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Original research

The impact of exercise modality on exercise-induced gastrointestinal syndrome and associated gastrointestinal symptoms

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ABSTRACT

Objectives: This study aimed to determine the impact of running and cycling exercise modalities on the magnitude of exercise-induced gastrointestinal syndrome (EIGS) and associated gastrointestinal symptoms (GIS).
Design: Parallel group trial design.
Methods: Twenty-eight endurance athletes (male $n = 14$, female $n = 14$) completed 2 h running at 55 % of maximal oxygen uptake or cycling at 55 % of maximal aerobic power in T_{amb} 35 °C and 22 % RH. Pre- and post-exercise blood samples were collected and analysed for markers of intestinal epithelial integrity perturbations (i.e., plasma intestinal fatty acid protein (I-FABP), soluble (s)CD14, and lipopolysaccharide binding protein (LBP)) and systemic inflammatory cytokines (i.e., plasma IL-1β, TNFα, IL-10, and IL-1ra). GIS were assessed pre-exercise and every 10 min during exercise.
Results: Exercise-associated Δ for plasma I-FABP (191 and 434 pg·ml^{-1}) and LBP (−1228 and 315 ng·ml^{-1}) did not differ between running and cycling, respectively; however for sCD14 was higher ($p = 0.030$) on cycling (116 ng·ml^{-1}) vs running (96 ng·ml^{-1}). There were no differences in absolute pre- and post-exercise systemic inflammatory cytokine concentration, with large individual variation observed. Exercise-associated plasma TNF-α, ($p = 0.041$) and IL-10 ($p = 0.019$) responses were greater in running than cycling, but did not lead to a greater systemic inflammatory response profile ($p = 0.305$) between running (5.0arb.units) and cycling (−2.5arb.units). Although greater GIS incidence occurred in running (44 %) compared with cycling (25 %), there was no difference between groups for GIS severity.
Conclusions: When running and cycling exercise is performed with similar duration, intensity, ambient conditions, and with confounder control, the exercise modality does not substantially impact the magnitude of EIGS or associated GIS severity.

Practical implications

- Moderate intensity endurance running and cycling exercise does not substantially differ in the presentation of exercise-induced gastrointestinal syndrome (EIGS) or associated gastrointestinal symptoms (GIS) when matched for exercise load, experimental conditions, and controlled for confounding factors.
- Considering individual variation observed, the modality of exercise may play a role in amelioration or exacerbation EIGS, and therefore should be a consideration within the pathophysiological and exacerbation assessment procedures of athlete reporting exercise-associated GIS.
- Future research is warranted to justify and establish the extent to which feeding during running and cycling exacerbates any potential GIS and feeding intolerance aligned with exercise body position, body movement, and its impact on mechanism factors of EIGS. Additionally, exercise types that have not yet been explored for magnitude of EIGS with associated GIS and feeding tolerance, but have body position, movement, and thermoregulatory implications (e.g., open water swimming, open water endurance paddle-sports (rowing, kayaking) and sailing, and enduro-motorsports) need exploration.

1. Introduction

It is now well established that at the onset of exercise the gastrointestinal tract undergoes several alterations that results in compromised gastrointestinal integrity and function, termed 'exercise-induced gastrointestinal syndrome (EIGS)'.[1,2] The aetiology and pathophysiology of

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EIGS has previously been described and updated.[1–4] In short, such alterations include: 1) A redistribution of blood flow to working muscles and peripheral circulation (circulatory-gastrointestinal pathway), which may result in intestinal epithelial cell injury and hyperpermeability, leading to translocation of pathogenic microbial agents from to lumen into circulation and resultant widespread systemic inflammatory responses. 2) An increase in sympathetic drive that suppresses enteric nervous activity (neuroendocrine-gastrointestinal pathway), which may result in impaired gastrointestinal motility, digestion, and/or nutrient absorption. In addition, it has been proposed that certain mechanical factors aligned with exercise (e.g., jarring, jolting, and impact) may also contribute to disturbing the integrity and function of the gastrointestinal tract.[5,6] These pathophysiological pathways that compromise the integrity and function of the gastrointestinal track in response to exertional stress are highly linked with the instigation of gastrointestinal symptoms (GIS) with the onset of exercise and as it prolongs.[1,2]

Evidence is now available to confidently show that heat stress is a prime extrinsic exacerbation factor for EIGS and associated GIS.[7–13] A substantial exposure to exertional-heat stress promotes greater intestinal epithelial cell injury, bacterial endotoxin translocation, and systemic immune responses, and GIS, compared with exertional stress of varying intensities in temperate ambient conditions. For example, Δ pre- to post-exercise plasma I-FABP concentration (i.e., a surrogate marker for enterocyte damage) is consistently apparent ≥1000 pg·ml^{-1}, systemic endotoxemia (i.e., plasma LPS, LBP, and/or sCD14 concentration) is greatly enhanced, and/or systemic inflammatory response profile (SIR-Profile: representing the combined peak post-exercise Δ for 6-plex systemic inflammatory cytokines, as previously described by Bennett et al.[13]) is consistently ≥100arb.units, in response to 2 h running at 60 % of maximal oxygen uptake ($\dot{V}O_{2max}$) in an ambient temperature (T_{amb}) of ~35.0 °C resulting in ≥39.0 °C core body temperature.[8,11–13] In comparison with 2-3 h running ranging between ≥55–80 % $\dot{V}O_{2max}$ in T_{amb} ~ 20.0 °C resulting in <39.0 °C core body temperature, and reporting of pre- to post-exercise magnitude of change for plasma I-FABP concentration consistently ≤1000 pg·ml^{-1}, no to modest systemic endotoxemia, and/or 6-plex SIR-Profile ≤50arb.units.[7,9,10] Such previous research outcomes clearly present an argument for exertional-heat stress exacerbating EIGS and potentially impacting associated GIS.

In addition, previous laboratory controlled and field-based research (i.e., competitive events) has also proposed that exercise modality may contribute to the magnitude of gastrointestinal perturbations and GIS in response to exercise.[14,15] For example, running exercise has been reported to create greater exercise-associated disturbances to intestinal integrity (e.g., epithelial permeability) and gastrointestinal function (e.g., orocecal motility) compared with cycling exercise[15]; although such outcomes in regards to gastrointestinal function has not always been consistent.[16] Nevertheless, exercise-associated GIS incidence and severity are consistently shown to be higher during competitive running events compared with competitive cycling events, and/or the running segment compared with the cycling segment of triathlon events.[17–19] The proposed mechanisms by which running exercise, over cycling, may create a greater burden on gastrointestinal status includes, but is not limited to: 1) greater splanchnic hypoperfusion and sympathetic drive linked to higher whole-body physiological and thermal strain, and/or 2) greater biomechanical vibration.[6,20,21] Such mechanisms are aligned with the aetiology of exercise-associated GIS.[1–4]

Although previous laboratory-controlled research has highlighted the potential role of exercise modality on exacerbating EIGS and GIS, these have generally been of modest duration (e.g., <2 h) and without heat stress,[15,16] and not synonymous with exercise bouts showing high rates of participants reporting signs and/or symptoms of substantial gastrointestinal disturbance.[1–4] Conversely, field-based research that have reported high GIS incidence rates amongst participants have not comprehensively measured gastrointestinal integrity, functional, and/or mechanical markers in adjunct.[14,17,19] However, a recent laboratory-controlled study recruited participants to perform 45 min of running and cycling exercise at 70 % $\dot{V}O_{2max}$ (i.e., T_{amb} 21 °C, 40 % relative humidity (RH)), in which cycling resulted in greater intestinal epithelium injury (plasma I-FABP: +447 pg·ml^{-1}) versus running (+144 pg·ml^{-1}), but running resulted in more mild GIS incidence rates (45 %) versus cycling (27 %).[22] It is important to highlight that such findings are likely attributed to the artefact of greater physiological strain and dehydration reported in cycling, and/or possibly the lack of adequate dietary control or monitoring, and not necessarily the impact of modality per se.[7,8,23,24]

With this in mind, the aim of the current study was to determine the impact of running and cycling exercise modalities on the magnitude of EIGS and associated GIS, using a parallel group design. Based on the current literature, it was hypothesised that running exercise would result in greater intestinal epithelium injury, endotoxin translocation, systemic inflammatory response, and GIS, compared to cycling exercise at a similar workload and environmental conditions.

2. Methods

Twenty-eight non heat-acclimatized, endurance-trained runners, cyclists, and triathletes volunteered to participate in this study (Supplementary Table 1). Trials for female athletes were scheduled during the early-mid follicular phase of their menstrual cycle. Resting estrogen levels (DKO003/RUO; DiaMetra, Italy) were measured for verification, were within normal reference range, and did not differ between exercise modalities (15.3(35.1)pg·ml^{-1}; $p = 0.581$). Participants opted to either complete the experimental procedure cycling or running depending on their usual sporting participation and personal preference. This study conformed to the standards set by the Declaration of Helsinki, and was approved by the local ethics committee (CF16/1125-2016000598). All participants gave written informed consent prior to participating in the study. Participants then completed the initial assessment and familiarisation trial, as previously described.[25]

The experimental procedures for the parallel group trial design are schematically illustrated in Supplementary Fig. 1. Participants completed the experimental trial consuming their usual free-living diet, and recorded all food and fluid intake for three days prior the experimental trial in a food-fluid log. Energy and nutritional intake during this monitoring period was analysed in accordance with previously described procedures (Supplementary Table 1).[26] Participants arrived at the laboratory fasted (0830–0930 h). Upon arrival, they were provided with a standardised breakfast [Mean (SD): Energy 2313(318)kJ (33 kJ·kg^{-1}), carbohydrate 104(14)g (1.5 g·kg^{-1}), protein 17(2)g (0.25 g·kg^{-1}), fat: 7(1)g (0.1 g·kg^{-1}), water provision 250 ml]. Prior to exercise commencement and after voiding, total body water (TBW) was measured (Seca 515 MBCA; Seca Group, Hamburg, Germany), and blood was collected by venepuncture from the antecubital vein into lithium heparin (6 ml, 1.5 IU·ml^{-1} heparin) and EDTA (4 ml, 1.6 mg·ml^{-1} K3EDTA) vacutainers (BD, Oxford, UK). To monitor rectal temperature (T_{re}) during exercise, participants inserted a thermocouple 12 cm beyond the external anal sphincter (Grant REC soft insertion probe thermocouple; Grant 2010 Squirrel data logger, Shepreth, UK).

The experimental protocol consisted of 2 h running in a on a motorized treadmill (Forma Run 500; Technogym, Seattle, WA, USA) at 55 % of ($\dot{V}O_{2max}$) or cycling at 55 % maximal aerobic power (MAP) on participants own bicycle attached to a Wahoo KICKR cycle ergometer (Wahoo Fitness, Atlanta, GA, USA),[27] within an environmental chamber in hot ambient conditions (T_{amb} 35.3 °C and 21.7 % RH, and T_{amb} 35.3 °C and 21.1 % RH, respectively; supplementary Table 1). With the focus of replicating airflow expected during the different exercise modalities, a fan was placed in front of the participant, with fan airspeed of ~10.6 km·h^{-1} for running or ~19.5 km·h^{-1} for cycling. Participants consumed water (~23 °C) of the same quantity and timing in both trials,[7,12] intended to limit body mass loss to 1.5 %, based on sweat rate calculated during the familiarisation. T_{amb} and T_{re} was recorded every 5 min throughout exercise, and rating of perceived exertion (RPE), thermal

comfort rating (TCR), and GIS every 10 min. As part of a previously published research study aimed at investigating sweat rate and composition during running and cycling,[25] following completion of the first hour of exercise, participants ceased exercising, and left the environmental chamber for 5 min to apply a second set of sweat patches. A second blood sample was collected immediately post-exercise, as previously described, mirroring pre-exercise procedures.

Whole-blood hemoglobin (Hb) (Hb201+, Hemocue AB, Ängelholm, Sweden) and hematocrit were used to calculate changes in plasma volume (P_v) relative to baseline, and to correct plasma variables. Remaining blood samples were centrifuged at 4000RPM and 4 °C for 10 min, within 15 min of collection. Plasma was aliquoted into 1.7 ml micro-storage tubes and frozen at −80 °C until analysis, except for 100 μl (2 × 50 μl) that was used to determine plasma osmolality (P_{Osmol}), in duplicate (CV 0.8 %), by freeze-point osmometry (Osmomat 030; Gonotec, Berlin, Germany). Plasma concentration of cortisol (IBL International, Hamburg, Germany), I-FABP, sCD14, and LBP (Hycult, Uden, Netherlands) were determined by ELISA, as per manufacturer's instructions. Plasma concentrations of pro-inflammatory cytokines IL-1β and TNF-α, and anti-inflammatory cytokines IL-10 and IL-1ra, were determined by multiplex ELISA (HCYTOMAG-60 K, EMD Millipore, Darmstadt, Germany). All variables were analysed in duplicate as per manufacturer's instructions, with standards and controls on each plate, and each participant assayed on the same plate. The CV for ELISA analysis was ≤10.4 %.

Based on the statistical test, mean, standard deviation, and effect of previously established exercise stress models, with and without additional heat stress that induce gastrointestinal integrity perturbations and GIS,[7–12] and applying a standard alpha (0.05) and beta value (0.80), the current participant sample size within the parallel group design is estimated to provide adequate statistical power (power* 0.80–0.99) for detecting significant modality differences (G*Power 3.1, Kiel, Germany) in the primary variables. Data in the text and tables are presented as either mean ± SD (descriptive data) or mean and 95 % confidence interval (CI) (primary or secondary variables), as indicated; and accumulative score (total and corrected) and individual participant range for GIS. For clarity, data in figures over a timeline are presented as mean ± standard error of the mean (SEM), and considering the common individual variation reported in exercise gastroenterology research,[8–21] pre- to post-exercise gastrointestinal integrity and systemic markers data are presented as 'box & whisker' format. Prior to data analysis, outlying values for all variables were detected through box-plot analysis (v.27.0, IBM SPSS Statistics, IBM Corp., Armonk, NY, USA). Participants that presented outlying values were removed before application of statistical analysis. All data were checked for normal distribution (Shapiro-Wilks test of normality) by calculating skewness and kurtosis coefficients, prior to applying appropriate parametric or non-parametric statistical tests. General linear mixed model with post hoc analysis was used to determine differences in physiological strain markers. Primary and secondary variables with singular data points were examined using independent sample *t*-tests or non-parametric equivalent Mann-Whitney *U* tests, where appropriate. Variables with multiple data points were examined using a two-way (group*time) ANOVA (or non-parametric Kraskal-Wallis test, where appropriate). Significant main effects were analysed using a post hoc Tukey's HSD test. Statistics were analysed using SPSS statistical software (v.27.0, IBM SPSS Statistics) with significance accepted at $p < 0.05$.

3. Results

Energy and macronutrient intake during the monitoring period is depicted in Supplementary Table 1, and did not differ between the running and cycling groups. Pre-exercise total body water [57.7 (56.0 to 59.3)%] did not differ between groups ($p = 0.697$), and was indicative of euhydration. Pre- and post-exercise P_{Osmol} [294 (292 to 297) and

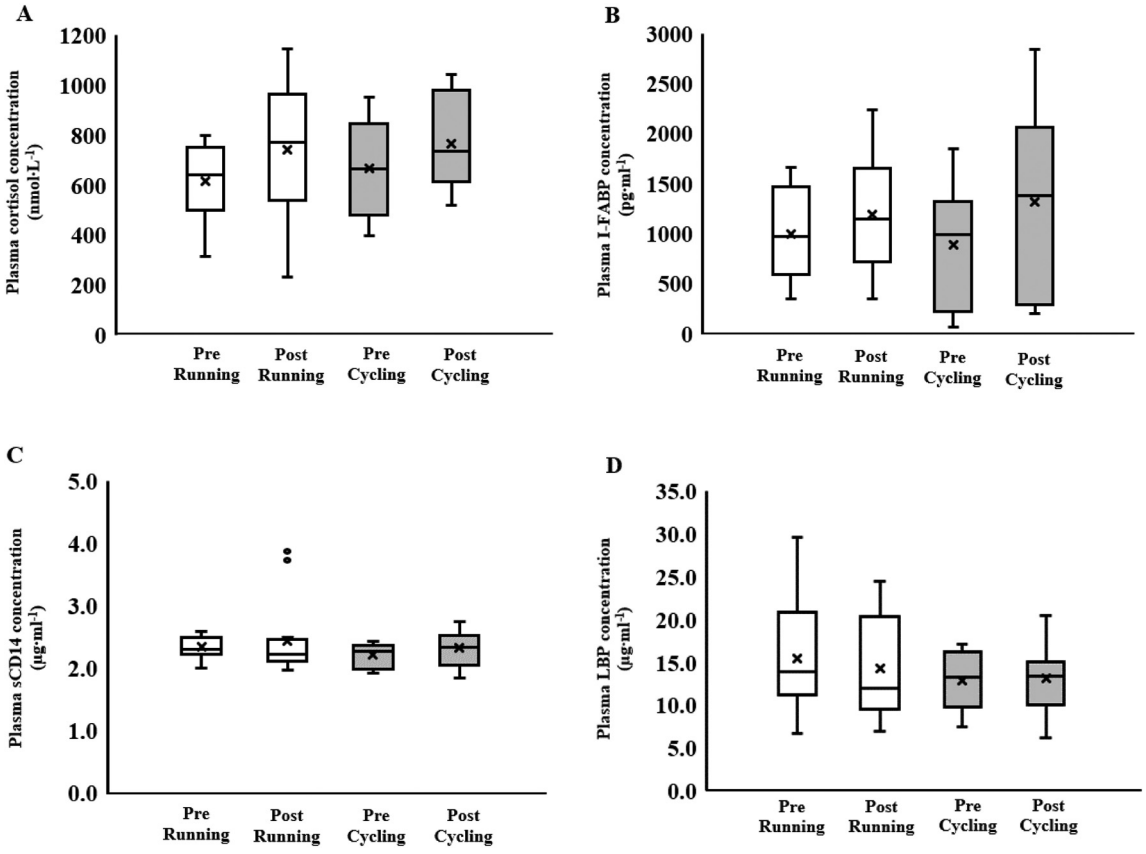

Fig. 1. Plasma stress response and gastrointestinal integrity biomarker concentration (A: cortisol, B: I-FABP, C: sCD14, and D: LBP) in response to 2 h of running at 55 % $\dot{V}O_{2max}$ (□) and cycling at 55 % MAP (■), in hot ambient conditions. Box & Whisker plots (n = 25–28, removal of outliers prior to analysis) with **X** indicative of mean.

295 (293 to 298)mOsmol·kg^{-1}, respectively] did not differ between groups ($p = 0.892$ and $p = 0.889$, respectively), and was indicative of euhydration. Pre-exercise BM [69.2 (65.7 to 75.6)kg] and water intake during exercise [571 (513 to 613)ml·h^{-1}] did not differ between groups ($p = 0.658$ and $p = 0.124$, respectively). Exercise-associated BM loss was greater ($p = 0.008$) in running (1.9 %) than cycling (1.2 %), but both values were within euhydration status criteria. Exercise-associated Δ P_v [−5.0 (−7.2 to −2.8)%] did not differ ($p = 0.227$) between groups.

No significant difference between running and cycling for HR, RPE, TCR, and T_{re} was observed throughout the 2 h exercise trial ($p > 0.05$), as depicted in Supplementary Fig. 2. Peak T_{re} for running at 55 % $\dot{V}O_{2max}$ and cycling at 55 % MAP was 38.6 (38.2 to 38.7)°C and 38.4 (38.2 to 38.5)°C, respectively; and did not significantly differ ($p = 0.227$). Absolute plasma cortisol concentration was observed to increase pre- to post-exercise in both modalities, but this increase failed to reach significance ($p = 0.063$). There was no significant difference in absolute pre- and post-exercise plasma cortisol concentrations ($p = 0.813$) between running and cycling (Fig. 1A). Additionally, there was no significant difference ($p = 0.759$) in the exercise-associated change (Δ pre- to post-exercise) in plasma cortisol response between running (110 nmol·L^{-1}, equivalent to +20 %) and cycling (98 nmol·L^{-1}, equivalent to +15 %).

There was no significant difference in absolute pre- and post-exercise plasma I-FABP ($p = 0.474$), sCD14 ($p = 0.993$), and LBP ($p = 0.578$) concentrations between running and cycling (Fig. 1B to D). Additionally, there was no significant difference in the exercise-associated change in plasma I-FABP [191 (−75 to 543) and 434 (−3 to 871)pg·ml^{-1}; $p = 0.337$] and LBP [−1228 (−2615 to 160) and 315 (−1417 to 2048)ng·ml^{-1}; $p = 0.086$] responses between running and cycling, respectively. However, a modestly higher exercise-associated increase in sCD14 ($p = 0.030$) was observed in cycling (116 (20 to 211)ng·ml^{-1}, +5.2 %) compared with running (96 (−145 to 337)ng·ml^{-1}, +4.1 %).

There was no significant difference in absolute pre- and post-exercise plasma IL-1β ($p = 0.598$), TNFα ($p = 0.210$), IL-10 ($p = 0.108$), and IL-1ra ($p = 0.962$) concentrations between running and cycling (Fig. 2). There was a trend in the exercise-associated change of plasma IL-1β [0.1 (−0.2 to 0.4) and −0.3 (−0.6 to 0.0)pg·ml^{-1}; $p = 0.066$], but not for IL-1ra [2.1 (−2.9 to 7.1) and −0.2 (−9.7 to 9.3)pg·ml^{-1}; $p = 0.306$] responses between running and cycling, respectively. Exercise-associated change in plasma TNFα ($p = 0.041$) and IL-10 ($p = 0.019$) for running [0.1 (−1.4 to 1.6) and 3.7 (0.3 to 7.0)pg·ml^{-1}, respectively] was greater than cycling [−2.1 (−3.2 to −1.0) and −1.3 (−3.7 to 1.1)pg·ml^{-1}, respectively]. Although exercise-associated SIR-Profile of the 4-plex was lower on cycling (−2.5 (−15.0 to 9.9)arb. units), values were not significant different ($p = 0.305$) from running (5.0 (−2.9 to 12.9)arb.units).

The incidence and severity of GIS as a result of the running and cycling exercise protocol are presented in Table 1. Despite running resulting in greater reports of GIS (i.e., lower-GIS and nausea), this did not translate into significantly greater GIS severity between the exercise modalities.

4. Discussion

The aim of the current study was to determine the impact of running and cycling exercise modalities on the magnitude of EIGS and associated GIS, using a parallel group design. Running exercise at 55 % $\dot{V}O_{2max}$ or cycling exercise at 55 % MAP in hot ambient conditions resulted in a modest disturbance to EIGS markers of intestinal integrity and systemic inflammation, and low incidence and severity of GIS. In contrast to our hypothesis, there was no substantial difference in intestinal integrity perturbations between modalities. Whereas, in accordance with our hypothesis, systemic inflammatory cytokine response was greater in running, but was modest in nature, and appears not to be fully and directly

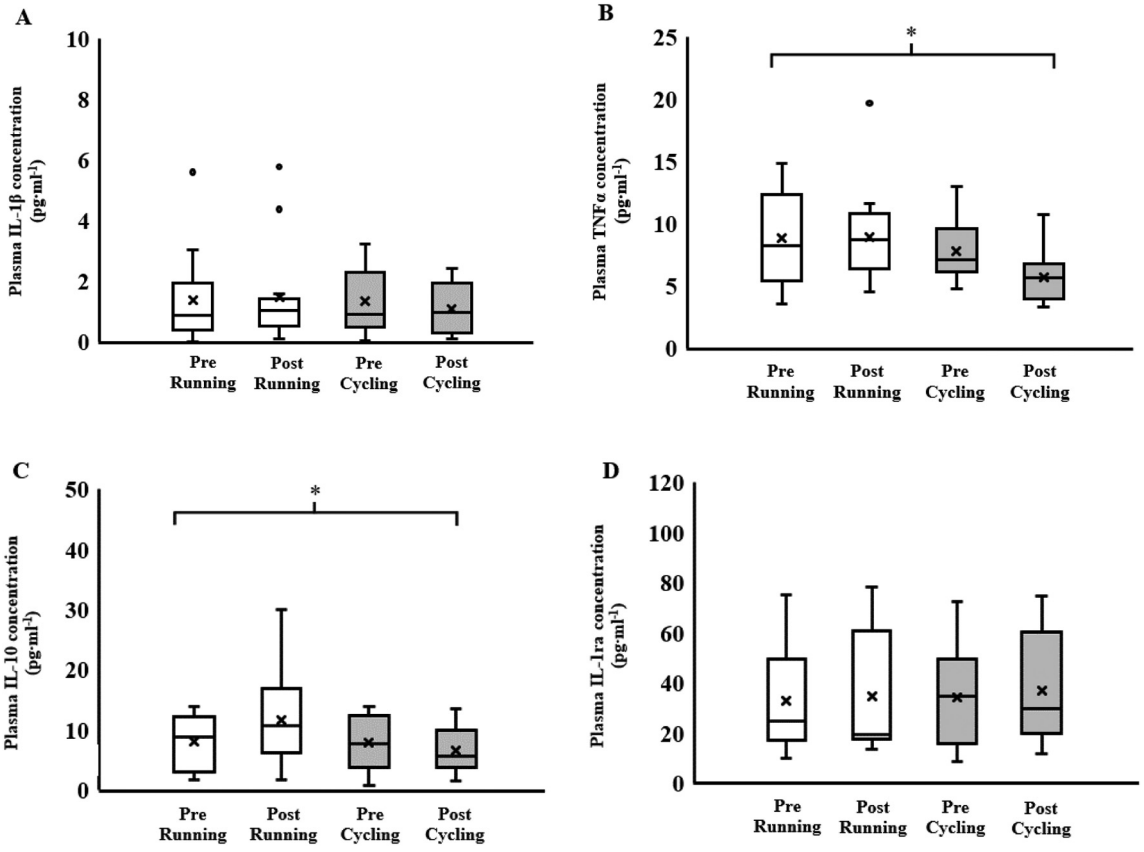

Fig. 2. Systemic inflammatory cytokine concentration (A: IL-1β, B: TNFα, C: IL-10, and D: IL-1ra) in response to 2 h of running at 55 % $\dot{V}O_{2max}$ (□) and cycling at 55 % MAP (■), in hot ambient conditions. Box & Whisker plots ($n = 25$–28, removal of outliers prior to analysis) with **X** indicative of mean: * $p < 0.05$ group difference for exercise-associated response.

Table 1
Gastrointestinal symptoms (GIS) in response to 2 h of running at 55 % $\dot{V}O_{2max}$ and cycling at 55 % MAP, in hot ambient conditions ($n = 28$).

	Running	Cycling	p
Incidence[b]			
Gut discomfort	NA	NA	NA
Total-GIS[c]	44 %	25 %	NA
Upper-GIS[d]	25 %	25 %	NA
Lower-GIS[d]	19 %	8 %	NA
Nausea	6 %	0 %	NA
Severity[b]			
Gut discomfort	2 (2−10)	3 (2−22)	0.401
Total-GIS[a]	2 (2–10)	4 (2−23)	0.448
Upper-GIS[b]	1 (2–7)	3 (2–22)	0.879
Lower-GIS[b]	1 (3–4)	1 (9)	0.517
Nausea	1 (8)	0 (0–0)	0.386

[a]Incidence: Total number (%) of participants reporting GIS ≥1 on the mVAS for any GIS type during the 120 min of running or cycling exercise. [b] GIS severity: overall participant summative accumulation of rating scale point score of measured time periods and individual participant range of those reporting symptoms; Upper-GIS (gastroesophageal): belching, heartburn, upper abdominal bloating, upper abdominal pain, urge to regurgitate, and/or regurgitation. Lower-GIS (intestinal): flatulence, lower abdominal bloating, lower abdominal pain, urge to defaecate, and/or abnormal defaecation (loose or watery stools, diarrhoea, or faecal blood loss). [c] summative accumulation of upper, lower, and other GIS; [b] summative accumulation of upper- or lower-GIS. GIS assessment tool: mVAS (10-point rating scale, each point indicative of 10 mm). 1–4 indicative of mild GIS (i.e., sensation of GIS, but not substantial enough to interfere with exercise workload) and increasing in magnitude, 5–9 indicative of severe GIS (i.e., GIS substantial enough to interfere with exercise workload), and 10 indicative of extremely severe GIS warranting exercise reduction or cessation. If no specific GIS was reported, this was indicative of 0, and subsequently no rating was warranted. Considering GIS, such as regurgitation and defecation, results in complete or temporary reduction or cessation of exercise, these GIS are presented as 0 and 10 rating only.[31] NA: not applicable.

related to perturbations in intestinal integrity (e.g., immune activation in response to luminal originated pathogenic agent translocation into systemic circulation). Considering previous laboratory-controlled and field-based research has provided some insinuation that running results in greater gastrointestinal perturbations and GIS compared to cycling; taken together, the findings from the current study suggest any potential exercise-associated perturbations to intestinal integrity, systemic inflammatory response, and GIS are similar between running and cycling exercise when assessed against a similar workload, environmental conditions, and experimental confounder control.

The increase in exercise-associated intestinal epithelial injury in response to running at 55 % $\dot{V}O_{2max}$ (191 pg·ml^{-1}, +19 %) and cycling at 55 % MAP (434 pg·ml^{-1}, +49 %) with environmental heat exposure (T_{amb} 35.3 °C), as measured by plasma I-FABP concentration, was observed to be modest in nature. Responses appeared to be substantially lower than previous exertional-heat stress models using slightly higher exercise intensities (e.g., 60 % $\dot{V}O_{2max}$) resulting in a Δ >1000 pg·ml^{-1},[8,11,12] a value indicative of potential clinical and performance relevance in translational practice.[2,28–30] It is likely that the lower maximum T_{re} observed in the current study (i.e., 38.4 °C), linked with the lower exercise intensity, and break in exercise at 60 min aligned with sweat collection and analysis procedures previously described,[25] contributed to these outcomes. Indeed, exertional-heat stress models that attain core body temperature ≥ 39.0 °C are synonymous with substantial pre- to post-exercise increase I-FABP, irrespective of exercise intensity and duration.[3,4,7–12] This highlights the importance of core body temperature in assessing the magnitude of perturbations to intestinal epithelial integrity using exercise models.

Considering the mild intestinal epithelial injury observed, it is not surprising that the current exercise model did not result in any substantial pre- to post-exercise changes in sCD14 [running: 96 ng·ml^{-1} (+4 %) and cycling: 116 ng·ml^{-1}, +5 %] and LBP [running: -1228 ng·ml^{-1} (−8 %) and cycling: 315 ng·ml^{-1}, +3 %], which are surrogate markers of systemic endotoxaemia. It is suggested that sCD14 and LBP generally increase proportionally in response to luminal originated bacterial endotoxin translocation into systemic circulation, and subsequently results in a proportional increase in systemic inflammatory cytokines.[4,7–10] In certain cases (e.g., rate of luminal to systemic circulation LPS translocation overrides in situ LBP baseline levels and synthesis capacity), like substantial exercise stress (e.g., prolonged duration), a reduction in LBP may be observed.[9] However, an exercise-associated reduction in LBP may simply represent a migration and translocation into cellular compartments.[8,9,31,32] Therefore, caution is needed when interpreting directional change in plasma LBP concentration in response to exertional or exertional-heat stress. These mechanisms may possibly provide some explanation for the differences in sCD14 ($p = 0.030$) and LBP ($p = 0.086$) response observed between running and cycling. Although bacterial endotoxin translocation biomarker responses were low on this occasion, they were consistent with previous exertional stress models of similar core body temperature values and using temperate ambient conditions,[7,10] but lower perturbations compared with exertional-heat stress models with mean peak core body temperature reaching 38.9 °C.[8] These findings suggest that luminal originating bacterial endotoxin translocation into systemic circulation is not substantially exacerbated by the exercise modality (i.e., running vs cycling).

An interesting finding in the current study was the significant difference in systemic inflammatory cytokines between running and cycling exercise. The pre- to post-exercise change in plasma inflammatory cytokines in running was consistent with previous exertional stress models of similar duration and intensity.[7–12] These are characterised by a minimal increase in pro-inflammatory cytokines (i.e., IL-1β and TNFα), and more pronounced increases in anti-inflammatory cytokines (i.e., IL-10). However, the relative pre- to post-exercise change in systemic cytokine concentrations that allowed for a SIR-Profile determination, as described by Bennett et al., were substantially lower compared with exertional-heat stress models promoting greater T_{re} and reporting values >100arb.units.[13] It is acknowledged the 4-plex analysis in the current study, compared with the 6-plex analysis previously describe may account for the low SIR-Profile.[13] Additionally, the reduction in plasma concentrations of inflammatory cytokines pre- to post-exercise in cycling was unexpected, and likely associated with skeletal muscle signalling, rather than an immune response to luminal bacterial endotoxin translocation.[33] Another observation was the large individual variation in absolute plasma inflammatory cytokine concentrations, including outliers. These cytokine response patterns are consistently reported in similar exertional and exertional-heat stress models, and potentially suggest the insensitivity of plasma cytokine responses to modest exercise stress. Nevertheless, the findings from the current study suggest modality differences in systemic cytokine responses.

It is consistently suggested that running exercise results in greater GIS incidence and severity compared with cycling, especially in competitive events.[17–19] In the current study, under laboratory-controlled conditions, running exercise at similar duration and intensity did result in greater GIS incidence, due to more incidence of lower-GIS (intestinal originated symptoms), but GIS severity and overall gut discomfort was not difference between modalities, and there were no reports of severe GIS (i.e., ≥5.0 in mVAS).[34] The overall GIS incidence and severity in the current study, in both running and cycling, was far below previous exertional-heat stress models resulting in greater thermal strain (i.e., TCR ≥11 and T_{re} ≥ 39.0 °C), reporting >80 % GIS incidence and a large proportion reporting severe GIS; but also below 2 h exertional-stress models of higher exercise intensity presenting lower thermal strain (e.g., TCR ~9 and T_{re} ≤ 39.0 °C) and reporting >50 % GIS incidence.[7–12] Considering the current findings, it would be worth assessing the exercise-associated GIS differences between running and cycling using more prolonged and higher intensity exercise models, with and without feeding challenge that mimics real-world activity.

5. Conclusion

When exercise is performed with similar duration, intensity, ambient conditions, and with confounder control (e.g., diet and hydration),

exercise modality (i.e., at steady-state endurance exercise with heat stress) does not impact the magnitude of EIGS or associated GIS. Based on the current learning from the experimental procedure and previously reported high rates of GIS in field research, it is imperative to assess the impact of exercise modality (e.g., running vs cycling) on markers of EIGS and GIS using more prolonged exercise durations (e.g., ultra-endurance) and invoking $T_{re} \geq 39.0$ °C. Additionally, EIGS and GIS responses have not previously been comprehensively studied in a number of endurance and ultra-endurance sports (e.g., open water swimming, rowing and kayaking, adventure or obstacle racing, cross-country skiing, and endurance motorsport events), and thus warrant exploration.

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Declaration of Interest Statement

All authors have no conflicts of interest to declare.

Confirmation of Ethical Compliance

This study conformed to the standards set by the Declaration of Helsinki, and was approved by the Monash University Human Research Ethics Committee (MUHREC ethics approval number: CF16/1125-2016000598). All participants gave written informed consent prior to participating in the study.

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