FABP concentrations and $\dot{V}O_2$were normally distributed and analysed using a two-factor (trial x time) repeated measures ANOVA. If a significant main effect or interaction was present, post hoc testing with Bonferroni correction was used to determine changes between and within trials. When data were combined, heart rate, tympanic temperature, rectal temperature, thermal sensation and RPE were non-normally distributed, therefore data were split into individual trials (i.e. hot: 30°C, 40%RH or temperate: 20°C, 40%RH). Due to technical issues on three trials, complete rectal temperature data was collected (and is presented) for nine participants. Differences within trials were determined using a Friedman test with Dunn's post hoc or where individual trial data was normally distributed, a one-way repeated measures ANOVA with Bonferroni post hoc. Differences between trials were analysed with a Wilcoxon matched-pairs signed rank test, or where data were normally distributed, a paired t-test. To determine the effect of passive heat exposure on intestinal damage, data between and within trials was compared for only the five participants that had completed each trial. All statistical analyses were performed using GraphPad Prism version 7.02 for Windows (GraphPad software, La Jolla California, USA). Acceptance for significance was set at $p<0.05$. Data are presented as mean ± SD for I-FABP, $\dot{V}O_2$, body mass, haematocrit and haemoglobin, and presented as median ± 95%CI for heart rate, tympanic temperature, rectal temperature, thermal sensation and RPE, and as median and 5^{th} to 95^{th} percentile for GI symptoms. Assuming a post-exercise concentration of I-FABP ~ 850 ± 200 $pg \cdot mL^{-1}$

(determined from previous research in our laboratory) and being able to detect a difference of 20% in response to different environmental conditions, 11 participants were required to achieve a power of 0.8.

Results

Intestinal Fatty Acid-Binding Protein

Plasma I-FABP levels significantly increased post-exercise in both temperate temperate (pre-exercise: 571±175 pg·mL^{-1}, post-exercise:852±317 pg·mL^{-1}) and hot conditions (pre-exercise: 585±188 pg·mL^{-1}, post-exercise: 954±411 pg·mL^{-1}) (p=0.0001);; however there was no main effect of environmental temperature (p=0.586) (Figure 1). There was no significant change in plasma I-FABP concentration following 45min of passive heat exposure (p>0.591).

Rectal Temperature

Average rectal temperature throughout the 20°C, 40%RH trial was 37.7°C (95%CI 37.5 to 37.8°C) and throughout the 30°C, 40%RH trial was 37.7°C (95%CI 37.4 to 37.9°C) (p=0.973). Peak rectal temperature in the temperate trial was 38.2°C (95%CI 37.99 to 38.31°C) and 38.3°C (95%CI 37.96 to 38.6°C) in the hot trial. Rectal temperature significantly increased from pre to post in both the temperate trial (p<0.001) and hot trial (p<0.001) (Figure 2A). Rectal temperature averaged across each five min interval significantly increased throughout the temperate exercise trial (p<0.001) and the hot trial (p<0.001) (Figure 2A). There was no significant increase in rectal temperature during the passive hot trial (p=0.084) (Figure 2A).

Tympanic Temperature

Average tympanic temperature throughout the temperate trial was 38.6°C (95%CI 38.20 to 38.73°C), and throughout the hot trial was 39.1°C (95%CI 39 to 39.5°C). Tympanic temperature significantly increased pre to post-exercise in both the temperate trial (p=0.012) and hot trial (p<0.001) (Figure 2B). Tympanic temperature for each five min interval, except at 10min, was significantly higher during the hot trial, compared to the temperate trial (Figure 2B). There was no significant increase in tympanic temperature during the passive hot trial (p=0.062) (Figure 2B).

VO_2 and Heart Rate

As intensity was set at 70% $\dot{V}O_{2max}$, $\dot{V}O_2$ during the exercise trials was similar, as intended (temperate: 36.8±7.2 mL·kg·$^{-1}$min^{-1}, hot: 36.5±7.1 mL·kg·$^{-1}$min^{-1}; p=0.94) (Figure 2D). Average HR was also similar during the temperate trial (165 bpm, 95%CI 158 to 171 bpm) and the heat trial (163 bpm, 95%CI 158 to 173 bpm) (p=0.655). HR averaged across each five min interval was similar throughout the temperate exercise trial (p=0.095) while in the hot condition HR significantly increased across time (p<0.001) (Figure 2C). There was no significant increase in HR during the passive hot trial (p=0.948) (Figure 2C).

Body Mass, Haematocrit and Haemoglobin

There was no significant effect of environmental temperature (p=0.93) on body mass, and no significant interaction (p=0.89). Body mass was significantly different over time (pre to post exercise) (temperate: pre 81.5 ± 7.78 kg, post 81.35 ± 7.88 kg; hot: pre 81.8 ± 7.83 kg, post 81.64 ± 7.96 kg) (p=0.018), however post-hoc testing did not identify any significant differences.

Haematocrit was not significantly different pre to post-exercise (p=0.51) or between environmental conditions (p=0.52), and there was no significant interaction (p=0.26) (temperate: pre 47.6 ± 3.8%, post 48.3 ± 3.3%; hot: pre 47.1 ± 3.7%, post 47.0 ± 2.9%).

Haemoglobin was significantly different over time (p<0.0001), but there was no significant main effect of environmental condition (p= 0.68) and no significant interaction (p=0.70). Post-hoc testing did not identify any significant differences pre to post exercise (temperate: pre 154 ± 13 g·L^{-1}, post 158 ± 14 g·L^{-1}; hot: pre 151 ± 11 g·L^{-1}, post 156 ± 11 g·L^{-1}).

Gastrointestinal Symptoms

During the temperate trial the frequency of symptom severity was significantly different (p=0.0019), however, post-hoc analysis did not reveal any significant differences across the severity categories of none (zero) to 4. During the hot and passive trials, the frequency of GI symptoms was significantly different across severity ratings (p=0.0004 and p=0.0008, respectively) with severity ratings of 3 or 4 reported significantly less than no symptoms at all (zero) (p<0.05). There were no significant

differences between the temperate and hot trials within each severity rating (0-4) (all $p>0.40$). There were no significant differences between the hot and passive trials within each severity rating (0-4) (all $p>0.58$).

Rating of Perceived Exertion and Thermal Sensation Scale

Perceived exertion increased during both the temperate and hot trials ($p<0.001$) (Table 1). Thermal sensation increased throughout all three trials ($p<0.001$), where thermal sensation peaked at 2, 3 and 1.5 for the temperate, hot and passive trials, respectively (Table 1).

Discussion

The primary finding of this study is that when exercise is performed at the same relative intensity, the magnitude of exercise induced intestinal damage is similar between conditions of compensable heat stress (30°C/40%RH) and a more temperate environment (20°C/40%RH). In addition, short term passive heat exposure alone did not increase intestinal damage, indicating that when exercising in conditions of compensable heat stress, intestinal damage is predominantly attributable to the exercise component rather than environmental heat.

Literature that is able to differentiate between the impact of environmental temperature and exercise intensity on associated intestinal damage is scarce. Previously, it has been shown that when exercising at the same absolute intensity, increasing environmental temperature is associated with greater increases in intestinal damage (Snipe et al. 2016; Snipe et al. 2018a). Snipe et al. (2016) reported a greater increase in I-FABP level when running at the same absolute speed in 30°C compared to 22°C; however, it was unknown whether this increase in damage was attributable to the greater exercise strain that accompanies exercise in the heat at the same absolute intensity, or to the increase in environmental temperature. It has been established that as exercise intensity increases there is a redistribution of blood flow from the splanchnic area to the active muscles and skin to dissipate heat to the environment in an act of thermoregulation (Rowell et al. 1965). This can lead to intestinal hypoxia (Hall et al. 1999) and likely results in ATP depletion and acidosis, in addition to inducing oxidative and nitrosative stress (Hall et al. 1994; Hall et al. 2001), which together can accumulate in damage to the cell membrane and tight junctions, consequently increasing intestinal

damage. A limitation of the present study is that we did not systematically capture data on absolute workload. While we cannot provide an exact magnitude of difference it is likely that power was reduced when cycling in the heat. For the same exercising heart rate (similar to the current study where heart rate and $\dot{V}O_2$ were the same between environmental conditions), we have previously shown a 30% reduction in cycling power output in the heat compared to cool environmental conditions (Philp et al. 2017). In the present study when exercise intensity was comparable (70% $\dot{V}O_{2max}$), the increase in I-FABP concentration was similar between temperate and hot conditions (*d*=0.18), suggesting that factors other than environmental heat may be greater contributors to intestinal damage.

Reduced blood flow to the gut over an extended period may be more influential for damage than core temperature. Indeed Morrison et al. (2014) have reported an increase in I-FABP of 563% following 90 min of cycling and running in 30°C with an end core temperature of 38.75°C, while Barberio et al. (2015) reported an increase of only 46% following 26 min of running in 40 degrees with an end core temperature of 39.0°C at a similar intensity. In support of the hypothesis that the magnitude of exercise induced intestinal damage is not related to environmental temperature we have combined the present group mean data with data from other studies that simultaneously report the post-exercise change in I-FABP in combination with end exercise core temperature, participants $\dot{V}O_{2max}$ (indicative of training status), and exercise duration and intensity (Morrison et al. 2014; Barberio et al. 2015; March et al. 2017; McKenna et al. 2017; Snipe et al. 2018a). There was a significant correlation between environmental temperature (range 22°C to 40°C) and end exercise core temperature (range 38.2°C to 39.6°C) (r=0.79, p=0.011), however; there was no significant correlation between the magnitude of intestinal damage and core temperature (r=0.3, p=0.43), suggesting that other factors contribute to the extent of damage. End exercise core temperature does not appear to be the best predictor of exercise induced intestinal damage.

The inclusion of the passive trial in the present study was to determine the magnitude of intestinal damage that could be solely attributable to the hot environment, as compared to exercise in the heat. The increase in I-FABP level following passive heat exposure was very small (*d*=0.12), compared to a large increase following exercise in the heat (*d*=1.12). While only a subset of participants completed

the passive trial, it is a most likely conclusion that the increase in I-FABP concentration in the present study following exercise can be predominantly attributed to the exercise component rather than the environmental conditions. While passive exposure to 30°C did not significantly increase core temperature, it is possible that higher environmental temperatures and longer duration exposures that elicit a greater increase in core temperature may increase intestinal damage at rest. Mice exposed to an external temperature of 39°C for two hours had a core temperature of 41°C and increased permeability (Soares et al. 2014), while prolonged heat exposure to induce a core temperature of 42.4°C significantly damaged mice intestine (Phillips et al. 2015). In humans, the core temperature required to induce intestinal damage following passive heat exposure is yet to be determined. Given the potentially large increase in core temperature required to damage the intestine with passive heat exposure (Soares et al. 2014), it may be hypothesised that the redistribution of blood flow is more influential than core body temperature in eliciting intestinal damage; however, future research is needed to investigate and contrast these two parameters.

The magnitude of intestinal damage can be influenced by an array of exercise factors. The present study reports a significant increase in I-FABP during both exercise trials which as previously outlined can be predominantly attributed to the work being done during exercise rather than the environmental conditions. However, there are a range of other factors which can also amplify intestinal damage, and the present study aimed to minimise the influence of these factors on the extent of intestinal damage. Lambert et al. (2008) previously reported that withholding fluid significantly increased intestinal permeability, determined via urinary sugars (lactulose to rhamnose ratio), compared to the ingestion of water and for this reason fluid was provided during exercise in the present study. Additionally, a cycling modality was chosen to reduce intestinal damage during exercise as van Niewenhoven et al. (2004), have previously shown that running and its associated mechanical agitation on the intestines increases intestinal permeability in comparison to cycling. Interestingly, Sessions et al. (2016) have reported a considerably lower post-exercise I-FABP concentration in highly trained athletes after 60min running at 70% VO_{2max} in comparison to the present study which employed 45min of cycling at 70% VO_{2max} (337.9 ± 207.4 $pg \cdot mL^{-1}$ vs 926.8 ± 427.2 $pg \cdot mL^{-1}$). Of note, Sessions et al. (2016) measured I-FABP 20min after exercise cessation and given that I-FABP has a short half-life (Strang et al. 2015), it is expected that this concentration would have been higher immediately post exercise.

While further research is required to explore the influence of exercise mode on intestinal damage, the relationship between training status and intestinal damage also requires investigation.

Gastrointestinal disturbances are rarely severe enough to force an individual to cease exercise (ter Steege et al. 2008); however, they can often result in a reduced ability to perform optimally. Exercise-induced GI symptoms commonly arise due to changes in GI motility, mechanical factors and hormone secretions (Gil et al. 1998). Ischaemia of the GI tract is largely considered to be a significant factor of exercise-induced GI symptoms (Moses 2005). Exercise and heat exposure have both been found to reduce splanchnic blood flow significantly to compensate for the skeletal muscle activity and heat dissipation (Rowell et al. 1965; Hales 1997; Dokladny et al. 2016). It has previously been found that GI ischemia during exercise can result in symptoms such as nausea, vomiting, abdominal pain and diarrhea, all of which will disrupt performance (Moses 2005), although other studies suggest no correlation of GI damage and GI symptoms (Karhu et al. 2017; Snipe et al. 2017). In the present study, GI symptoms were apparent in both exercise trials and the passive trial; however, the incidence and severity did not differ between exercise trials or between the hot and passive trials, suggesting that exercise intensity nor duration was not sufficient to induce GI symptoms. However, we should note that exercise associated GI symptoms may not manifest until after exercise (Moses 1990) so symptom recording for 24 hours post-exercise should be incorporated in future studies investigating the relationship between exercise, intestinal damage and GI symptoms.

In summary, this is the first study to report similar levels of intestinal damage when exercising at the same relative intensity in a hot environment and a temperate environment. Additionally, short-duration passive heat exposure did not increase intestinal damage indicating that exercise workload (or other factors), rather than environmental heat, are greater determinants of intestinal damage in a model of compensable heat stress.

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Conflict of Interest

The authors have no conflicts of interest to report.

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Tables

	Time (min)								
	5	10	15	20	25	30	35	40	45
RPE									
Temperate*	14.5	14.5	15.5	15	16	15.5	15.5	15.5	16
	(12.9-15.2)	(13.7-15.5)	(14.3-15.7)	(14.3-15.9)	(14.3-16.1)	(14.4-16.1)	(14.4-16.1)	(14.8-15.9)	(14.9-16.2)
Hot*	14.5	15	15	15.5	16	15.5	16	15	16
	(13.6-14.8)	(13.8-15.4)	(14.3-15.8)	(14.5-16.5)	(14.9-16.7)	(14.9-16.6)	(15.1-16.4)	(14.9-16.2)	(14.9-16.5)
Passive	6 (6-6)	6 (6-6)	6 (6-6)	6 (5.6-6.8)	6 (5.6-6.8)	6 (5.6-6.8)	6 (5.6-6.8)	6 (6-6)	6 (6-6)
TSS									
Temperate#	1.5 (1.2-1.7)	1.5 (1.4-1.9)	2 (1.5-2.1)	2 (1.4-2.3)	2 (1.5-2.1)	2 (1.5-2.2)	2 (1.4-2.2)	2 (1.5-2.4)	2 (1.6-2.3)
Hot#	2 (1.7-2.2)	2 (1.9-2.5)	2.5 (2.2-2.6)	2.5 (2.2-2.9)	3 (2.1-3.1)	3 (2.3-3.2)	3 (2.5-3.2)	3 (2.5-3.2)	3 (2.5-3.2)
Passive#	1 (0.6-1.8)	1 (0.4-1.4)	1.5 (0.6-1.8)	1.5 (0.6-1.8)	1.5 (0.9-1.6)	1 (0.9-1.5)	1 (0.9-1.5)	1 (0.9-1.5)	1 (0.9-1.5)

Table 1: Median (95% CI) of rating of perceived exertion (RPE) and thermal sensation scale (TSS) throughout the temperate, hot and passive trials (n=5). * significant increase in RPE across the temperate trial ($p<0.001$) and hot trial ($p<0.001$). # significant increase in TSS across all trials ($p<0.031$). Passive $n = 5$.

List of Figures

Figure 1: Intestinal fatty-acid binding protein (I-FABP) levels pre and post exercise in 20°C, 40%RH (Temperate) or 30°C, 40%RH (Hot) (n=12) and pre and post seated rest in 30°C, 40%RH (Passive) (n=5). * Post-exercise significantly increased from pre-exercise for Temperate and Hot ($p<0.05$). Data are mean ± SD.

Figure 2: A) Rectal temperature for each five min interval during the temperate (n=9), hot (n=9) and passive trials (n=5). * significantly different from 25, 30, 35, 40 and 45 min intervals during the hot trial and from 30, 35, 40 and 45 min intervals during the temperate trial ($p<0.05$). B) Tympanic temperature for each five min interval during the temperate, hot and passive trials. * significantly different from 30, 35, 40 and 45 min intervals during the hot trial ($p<0.05$). # significantly different from 20, 35, 40 and 45min intervals during the temperate trial ($p<0.05$). C) Heart rate for each 5 min interval during the temperate, hot and passive trials. * significantly different from 20, 25, 30, 35, 40 and 45 min intervals during the hot trial ($p<0.05$). D) $\dot{V}O_2$ for each five min interval during the temperate and hot trials. Data for each trial were collected at the same time points however data for the hot trial is staggered to enable differentiation of data points. Data are median and range for A) to C) and mean ± SD for D).

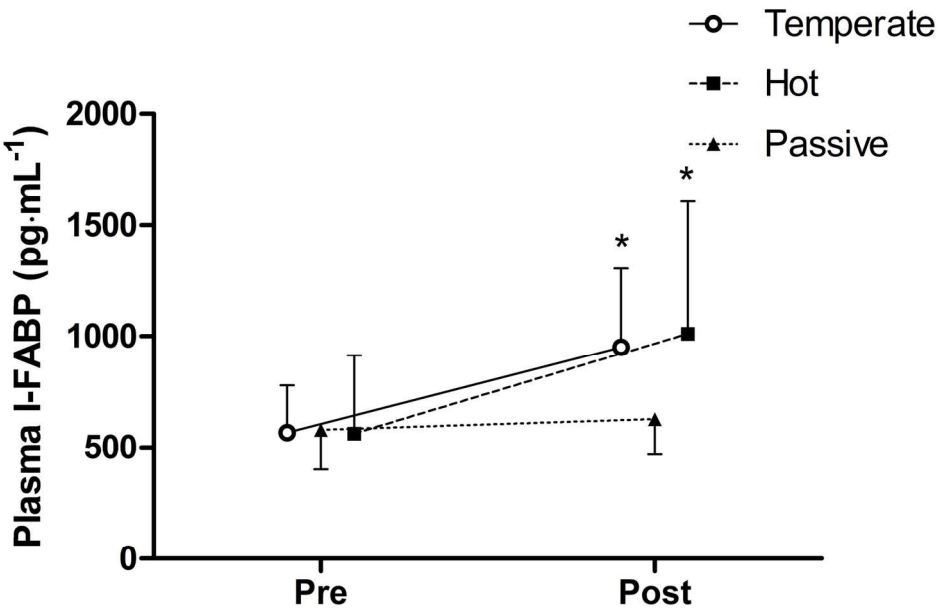

Figure 1

76x51mm (600 x 600 DPI)

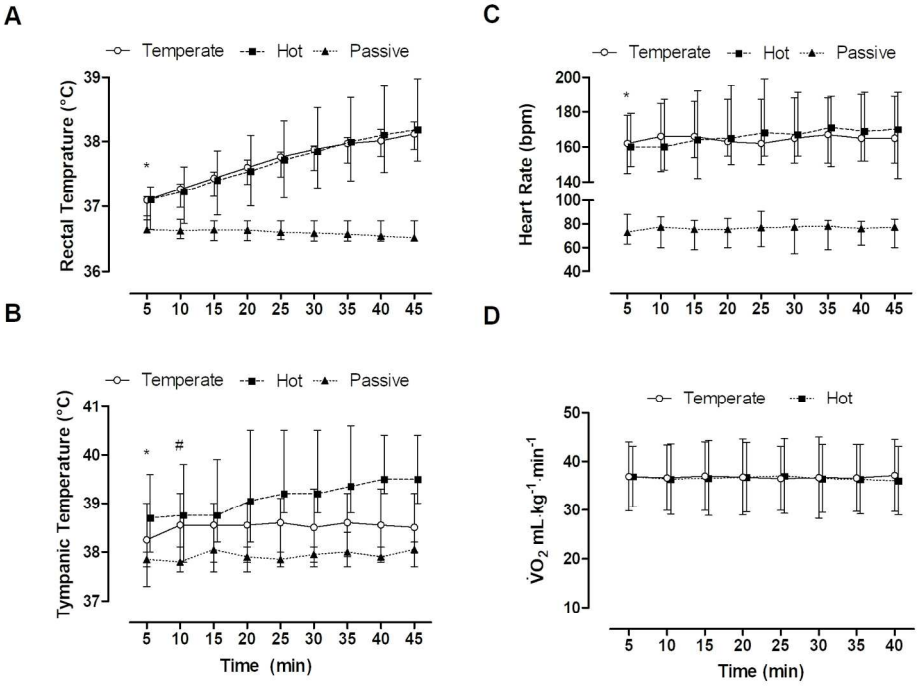

Figure 2

168x124mm (300 x 300 DPI)