Sports Medicine - Open

ORIGINAL RESEARCH ARTICLE

Open Access

Exploration of Exercise-Induced Gastrointestinal Syndrome in Endurance-Trained Adolescents: Is There Cause for Concern in Regard to Ultra-endurance Event Participation

Pascale Young[1], Volker Scheer[2], Zoe Davidson[1], Jessica R. Biesiekierski[1] and Ricardo J. S. Costa[1*]

Abstract

Background Exercise-induced gastrointestinal syndrome (EIGS) in response to prolonged exercise is well-established in adult athletes but no study has investigated how EIGS presents in adolescent athletes despite an increase in youth participation in ultramarathon events. This study aimed to determine the impact of prolonged exercise on gastrointestinal integrity, functional responses, systemic immune profile, and gastrointestinal symptoms (GIS) in youth (YOUTH) vs adult (ADULTS) athletes.

Methods Twelve youth (≤ 18y) and twelve adults (≥ 30–50y) completed 3 h treadmill running at 60% $\dot{V}O_{2max}$ (22.9˚C ambient temperature), followed by 2 h seated recovery. Venous and finger-prick blood samples were collected pre-, during-, post-exercise, and during recovery to determine leukocyte counts, plasma concentrations of cortisol, *E.coli* lipopolysaccharide-stimulated elastase, I-FABP, sCD14, TNF-α and IL-10. Functional gastrointestinal response [i.e., orocecal transit time (OCTT)], GIS [via modified visual analogue scale (mVAS) quantifying self-reported GIS], feeding tolerance and physiological strain variables were assessed pre-, during-, and in recovery from exercise.

Results There was no difference in pre-to-post-exercise concentrations of plasma cortisol, elastase, I-FABP, sCD14, TNF-α, or IL-10 between YOUTH and ADULTS ($p > 0.05$). YOUTH had higher total leukocyte and neutrophil counts ($p < 0.05$) in response to exercise [mean (95% CI) 13.1 (10.6 to 15.5) × 10^9/L, and 8.9 (7.0 to 10.8) × 10^9/L, respectively] vs ADULTS [9.4 (7.6 to 11.2) × 10^9/L, and 6.4 (5.0 to 7.7) × 10^9/L, respectively]. ADULTS reported higher (p = 0.024) upper-GIS severity during exercise [summative accumulation (range of GIS): 252 (6–67)] compared with YOUTH [96 (4–19)]. No other differences were observed in exercise-associated gastrointestinal symptoms (Ex-GIS) or OCTT between groups.

Conclusions The pathways of EIGS in response to exercise do not substantially differ in youth vs adult athletes. Therefore, EIGS and Ex-GIS management strategies for adult athletes are likely transferable to youth athletes. Further studies investigating the use of these specific strategies in youth athletes are required to confirm this.

*Correspondence:
Ricardo J. S. Costa
ricardo.costa@monash.edu
Full list of author information is available at the end of the article

Keypoints

- Adolescent age does not appear to exacerbate the incidence and severity of EIGS and Ex-GIS during prolonged endurance running.
- Regular carbohydrate feeding for adolescents during endurance activity appears effective at mitigating gastrointestinal perturbations, suggesting current feeding guidelines aimed at attenuating severity of EIGS in adults are transferrable to adolescents
- The late-to-end pubertal stage of youth athletes in the current study may contribute to the absence of age-related differences in gastrointestinal responses, with the recognition that physiological responses to exercise closely correlate to maturation.

Keywords Intestinal fatty-acid binding protein, Bacterial endotoxin, Inflammatory cytokines, Elastase, Orocecal transit, Gastrointestinal symptoms

Introduction

The popularity of ultramarathon running has grown over the last 2 decades, with a > 35% rise in the number of events, attracting athletes of varying ages, biological sexes, and athletic ability [1]. Ultramarathons, classified as events exceeding marathon length (> 42.195 km) or ≥ 6 h duration, span from single-stage races (~ 50 to 161 km) to ≥ 24 h continuous or multi-day competitions [2]. Challenging environmental conditions (i.e., high ambient temperature and/or altitude) and demanding topographies place significant physiological stress on the body [2]. Well-established is the impact on the gastrointestinal system, with activation of a cascade of reversible perturbations, termed exercise-induced gastrointestinal syndrome (EIGS) [3]. The pathophysiological pathways of EIGS have been extensively discussed previously [3, 4]. In short, EIGS is characterised by: (1) the redistribution of blood away from the gastrointestinal tract during exercise, compromising epithelial cell integrity and (2) an increase in sympathetic drive that initiates a reduction in gastrointestinal tract function [3]. These disruptions can result in a systemic inflammatory response due to potential translocation of lumen-originating pathogenic agents into systemic circulation and exercise-associated gastrointestinal symptoms (Ex-GIS) [3, 5]. Such symptoms include those of the upper-gastrointestinal tract (i.e., belching, upper abdominal bloating, regurgitation or projectile vomiting), lower-gastrointestinal tract (i.e., flatulence, lower abdominal bloating, urge to defecate) and other related gastrointestinal symptoms (i.e., nausea). Symptoms of the upper-gastrointestinal tract are the most commonly reported GIS amongst adult endurance athletes [6]. Ex-GIS have been associated with reduced performance (i.e., inability to fuel and rehydrate, exercise cessation and/or withdrawal from activity) and clinical consequences (e.g., gastroparesis, reversable acute colitis, and/or sepsis) [7–10].

Several intrinsic (i.e., biological sex, individual feeding tolerance, hydration status, gastrointestinal disease/disorder diagnosis, and/or predisposition) and extrinsic (i.e., exercise intensity, duration, heat exposure, dietary modification) factors have been identified to exacerbate gastrointestinal disturbances [4, 11, 12]. Recently, age as an intrinsic exacerbation factor has garnered interest, attributable to a number of youth athletes (< 19 years) participating in ultra-endurance running (e.g., 7775 adolescent finishes between 1960 and 2017) [13]. While the exploration of EIGS and Ex-GIS, has been extensive in adults, there is limited research investigating the presentation of EIGS in adolescent athletes [14]. One recent case series explored the gastrointestinal integrity, systemic immune response and Ex-GIS in three youth athletes competing in an ultramarathon and observed changes in EIGS biomarkers similar to that observed in adult ultramarathon runners [15]. However, the limited sample size and the inability to control for potential confounding factors in field-research greatly impacts the generalizability of observed results. Consequently, adult-based management guidelines [16] are often extended to youth athletes. This could be problematic as age-related physiological differences may affect guideline applicability [17].

There appear to be some differences in gastrointestinal physiology and function in paediatric populations that become similar to adults with maturation, such as motility, gastric fluid acidity, digestive enzyme activity and microbiota [18–20]. However, research in changes in intestinal epithelial integrity, which is most relevant to EIGS, is scarce. Literature has, however, highlighted a disparity in immune response to exercise between children, adolescents, and adults. For example, smaller increases in leukocyte counts (28% vs 38%), and systemic IL-6 (11% vs 18% increase) has previously been observed in children (~ 9.8 y) vs adult (~ 22.1 y) men following 60 min of cycling at 70% $\dot{V}O_{2max}$. [21]

Similarly, a smaller elevation in lymphocyte and IL-6 was observed in young girls (12 y) vs adolescent girls (14 y) when following the same exercise protocol [22]. These results suggest moderated immunological perturbations in youth that may be attributable to the immaturity of tissue cells, lending to inefficiency in immune cell response to exercise [23]. It is plausible that a similar pattern may present itself when youth athletes engage in prolonged exercise. However, issues affecting interpretability include a limited subset of immune biomarkers known to present none-to-modest response to exercise (i.e., IL-6), inappropriate sample timing, lack of experimental and biomarker confounder control (i.e., dietary provisions), and an exercise protocol inadequate in inducing clinically relevant changes to concentrations of immune markers [24, 25]. As such the outcomes are not applicable to professional practice.

To address the existing evidence gaps and methodological limitations, the aim of this study was to determine the impact of prolonged exercise on gastrointestinal integrity, functional responses, systemic immune profile and Ex-GIS in youth vs adult athletes. Considering the subtle differences in gastrointestinal structure and function in the developing gastrointestinal tract, it was hypothesised that youth athletes would experience greater gastrointestinal disturbances compared to adults.

Methods

Study Design

This was a between-group experimental trial comparing the gastrointestinal response to endurance exercise of adolescent athletes to adult controls. Participants were required to visit the laboratory on two occasions. First, the pre-assessment preliminary visit to determine eligibility, body composition, fitness level and to familiarise participants with the experimental trial. Second, for the experimental procedure.

Subjects

Twelve exercise-trained youths (YOUTH) and twelve exercise-trained adults (ADULTS) participated in the study (Table 1). Participants were recruited using hard copy and digital advertising materials, targeted advertising on social media platforms and community connections. At the preliminary visit, participants gave written informed consent, approved by the local ethics committee and adhering to the 2013 Helsinki Declaration for Human Research Ethics (Monash University Human Research Ethics Committee: 28696). Participants were excluded from the study if they confirmed having gastrointestinal infections, diseases, disorders, and/or previous gastrointestinal surgical procedures. In addition, participants were excluded if, within 1 month before the experimental protocol, they consumed potential modifiers of gastrointestinal integrity such as prebiotics, probiotics, and/or antibiotics or were adhering to gastrointestinal-focused dietary regime/s such as FODMAP (fermentable oligo-di-mono- and polyols), fibre-modified, or gluten-free diets; consumed non-steroidal anti-inflammatory medications, and/or stool altering medications such as laxatives and anti-diarrhoeal medication/s. Participants who presented with soft tissue injuries within 1 month prior to participation were also excluded from the study. Adolescents who had 4 or more dual-energy X-ray absorptiometry (DEXA) procedures for the purpose of assessing body composition within any 12-month period were also excluded.

Preliminary Measures

Within 1 month prior to the trial, participants visited the laboratory for height, body mass and body composition measures. Participants completed a pre-study screening questionnaire to ensure they were free of chronic

Table 1 Participant characteristics

	YOUTH	ADULTS	*p*
n = males	9	9	
n = females	3	3	
Age (years)	16.8 (15.9 to 17.6)	36.8 (32.8 to 40.9)	< .001
Height (cm)	176 (171 to 181)	171 (164 to 179)	.271
Body mass (kg)	64.0 (60.0 to 68.0)	68.3 (58.9 to 77.6)	.376
Fat mass (%)	15.6 (11.1 to 20.0)	19.8 (15.0 to 24.6)	.171
$\dot{V}O_{2max}$ (mL/kgBM/min)	62.1 (55.1 to 64.0)	54.6 (49.1 to 60.1)	.101
Weekly training volume (min/week)	496 (346 to 647)	376 (248 to 504)	.192
Trial running speed (km/h)	9.1 (8.4 to 9.9)	8.7 (8.0 to 9.3)	.285

Values shown are mean and 95% CI

and acute illness and injury, and met a minimum weekly exercise frequency (>4–5 times per week). YOUTH self-report pubertal development using Marshall–Tanner criteria (Tanner Stage) [26, 27]. ADULT body composition was measured via multi-frequency bioelectrical impedance (mBIA) (Seca 515 MBCA, Seca Group, Hamburg, Germany), while YOUTH were measured via DEXA (GE Lunar encore V18, GE Healthcare, Madison, WI United States) due to age limitations within the SECA analysis system. In-house mBIA and DEXA measurements have previously been validated for acceptance [28].

Maximal oxygen uptake ($\dot{V}O_{2max}$; Vyntus, Vyaire Medical, Mettawa, Illinois, USA) was measured by means of continuous incremental exercise test to volitional exhaustion on a motorised treadmill (Pulsar, h/p/cosmos, Munich, Germany). The exercise test began with a treadmill speed of 8 km/hr and 1% inclination. Speed was increased by 2 km/hr every 3 min until reaching 16 km/hr, at which point inclination was increased by 2.5% every 3 min until the participant reached volitional exhaustion. Criteria for attaining $\dot{V}O_{2max}$ included the participants reaching volitional exhaustion, (Borg Scale 19–20), heart rate (HR) within 10 beats/min of HR_{max}, RER ≥ 1.10 at exercise cessation, and/or $\dot{V}O_2$ plateau. To determine running speed for the exercise trials, the speed at approximately 60% $\dot{V}O_{2max}$ and 1% gradient was determined and verified from the $\dot{V}O_2$–work rate relationship (Table 1).

Experimental Procedure

Participants were provided with an individualised low FODMAP diet for the 24 h period before each experimental trial [mean (SD): 12 (1.4) MJ/day, 489 (85) g/day carbohydrate (~8 g/kg body mass/day), 116 (20) g/day protein, 43 (6.3) g/day fat, 31 (3.1) g/day fibre, and 3.4 (2.0) g/day FODMAP] to limit any confounding factors associated with the lead-in diet [11]. Participants were asked to refrain from consuming additional high FODMAP foods, alcohol, and caffeinated beverages during the diet-controlled period, and avoid strenuous exercise during the 24 h period before each experimental trial. Compliance was determined by an exercise and diet log.

On trial day, participants arrived at the laboratory at 0800 h following consumption of a low FODMAP breakfast [4.1 (1.0) MJ, 63 (6.0) g carbohydrate (~1 g/kg body mass), 16 (1.0) g protein, 10 (0.2) g fat, 3.1 (0.5) g fibre, and 1.0 (1.0) g FODMAP] at 0700 h. Participants voided before total body water (Seca mBCA 515, Seca Group, Hamburg, Germany) and nude body mass (NBM) measurements. Blood was collected by venepuncture from the antecubital vein into 2 separate sterile vacutainers (6 mL lithium heparin and 4 mL K_3EDTA) (Becton Dickinson Pty Ltd, Macquarie Park, New South Wales, Australia). A lancet (21G Unistik 3, Owen Mumford Ltd, Oxfordshire, United Kingdom) was used for a finger-prick blood sample at blood sampling timepoints, collected in a HemoCue WBC micocuvette and inserted into HemoCue WBC DIFF (HemoCue AB, Ängelholm, Sweden) to provide total and differential leukocyte counts (neutrophils, lymphocytes, and monocytes). An exercise specific modified visual analogue scale (mVAS) quantified self-reported GIS (Wagner Analysen Technick, Bremen, Germany) [29]. Trials for female athletes were scheduled during the follicular phase ($n=6$) or when taking the active medication of the oral contraceptive pill ($n=1$). Resting estrogen levels (DKO003/RUO; DiaMetra, Italy) were measured and were within normal reference range [mean (SD): 62.8 (59.7) pg/mL].

The experimental trial consisted of 3 h (initiated at 0900 h) running on a motorised treadmill at the previously determined speed that elicited 60% $\dot{V}O_{2max}$ at 22.5 (0.7) °C ambient temperature, 50 (0) % relative humidity (RH), and dual fan wind speed at 10.6 km/h. Participants consumed an in-house formulated carbohydrate solution at room temperature (22 °C) (15 g dextrose monohydrate, 10% *wv*) every 20 min for the first 2 h, with water allowed *ab libitum* (mean: YOUTH 409 mL, ADULTS 351 mL). Carbohydrate feeding during exercise was applied to support participants completion and ceased at 2 h into exercise to avoid interference with orocecal transit time (OCTT) testing. To determine OCTT, 150 mL solution containing 30 g lactulose (Actilax; alphapharm, QLD, Australia) was provided to participants 150 min into exercise. Water was withheld in the final 30 min of exercise to avoid inter-reference with OCTT testing. Breath samples were collected every 30 min during exercise after lactulose administration, and every 15 min throughout recovery. Time between ingestion of lactulose solution and a rise in breath hydrogen (H_2) of 10 ppm, with at least 2 consecutive readings above basal, was used as a measure of OCTT [30]. HR, Borgs rating of perceived exertion (RPE), thermal comfort rating (TCR), and GIS were measured every 15 min during exercise. Breath-by-breath indirect calorimetry (Vyntus, Vyaire Medical, Mettawa, Illinois, USA) was used to determine $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), and respiratory quotient for 5 min continuously every 30 min during exercise. Venous and finger-prick blood samples were collected at the 2 h timepoint during exercise, immediately post-, 1 h and 2 h post-exercise cessation. Any urine output during exercise was collected and accounted for in pre-to-post-exercise NBM measurements. Immediately following cessation of exercise, NBM was recorded. GIS were recorded every 15 min during the 2 h recovery period. Participants remained seated and drank room temperature water *ab libitum* during recovery.

Sample Analysis

Blood glucose [coefficient of variance (CV): 5.9%)] and haemoglobin (CV: 1.2%) were determined by HemoCue systems (Glucose 201+, Hb201, HemoCue AB, Ängelholm, Sweden) in duplicate from heparinised whole blood samples. Total and differential leukocyte counts were determined by HemoCue system (WBC DIFF; HemoCue AB, Ängelholm, Sweden) in duplicate (CV: 5.8%). Capillary method was used to determine haematocrit in triplicate from heparin whole blood samples using a microhematocrit reader (CV: 0.8%) (Thermo Fisher Scientific). Haemoglobin and haematocrit values were used to estimate changes in plasma volume relative to baseline, to correct plasma variables [31]. Determination of in vitro bacterially stimulated elastase release followed protocols and storage described in Costa et al., 2019 [32]. Remaining heparin and K_3EDTA whole blood samples were centrifuged at 4000 rpm for 10 min within 15 min of collection. Plasma was aliquoted into eppendorfs and frozen at −80 °C, except two 50 µL that was used to determine plasma osmolality (P_{Osmol}), in duplicate (CV: 1.0%), by freezepoint osmometry (Osmomat 030, Gonotec, Berlin, Germany). Breath samples (20 mL) were analysed in duplicate (CV: 2.0%) for H_2 content using a gas-sensitive analyser (Breathtracker Digital Microlyzer, Quintron, Milwaukee, WI, United States). Plasma concentrations of cortisol (DKO001, DiaMetra, Spello, Italy), intestinal fatty-acid binding protein (I-FABP; HK406, Hycult Biotech, Uden, Netherlands), soluble CD14 (sCD14; HK320, Hycult Biotech, Uden, Netherlands), bacterially stimulated elastase (HK319, Hycult Biotech, Uden, Netherlands), and cytokines TNF-α and IL-10 (Bender MedSystems GmbH, Vienna, Austria) were measured by ELISA with CVs of 2.4%, 4.4%, 3.6%, 11.6%, 6.5%, and 5.9%, respectively. Biomarkers were analysed as per manufacturer's instructions on the same day, with standards and controls on each plate and each participant assayed on the same plate.

Statistical Analysis

Based on statistical test, mean, standard deviation, and effect size of 1.283 to the impact of exercise on Δ pre-to-post-exercise plasma I-FABP concentration, [11, 12] indicative of intestinal epithelial cell injury, and applying a standard alpha (0.05) and beta value (0.95) the current participant sample size ($n=12$ parallel group design) is estimated to provide adequate statistical power (power* 0.80–0.99) for detecting significant within- and between-group differences (G*Power 3.1, Kiel, Germany). Data in text and tables are presented as mean and 95% confidence interval (CI), unless otherwise indicated. Data in figures are presented as mean and individual responses or mean ± standard error of mean (SEM) to provide clarity. Subjective GIS data are reported as incidence (%), reflecting total proportion of participants reporting GIS ≥ 1 on the mVAS (range 0 to 10) for any GIS type with 1–4 indicative of mild GIS, 5–9 indicative of severe GIS, and 10 indicative of extremely severe GIS [29]. Severity indicative of mean overall summative accumulation of rating scale point score of measured time periods and individual participant range of those reporting and rating GIS. All data were checked for normal distribution using skewness and kurtosis coefficients before comparative data analysis was performed. Within-group differences in GIS along timepoints during exercise and in recovery were analysed using non-parametric Friedman Test. Age-group comparison data were analysed using independent sample t test or Mann–Whitney U test non-parametric equivalent where appropriate, comparing single data points at pre-, immediately post-, 1 h post-, 2 h post-exercise and maximum magnitude response post-exercise. Cohen's d standardised measurement of effect size was applied where appropriate and corrected for low sample size bias risk with $d=0.20$, $d=0.50$, and $d=0.80$ for small, medium, and large. Exercise-associated perturbations to EIGS biomarkers within groups were analysed through one-way repeated measures ANOVA or Friedman non-parametric equivalent where appropriate. Significant main effects from pre-exercise were identified using a post hoc Tukey's test for parametric data and post hoc multiple Wilcoxon Signed Rank test where appropriate. Correction factors were applied for multiple comparisons tests of post hoc analysis. Statistics were analysed using SPSS statistical software (v.27.0, IBM SPSS Statistics, IBM Corp., Armonk, NY) with significance accepted at $p \leq 0.05$.

Results

Participant Characteristics

Participant characteristics are presented in Table 1. Nine out of twelve adolescents self-reported pubertal development using the Tanner Staging system with a frequency of 3/9 in Stage 4 and 6/9 in Stage 5 of development.

Physiological Strain

Physiological strain data in YOUTH and ADULTS are reported in Fig. 1. No difference was observed in pre-exercise variables between groups. At 180 min of the exercise protocol YOUTH presented a significantly higher peak HR (166 bpm; $p<0.001$) compared to ADULTS (147 bpm). Mean HR during exercise was also significantly higher in YOUTH (158 bpm; $p<0.001$) vs ADULTS (137 bpm) (Fig. 2A). YOUTH had a significantly higher peak (10; $p=0.041$) and mean TCR (9; $p=0.039$), compared with ADULTS (9 and 8, respectively) (Fig. 2C).

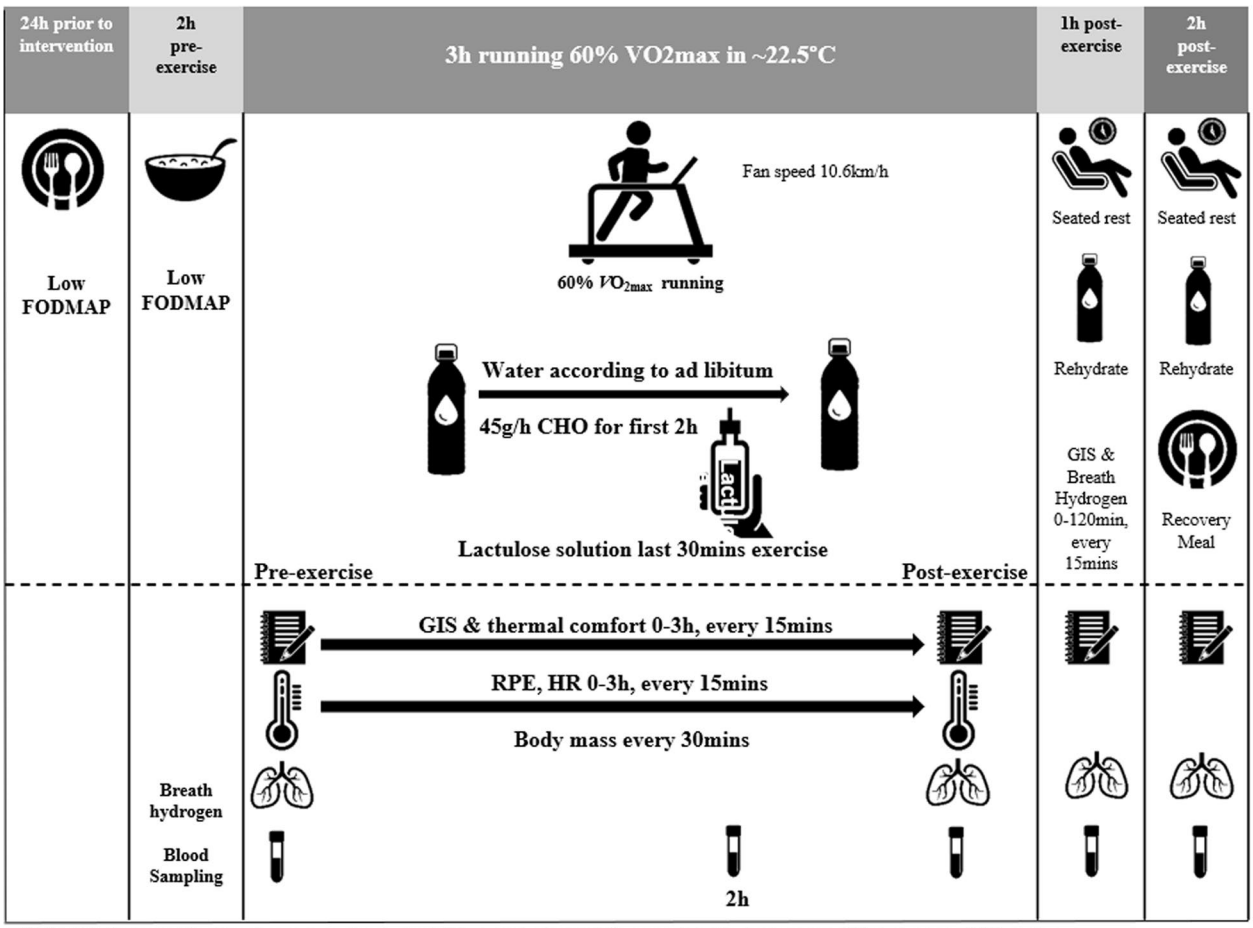

Fig. 1 Schematic illustration of the experimental design

Glucose Availability and Fuel Kinetics

An exercise effect was observed for carbohydrate and fat oxidation in both groups, whereby YOUTH had a significantly lower carbohydrate and higher fat oxidation rate at 160 min compared to 20 min into exercise and ADULTS at 180 min compared to 20 min into exercise (Fig. 2D, E). No significant difference was observed in pre- to peak Δ post-exercise for fat ($p = 0.345$) or carbohydrate ($p = 0.203$) oxidation between YOUTH and ADULTS.

An exercise effect was observed for plasma glucose in YOUTH ($p < 0.001$) and ADULTS ($p = 0.049$), whereby plasma glucose concentrations were significantly greater 2 h into exercise compared to pre-exercise. No significant difference was observed in pre- to peak Δ post-exercise plasma glucose concentrations between age groups ($p = 0.128$) with a medium effect size ($d = 0.58$) between groups.

Plasma Cortisol

An exercise effect was observed for plasma cortisol concentration in both groups, whereby concentrations were significantly higher immediately post- and 2 h post-exercise compared to pre-exercise in YOUTH and immediately post- and 1 h post-exercise in ADULTS (Fig. 3Ai). No significant difference between YOUTH and ADULTS in pre- to peak Δ post-exercise plasma cortisol concentration was observed ($p = 0.266$) (Fig. 3Aii) with a medium effect size between groups ($d = 0.70$).

Intestinal Epithelial Integrity

An exercise effect was observed for plasma I-FABP in YOUTH, whereby plasma concentrations were significantly higher 1 h post- and 2 h post-exercise compared to pre-exercise (Fig. 3Bi). An exercise effect was also observed for plasma sCD14 concentrations, whereby concentrations were significantly higher 1 h

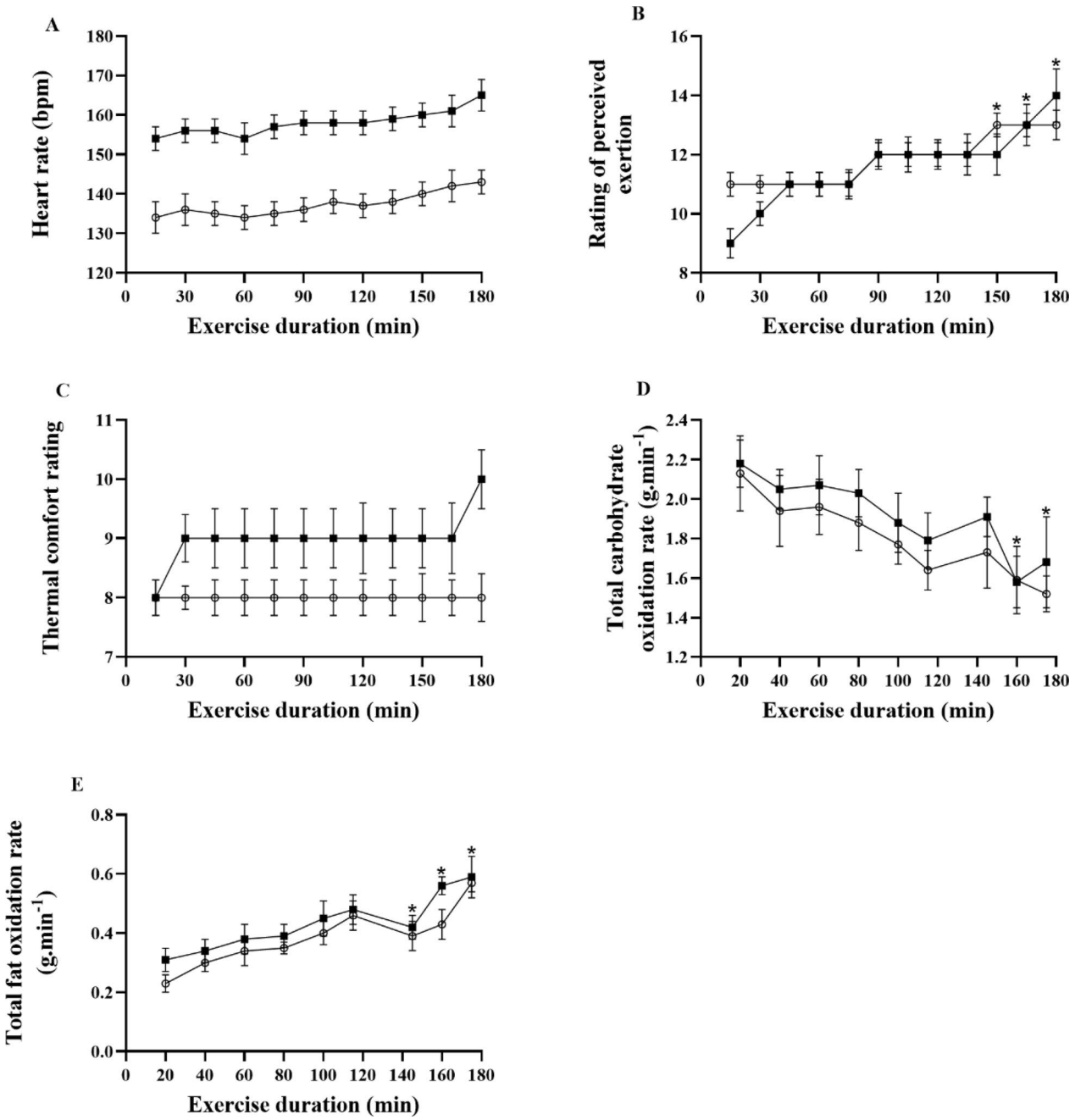

Fig. 2 Heart rate (**A**), rating of perceived exertion (**B**), thermal comfort rating (**C**), total carbohydrate (**D**) and fat oxidation rates (**E**) in response to 3 h running at 60% $\dot{V}O_{2max}$ in ambient conditions (T_{amb} 22.5˚C, RH 50%) in ADULTS (white circles, $n = 12$) and YOUTH (black squares, n = 12). Values are mean ± SEM: * $p < 0.05$ vs 15 min into exercise (**B**), vs 20 min into exercise (**E**)

post- and 2 h post-exercise in YOUTH but not ADULTS (Fig. 3Ci). No significant difference was observed in pre- to peak Δ post-exercise plasma I-FABP ($p = 0.744$) or sCD14 ($p = 0.865$) concentrations between YOUTH and ADULTS (Fig. 3Bii, Cii, respectively) with a medium effect size ($d = 0.26$) between groups for plasma I-FABP and a small effect size ($d = 0.15$) between groups for plasma sCD14 concentrations.

Immune Response

An exercise effect was observed for total circulating leukocyte, neutrophil, and monocyte counts in YOUTH and

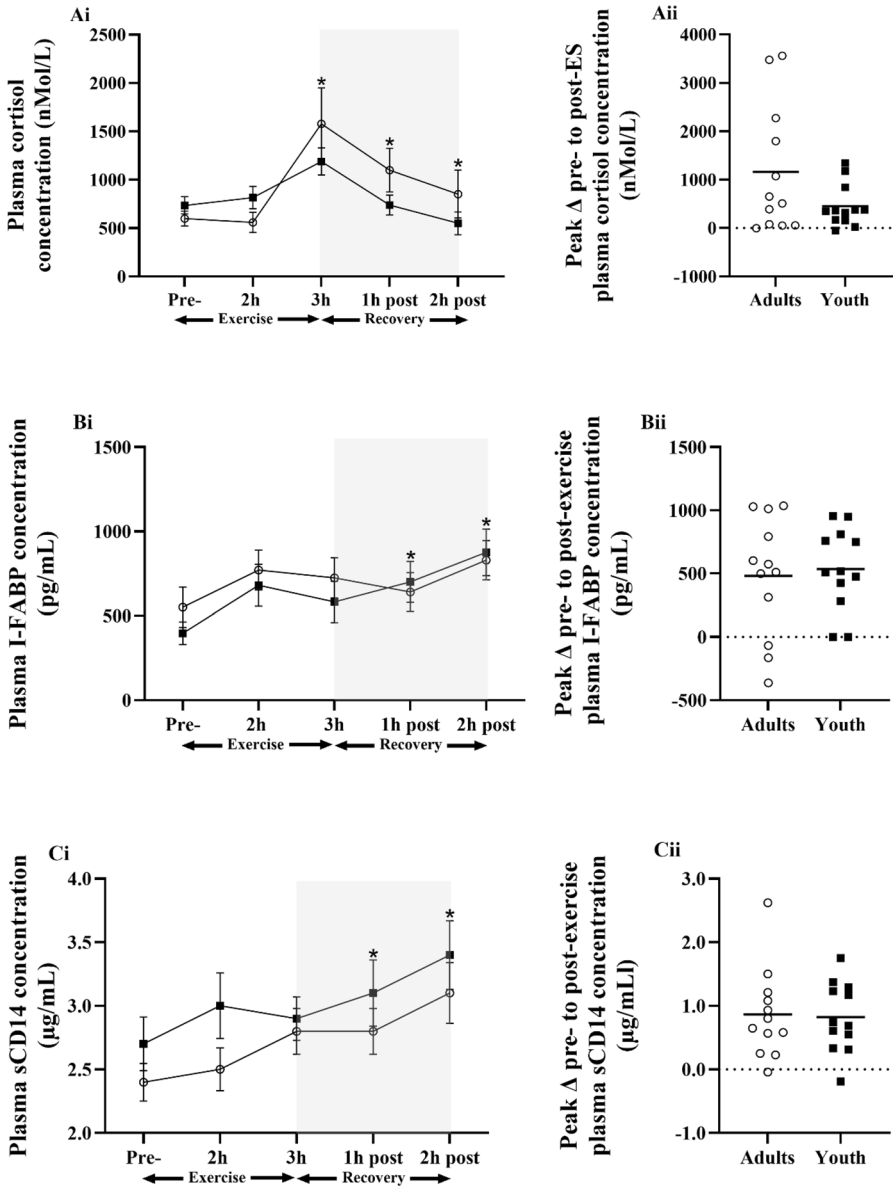

Fig. 3 Absolute (i) and peak magnitude pre-to-post-exercise change (ii) in plasma cortisol (**A**), I-FABP (**B**), sCD14 (**C**), bacterially stimulated elastase concentration (**D**) and per neutrophil (**E**), TNF-α (**F**) and IL-10 (**G**) in response to 3 h running at 60% $\dot{V}O_{2max}$ in ambient conditions (T_{amb} 22.5˚C, RH 50%) in ADULTS (white circles) and YOUTH (black squares). Shaded timepoints represent post-exercise recovery period. Values are mean ± SEM (i) and magnitude response in individual response (ii). Exercise effect: * $p < 0.05$ vs pre-exercise

ADULTS, whereby concentrations significantly increased immediately post-exercise and remained throughout recovery (Table 2). Circulating lymphocyte concentrations increased significantly post-exercise in ADULTS but not YOUTH (Table 2). An exercise effect for neutrophil-to-lymphocyte ratio was observed in YOUTH and ADULTS, whereby values increased in response to exercise and remained elevated throughout recovery (Table 2). A significant difference was observed between age groups for total leukocyte, neutrophil, and lymphocyte counts in response to exercise (Table 2). No significant difference between YOUTH and ADULTS in pre- to peak Δ post-exercise total leukocyte, neutrophil, lymphocyte or monocyte counts was observed.

An exercise effect was observed for bacterially stimulated elastase concentration in YOUTH and ADULTS (Fig. 3Di), whereby plasma concentrations were significantly higher during exercise and throughout recovery vs pre-exercise in YOUTH and plasma concentrations were significantly higher during and 2 h post-exercise vs

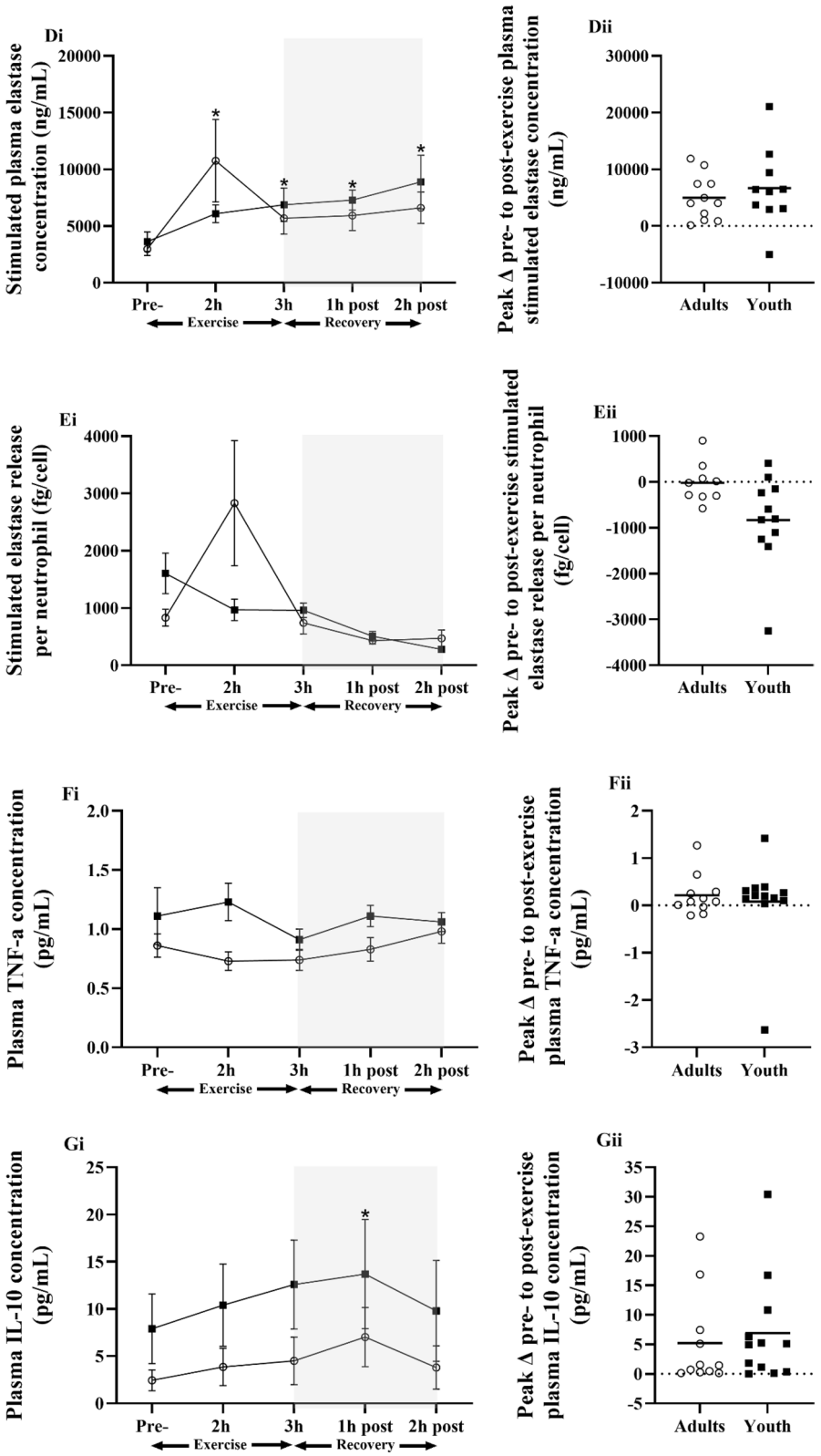

Fig. 3 continued

Table 2 Responses of circulating leukocyte, neutrophil, lymphocyte and monocyte count ($\times 10^9$/L) to 3 h running at 60% $\dot{V}O_{2max}$ in ambient conditions (T_{amb} 22.5˚C, RH 50%) in YOUTH ($n=12$) and ADULTS ($n=12$)

	Pre-ES	2 h during ES	0 h post	1 h post	2 h post
Leukocyte			**	**	**
YOUTH	4.9 (4.0 to 5.8)	6.7 (5.3 to 8.0)	9.6 (7.9 to 11.3) †	13.1 (10.6 to 15.5) †	12.6 (10.5 to 14.8)
ADULTS	4.0 (3.1 to 5.0)	5.1 (4.1 to 6.2)	**	**	**
			6.8 (5.6 to 7.9)	9.4 (7.6 to 11.2)	10.5 (8.8 to 12.2)
Neutrophil			**	**	**
YOUTH	2.3 (1.7 to 2.9)	3.7 (2.7 to 4.7)	5.9 (4.6 to 7.1) †	8.9 (7.0 to 10.8) †	8.8 (6.9 to 10.6)
ADULTS	2.0 (1.4 to 2.5)	2.7 (2.1 to 3.4)	**	**	**
			3.8 (3.1 to 4.4)	6.4 (5.0 to 7.7)	7.1 (5.6 to 8.6)
Lymphocyte					
YOUTH	2.0 (1.5 to 2.5)	2.4 (1.9 to 2.9)	2.9 (2.2 to 3.6)	2.9 (2.3 to 3.4) †	2.6 (2.0 to 3.1)
ADULTS	1.8 (1.4 to 2.1)	2.0 (1.6 to 2.4)	*	*	*
			2.5 (1.9 to 3.1)	2.5 (2.1 to 2.8)	2.6 (2.1 to 3.0)
Monocyte			*	*	*
YOUTH	0.3 (0.2 to 0.3)	0.4 (0.3 to 0.4)	0.6 (0.4 to 0.7)	0.7 (0.5 to 0.9)	0.7 (0.6 to 0.9)
ADULTS	0.3 (0.2 to 0.3)	0.4 (0.3 to 0.4)	*	*	*
			0.5 (0.4 to 0.5)	0.7 (0.6 to 0.7)	0.7 (0.6 to 0.8)
Neutrophil:lymphocyte			*	*	*
YOUTH	1.2 (0.8 to 1.6)	1.6 (1.2 to 2.0)	2.2 (1.7 to 2.7)	3.2 (2.5 to 4.0) *	4.5 (0.9 to 8.1) *
ADULTS	1.1 (0.8 to 1.4)	1.4 (1.1 to 1.7)	1.6 (1.3 to 2.0)	2.7 (2.1 to 3.3)	3.0 (2.1 to 3.8)

Values are mean (95% CI). †$p<0.05$ YOUTH vs ADULTS, and exercise effect * $p<0.05$ and **$p<0.001$ vs pre-exercise

ES exertional stress

pre-exercise in ADULTS. No other significant findings were observed for exercise effect on bacterially stimulated elastase release per neutrophil plasma concentrations (Fig. 3Ei) or in pre- to peak Δ post-exercise between YOUTH and ADULTS (Fig. 3Dii, Eii).

An exercise effect was observed for IL-10, whereby 1 h post-exercise plasma concentrations were significantly higher in YOUTH and ADULTS compared to pre-exercise (Fig. 3Gi). TNF-α:IL-10 ratio was significantly higher 1 h post-exercise compared to pre-exercise (p = 0.005) in ADULTS but not YOUTH. No significant difference was observed in pre- to peak Δ post-exercise plasma TNF-α (Fig. 3Fii), IL-10 (Fig. 3Gii) or TNF-α:IL-10 ratio concentrations between YOUTH and ADULTS with a small effect size ($d=0.18$) between groups for plasma TNF-α and a medium effect size ($d=0.28$) between groups for plasma IL-10 concentrations.

Gastrointestinal Function and Symptoms

H_2 responses in YOUTH and ADULTS significantly increased (turning point) at 60 min and 75 min after lactulose ingestion, respectively (Fig. 4A). A H_2 turning point was detected in all participants within the 150 min post-lactulose ingestion time except for 1 ADULT. There was no significant difference ($p=0.266$) in OCTT between ADULTS and YOUTH (Fig. 4B).

The incidence of total GIS during exercise and recovery for YOUTH and ADULTS is reported in Table 3. The predominate GIS type reported in both groups during exercise was belching. Upper-GIS severity was significantly higher during exercise in ADULTS [summative accumulation (range of GIS): 252 (6–67)] compared with YOUTH [96 (4–19), $p=0.024$]. ADULTS reported significantly higher belching during exercise vs YOUTH. Lower-GIS was more predominant during recovery in both age groups compared to upper-GIS (Table 3). There was no difference between YOUTH and ADULTS in total (during exercise and recovery) outcomes of GIS except belching, with ADULTS reporting significant higher severity throughout (Table 3). There was no within-group or between age-group differences in reporting of gut discomfort, total GIS, upper GIS, lower GIS or nausea at any timepoint during exercise and in recovery (Fig. 5).

Discussion

This study aimed to determine the impact of prolonged exercise on the gastrointestinal integrity, functional responses, systemic immune profile, and Ex-GIS of youth (≤18 years) vs adult (≥30–49 years) athletes (Fig. 5). No significant difference was observed between groups in markers of intestinal epithelial injury, bacterial endotoxin translocation, and immune response in response to 3 h

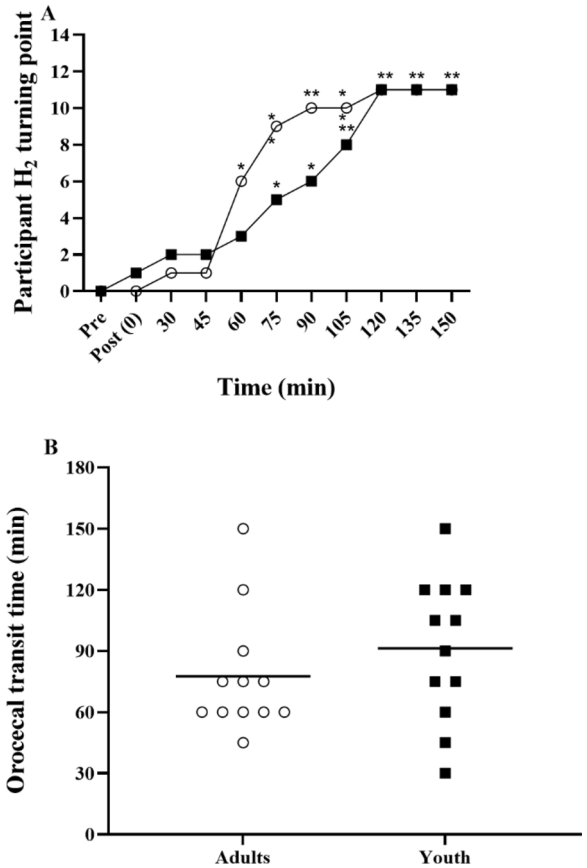

Fig. 4 Breath H_2 turning point (time between ingestion of lactulose and rise in breath hydrogen (H_2) ≥ 10 ppm, with two consecutive readings above basal) (**A**) and OCTT (**B**) in response to 3 h running at 60% VO_{2max} in ambient conditions (T_{amb} 22.5 °C, RH 50%) in YOUTH and ADULTS. Total participant numbers for H_2 turning point (YOUTH black squares; ADULTS white circles) and individual responses (black circles) for OCTT ($n = 24$). $^{**}p < 0.001$ and $^{*}p < 0.05$ vs 0 min

treadmill running. However, total leukocyte and neutrophil cell counts were greater in YOUTH than ADULTS during the recovery period but remained within normal physiological reference range for exercise responses previously observed in adult athlete studies [24, 32]. Functional gastrointestinal response (i.e., OCTT) and Ex-GIS were similar, except in upper-GIS, with higher incidence during exercise in ADULTS vs YOUTH. Despite contrasts in immune and GIS response to exercise between the age groups and contrary to the hypothesis, overall findings suggest that adolescence does not substantially exacerbate the incidence and severity of EIGS, and, therefore, is unlikely to exacerbate EIGS-associated performance and clinical implications more than adults.

Intestinal Epithelial Integrity

Due to its rapid release from mature enterocytes into systemic circulation upon epithelial cell damage, I-FABP is commonly utilised as a surrogate measure of intestinal cell injury related to the circulatory–gastrointestinal pathway of EIGS [24]. The change in circulating I-FABP concentrations between groups was comparable [YOUTH peak Δ 536 (329 to 744) pg/mL vs ADULTS peak Δ 481 (180 to 782) pg/mL] in response to exercise, with the increase not of clinical significance (i.e., ≥ 1301 pg/mL change) [24, 25].

Intestinal epithelial injury is typically associated with a more hyperpermeable gastrointestinal tract, [3] with sCD14 used as a surrogate biomarker of systemic endotoxemia, due to its role as a wide-ranging PAMP receptor [24]. Plasma sCD14 did increase significantly from pre-exercise to 1 h and 2 h post-exercise in YOUTH despite minimal changes in plasma I-FABP; however, the increase remained below clinical relevance reference values in both groups [25]. The modest increase in plasma I-FABP and sCD14 in this study is likely attributable to the carbohydrate feeding strategy, which was provided to ensure participants completed the 3 h exercise protocol and mimic real-world practice. Continual carbohydrate provisions during exercise in adult athletes is known to mitigate splanchnic perfusion dynamics, likely a result of increases in glucagon and noradrenaline in response to feeding [33]. In addition, stimulation of vasodilation due to carbohydrate presence in chyme supports blood flow to intestinal microvilli [3, 34], thus, preserving intestinal epithelial cell integrity and permeability dynamics [35].

Immune Markers

Significant increases in total leukocyte and neutrophil counts occurred in both YOUTH and ADULTS during the recovery period. However, in contrast to previous studies, no acute lymphopenia was observed within the 2 h recovery period; likely due to a lower steady-state exercise intensity (e.g., 60% VO_{2max} vs 75% VO_{2max}). [32] Of interest, the significant increase in total leukocyte and neutrophil counts immediately post- and 1 h post-exercise was greater in YOUTH compared to ADULTS; however, this did not translate to group differences in neutrophil-to-lymphocyte ratios. Findings from this study contradict previous research in which paediatric age groups experienced lower total increases in leukocyte trafficking following 60 min cycling at 70% VO_{2max} in comparison with adults [21, 22]. Preceding studies also suggest that carbohydrate feeding during exercise has a greater influence in the attenuation of exercise-induced leukocytosis in boys compared to men [21]. It is recognised that discrepancies in findings may be attributable to opposing maturation status of youth participants between studies, with the knowledge that various physiological responses to exercise are influenced by pubertal development stage [36]. However, the use of insufficient

Table 3 Incidence and severity of gastrointestinal symptoms and feeding tolerance during and in recovery following 3 h treadmill running at 60% $\dot{V}O_{2max}$ in ambient conditions (ambient temperature 22.9 °C) of ADULTS and YOUTH

	During exercise					Recovery					Total during exercise and recovery		
	ADULTS		YOUTH		*p*	ADULTS		YOUTH		*p*	ADULTS	YOUTH	*p*
	Incidence	Severity	Incidence	Severity		Incidence	Severity	Incidence	Severity		Severity	Severity	
Gut discomfort	–	5 (2–10)	–	4 (1–10)	.347	–	4 (1–10)	–	2 (1–8)	.291	9 (1–20)	6 (1–18)	.117
Total GIS[a]	100%	32 (6–82)	83%	28 (3–91)	.671	60%	23 (4–54)	50%	11 (3–66)	.410	55 (4–136)	39 (3–157)	.075
Upper GIS[b]	92%	21 (6–67)	75%	8 (3–19)	.033	33%	4 (4–20)	25%	2 (3–8)	.590	25 (4–87)	10 (3–27)	.075
Belching	83%	12 (5–30)	50%	4 (2–18)	.028	17%	1 (6–10)	0%	0 (0–0)	.514	13 (5–40)	4 (2–18)	.039
Heartburn	0%	0 (0–0)	17%	1 (1–11)	.514	0%	0 (0–0)	0%	0 (0–0)	1.00	0 (0–0)	1 (1–11)	.153
Stomach bloating	67%	7 (2–30)	42%	1 (2–4)	.052	25%	2 (3–13)	17%	1 (3–4)	.713	9 (2–43)	2 (2–8)	.071
Stomach cramps	25%	0 (1–2)	17%	1 (4–6)	.887	0%	0 (0–0)	17%	1 (3–4)	.514	0 (1–2)	2 (3–10)	.545
Urge to regurgitate	8%	0 (2–2)	8%	1 (9–9)	1.00	0%	0 (0–0)	0%	0 (0–0)	1.00	0 (2–2)	1 (9–9)	.976
Regurgitation	17%	1 (5–6)	0%	0 (0–0)	.514	0%	0 (0–0)	8%	0 (4–4)	.755	1 (5–6)	0 (4–4)	.523
Projectile vomiting	0%	0 (0–0)	0%	0 (0–0)	1.00	8%	1 (10–10)	0%	0 (0–0)	.755	1 (10–10)	0 (0–0)	.317
Lower GIS[b]	58%	9 (1–46)	58%	11 (2–51)	.977	50%	14 (4–49)	33%	7 (2–58)	.242	23 (1–95)	18 (2–109)	.336
Flatulence	33%	2 (1–19)	25%	1 (1–7)	.713	17%	1 (6–8)	17%	1 (5–10)	1.00	3 (1–27)	2 (1–17)	.705
Lower abdominal bloating	42%	4 (4–20)	42%	4 (1–23)	.887	17%	2 (6–15)	17%	2 (4–16)	1.00	6 (4–35)	6 (1–39)	.888
Urge to defecate	25%	3 (1–13)	33%	4 (2–21)	.932	33%	6 (4–43)	17%	3 (2–30)	.478	9 (1–56)	7 (2–51)	.546
Intestinal cramps	8%	0 (1–1)	25%	2 (3–16)	.443	17%	4 (12–36)	17%	2 (2–18)	.977	4 (1–37)	4 (2–34)	.445
Abnormal stools[c]	0%	0 (0–0)	0%	0 (0–0)	1.00	8%	1 (10–10)	0%	0 (0–0)	.755	1 (10–10)	0 (0–0)	.317
Nausea	8%	0 (4–4)	8%	1 (9–9)	.952	17%	3 (13–21)	8%	1 (6–6)	.713	3 (4–25)	2 (6–15)	.613
Dizziness	17%	1 (1–1)	33%	5 (2–37)	.590	8%	2 (19–19)	25%	0 (1–2)	.551	3 (1–20)	5 (1–39)	.287
Abdominal stitch	17%	1 (3–5)	50%	3 (2–12)	.178	0%	0 (0–0)	0%	0 (0–0)	1.00	1 (3–5)	0 (0–0)	.127
Feed tolerance													
Taste fatigue	–	13 (10–27)	–	20 (1–60)	.283	–	–	–	–		–	–	
I nterest in food	–	11 (1–34)	–	14 (4–35)	.432	–	–	–	–		–	–	
Interest in drink	–	23 (2–39)	–	29 (14–50)	.367	–	–	–	–		–	–	
Tolerance to food	–	58 (29–80)	–	49 (17–80)	.713	–	–	–	–		–	–	
Tolerance to drink	–	66 (32–80)	–	65 (42–80)	.974	–	–	–	–		–	–	
Appetite	–	28 (1–65)	–	28 (2–63)	.974	–	51 (37–80)	–	55 (25–79)	.622	79 (1–145)	83 (2–142)	.807
Thirst	–	42 (7–67)	–	49 (2–88)	.480	–	38 (17–54)	–	32 (18–65)	.551	80 (7–121)	81 (2–153)	.893

Values are presented as mean and range of participant reporting gastrointestinal symptoms (GIS) incidence. Incidence: total proportion of participants reporting GIS ≥ 1 on the modified visual analogue scale [mVAS: range 0–10)] for any GIS type from 15 to 180 min during exercise. 1–4 indicative of mild GIS, 5–9 indicative of severe GIS and 10 indicative of extremely severe GIS.[24] Severity indicative of mean overall summative accumulation of rating scale point score of measured time periods and individual participant range of those reporting and rating GIS (does not include those reporting no symptoms)

[a] Summative accumulation of upper, lower, and other GIS

[b] Summative accumulation of upper or lower GIS

[c] Abnormal stools referring to loose watery stools, diarrhoea and/or fecal blood loss

Mean significant difference between YOUTH vs ADULTS (analysed via Mann–Whitney *U* test): $p \leq 0.05$

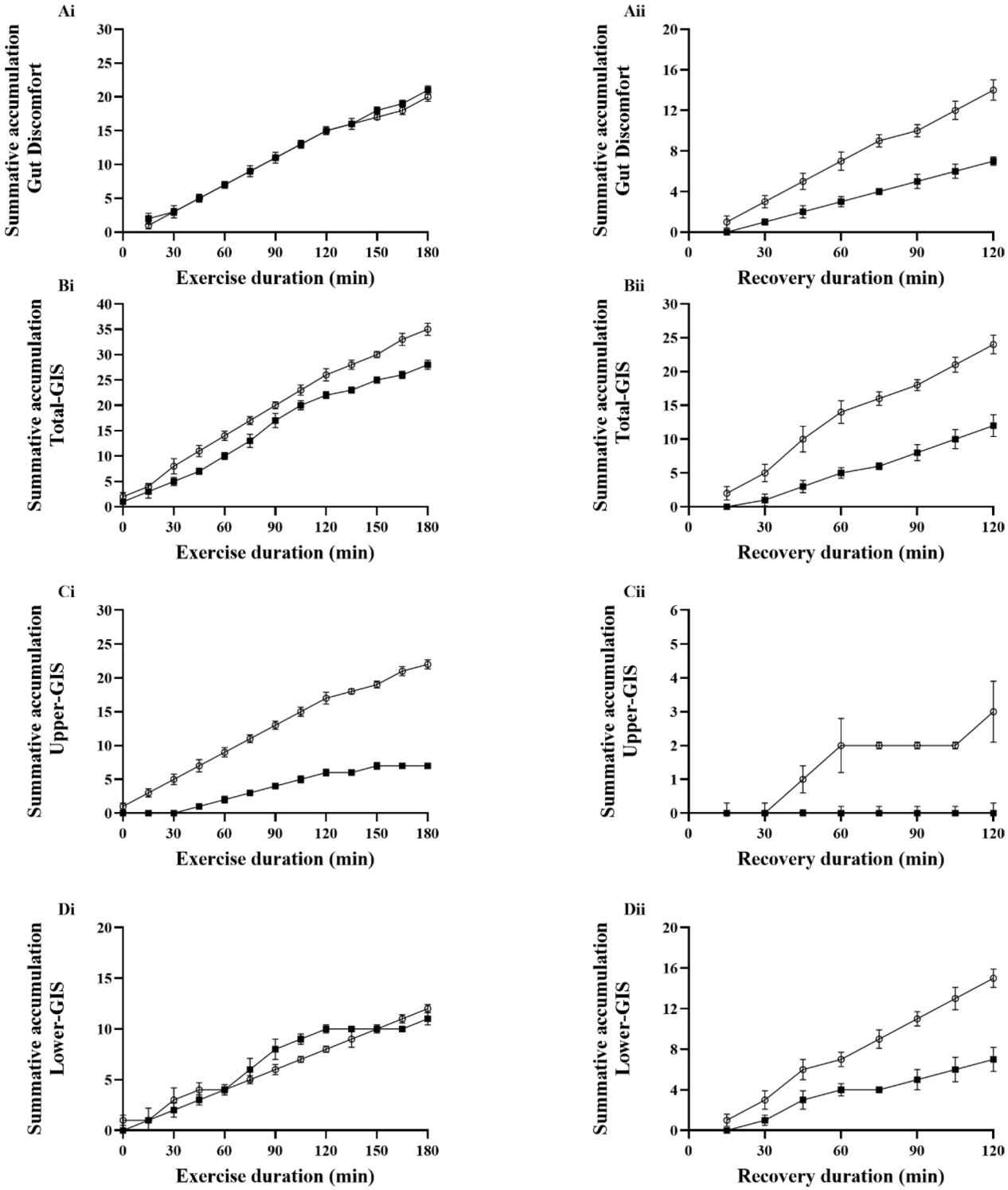

Fig. 5 (Supplementary). Summative accumulation during 3 h running at 60% $\dot{V}O_{2max}$ in ambient conditions (T_{amb} 22.5˚C, RH 50%) (i) and in 2 h seated recovery (ii) of gut discomfort (**A**), tota- GIS (**B**), upper-GIS (**C**), and lower-GIS *(D)* in ADULTS (white circles) and YOUTH (black squares). Values are mean ± SEM

exercise protocols to perturb immune markers to levels of clinical relevance in past research renders it challenging to compare findings [24]. In the current study, it is speculated that higher plasma leukocyte counts observed following exercise in YOUTH are a result of increased cardiovascular strain (i.e., higher mean HR), contributing to greater demargination of leukocyte cells from vascular walls [37]. In addition, greater plasma neutrophil counts during recovery in YOUTH may be in response to higher circulatory endotoxin load (i.e., sCD14 plasma concentrations) in YOUTH compared to ADULTS across the study protocol [32].

Prolonged strenuous exercise triggers the release of highly functional innate immune cells into circulation (i.e., neutrophils), with bacterially stimulated elastase release a marker of neutrophil degranulation activity and an important first-line functional response aiding in the clearance of systemic endotoxemia and/or bacteremia [38]. Mirroring findings from previous endurance exercise research [32], a decrease, albeit not significant, was observed in bacterially stimulated neutrophil degranulation in both groups throughout the 2 h recovery period. During recovery, the functional neutrophil response to potential luminal translocated pathogen (i.e., LPS) was comparable between YOUTH and ADULTS. Findings are novel, with previous research investigating the paediatric immune response to exercise being limited to cell counts and proportion of immune cells with no exploration into functional significance. Furthermore, consistent with previous research, [12] plasma TNF-α concentrations in the current study did not significantly change from pre- to post-exercise, with no difference in response observed between groups. This is not unexpected considering substantial increases in pro-inflammatory cytokines are recognised only to occur in application of extreme exertional stress (i.e., ultra-endurance exposure) [39]. Anti-inflammatory cytokine, IL-10, is more sensitive to lesser exertional stress with more considerable increases in previous studies observed following 2 h and 3 h treadmill running in ambient temperatures [32, 40]. In the current study, a change was observed in plasma concentrations from pre- to 1 h post-exercise in both groups with no difference in response between YOUTH and ADULTS. Taken together, findings indicate that adolescent age is not a substantial exacerbator of leukocytosis, impairment in bacterially stimulated neutrophil degranulation, and/or systemic inflammatory response associated with EIGS when carbohydrates are consumed regularly during prolonged endurance/ultra-endurance exercise.

Gastrointestinal Function and Symptoms

Stimulation of the neuroendocrine–gastrointestinal pathway of EIGS elicits alterations in gut motility, potentially delaying gastric emptying and intestinal transit time (e.g., OCTT) [40, 41]. In this study, changes in OCTT in response to prolonged exercise did not differ between age groups, with a turning point at 75 min for YOUTH and 60 min for ADULTS. In consideration of group similarities in plasma cortisol concentrations across the trial, it was not surprising that parallel gastrointestinal transit times were observed [33], suggesting adolescent age is not an exacerbating factor in the reduced gastrointestinal functional capacity often associated with prolonged exercise [40, 41].

Manifestation of GIS is closely linked to the pathophysiological processes of EIGS and can impact performance [3, 5, 9]. The severity of Ex-GIS can also reduce feeding tolerance, impacting the athlete's capacity to adhere to optimal fuelling guidelines during and post-exercise, and inhibiting the mitigation of gastrointestinal and immune disturbances that is recognised to occur as a result of regular feeding (i.e., carbohydrate) [16] In the current study, ADULTS reported significantly higher incidence and severity of upper-GIS during exercise compared to YOUTH. It is speculated that this difference is potentially attributable to the greater number of years ADULTS have engaged in endurance training, leading to a heightened sensitivity and/or awareness of GIS as opposed to a true age-related difference.

Limitations and Future Directions

It is plausible that the biological age of participants in the current study contributed to the lack of age-related differences in gastrointestinal responses, although evidence in gastrointestinal integrity changes from adolescent to adulthood remains limited. Differences between age groups in physiological response to exercise (i.e., thermoregulation, substrate oxidation) is shown to diminish as adolescents reach later stages [36, 42] of puberty and 9 of 12 adolescents in this study self-reported in Tanner Stage 4 or 5, with their body mass and height comparable to the adult group. However, given that most youth ultramarathon participants are aged 16–18, [13, 14] examining gastrointestinal responses in older adolescents is relevant. Investigating EIGS in youth athletes in early-to-mid puberty is justified if ultra-endurance participation increases in this group.

Future research is needed investigating the incidence and severity of EIGS and Ex-GIS in youth athletes competing in real-life ultramarathon events, with some key markers of EIGS rising to clinical significance in application of more extreme exercise stress (e.g., ultramarathons, 24-h or multistage events) [39]. Evidence from real-world events also suggests a rapid onset of Ex-GIS, and reduced feeding tolerance in exercise lasting ≥ 4 h [7, 39]. Finally, with dietary intake a central intrinsic factor

impacting EIGS, [5, 7, 9, 11, 43] insights into youth athletes nutrition practices during competition and the interplay with EIGS biomarkers is important in guiding management strategies to safeguard youth participation in the sport.

Conclusion

Adolescent age does not appear to be a potent intrinsic factor in the exacerbation of EIGS, with youth endurance athletes (≤18 years) responding to 3 h of prolonged exercise comparably to adult endurance athletes (>30–50 years) when regularly consuming carbohydrates. Therefore, current nutrition guidelines for mitigating gastrointestinal disruptions in adult endurance athletes is likely appropriate for adolescent athletes.

Abbreviations

BM	Body mass
bpm	Beats per minute
CHO	Carbohydrates
CRP	C-reactive protein
EIGS	Exercise-induced gastrointestinal syndrome
Ex-GIS	Exercise-associated gastrointestinal syndrome
FODMAP	Fermentable oligo-, di-, monosaccharides, and polyols
GIS	Gastrointestinal symptoms
HR	Heart rate
I-FABP	Intestinal fatty-acid binding protein
IL-6	Interleukin 6
LBP	Lipopolysaccharide binding protein
MJ	Megajoules
MUHREC	Monash University Human Research Ethics Committee
mVAS	Modified visual analogue scale
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
sCD14	Soluble CD14
$\dot{V}O_{2max}$	Maximum oxygen uptake

Acknowledgements

The authors would like to thank the Ultra Sports Science Foundation (USSF), 109 Boulevard de l'Europe, 69310 Pierre-Benite, France, for providing the funding for this study. The authors would like to show our appreciation to all participants that volunteered within the study, and would also like to thank the Monash University Sports Dietetics and Extremes Physiology Group for their assistance in the laboratory. The research study procedures were performed at the BASE Facility, Department of Nutrition, Dietetics and Food, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Australia.

Author Contributions

The author's contributions are as follows: RC was the Chief Investigator and contributed to conceptualisation, methodology, data analysis, resources, writing review and editing, supervision and funding acquisition. PY contributed to conceptualisation, methodology, data collection and analysis, data curation and writing of original draft, review and editing. VS provided funding for resources and contributed to writing review and editing. ZD contributed to conceptualisation, methodology, writing review and editing and supervision. JB contributed to writing review and editing and supervision. All authors read and approved the final version.

Funding

Open Access funding enabled and organized by CAUL and its Member Institutions. The study was supported by Ultra Sports Science Foundation (USSF), 109 Boulevard de l'Europe, 69310 Pierre-Benite, France.

Data Availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval and Consent to Participate

Participants gave written informed consent, approved by the local ethics committee and adhering to the 2013 Helsinki Declaration for Human Research Ethics (Monash University Human Research Ethics Committee: 28696).

Consent for Publication

All participants, and were appropriate their parents, gave written informed consent for their data results to be published in a research journal prior to participation in the study.

Competing Interests

RC and VS are members of the Ultra Sports Science Foundation (USSF) scientific committee. Pascale Young, Volker Scheer, Zoe Davidson, Jessica R. Biesiekierski and Ricardo J.S. Costa declare no other competing interest.

Author details

[1]Department of Nutrition, Dietetics and Food, Monash University, Level 1, 264 Ferntree Gully Road, Notting Hill, Melbourne, Victoria 3168, Australia. [2]Ultra Sports Science Foundation, 109 Boulevard de l'Europe, 69310 Pierre-Benite, Lyon, France.

Received: 1 December 2025 Accepted: 25 June 2026
Published online: 08 July 2026

References

1. Stöhr A, Nikolaidis PT, Villiger E, et al. An analysis of participation and performance of 2067 100-km ultra-marathons worldwide. Int J Environ Res Public Health. 2067;18(2):362. https://doi.org/10.3390/ijerph18020362.
2. Scheer V, Basset P, Giovanelli N, et al. Defining off-road running: A position statement from the ltra Sports Science Foundation. Int J Sports Med. 2020;41(5):275–84. https://doi.org/10.1055/a-1096-0980.
3. Costa RJS, Snipe RMJ, Kitic CM, et al. Systematic review: exercise-induced gastrointestinal syndrome-implications for health and intestinal disease. Aliment Pharmacol Ther. 2017;46(3):246–65. https://doi.org/10.1111/apt.14157.
4. Costa RJS, Gaskell SK, McCubbin AJ, et al. Exertional-heat stress-associated gastrointestinal perturbations during Olympic sports: management strategies for athletes preparing and competing in the 2020 Tokyo Olympic Games. Temperature. 2020;7(1):58–88. https://doi.org/10.1080/23328940.2019.1597676.
5. Scrivin R, Costa RJS, Pelly F, et al. An exploratory study of the management strategies reported by endurance athletes with exercise-associated gastrointestinal symptoms. Front Nutr. 2022;9:1003445. https://doi.org/10.3389/fnut.2022.1003445.
6. Costa RJ, Snipe R, Camões-Costa V, Scheer V, Murray A. The impact of gastrointestinal symptoms and dermatological injuries on nutritional intake and hydration status during ultramarathon events. Sports Med Open. 2016;2:16. https://doi.org/10.1186/s40798-015-0041-9.
7. Benmassaoud A, Kanber Y, Nawar J, et al. Exercise-induced ischemic colitis in an amateur marathon runner. Endoscopy. 2014;46:E480. https://doi.org/10.1055/s-0034-1377536.
8. Costa RJ, Snipe R, Camões-Costa V, et al. The impact of gastrointestinal symptoms and dermatological injuries on nutritional intake and hydration status during ultramarathon events. Sports Med Open. 2016;2:16. https://doi.org/10.1186/s40798-015-0041-9.
9. Gaskell SK, Rauch CE, Costa RJS. Gastrointestinal assessment and therapeutic intervention for the management of exercise-associated gastrointestinal symptoms: a case series translational and professional practice approach. Front Physiol. 2021;12:719142. https://doi.org/10.3389/fphys.2021.719142.

10. Grames C, Berry-Cabán CS. Ischemic colitis in an endurance runner. Case Rep Gastrointest Med. 2012;2012:356895. https://doi.org/10.1155/2012/356895.
11. Gaskell SK, Taylor B, Muir J, et al. Impact of 24-h high and low fermentable oligo, di-, monosaccharide, and polyol diets on markers of exercise-induced gastrointestinal syndrome in response to exertional heat stress. Appl Physiol Nutr Metab. 2020;45(6):569–80. https://doi.org/10.1139/apnm-2019-0187.
12. Snipe RMJ, Khoo A, Kitic CM, et al. The impact of mild heat stress during prolonged running on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profiles. Int J Sports Med. 2018. https://doi.org/10.1055/s-0043-122742.
13. Scheer V, Di Gangi S, Villiger E, et al. Age-related participation and performance trends of children and adolescents in ultramarathon running. Res Sports Med. 2020;28(4):507–17. https://doi.org/10.1080/15438627.2020.1781124.
14. Scheer V, Costa RJS, Doutreleau S, et al. Recommendations on youth participation in ultra-endurance running events: a consensus statement. Sports Med. 2021;51(6):1123–35. https://doi.org/10.1007/s40279-021-01441-w.
15. Young P, Gaskell SK, Mika A, et al. Exploration of gastrointestinal integrity, systemic immune response, and exercise-associated gastrointestinal symptoms in adolescent athletes in response to a single-stage ultramarathon: a case series approach. Wilderness Environ Med. 2025. https://doi.org/10.1177/10806032251401805.
16. Costa RJS, Gaskell SK, Henningsen K, et al. Sports Dietitians Australia and Ultra Sports Science Foundation joint position statement: a practitioner guide to the prevention and management of exercise-associated gastrointestinal perturbations and symptoms. Sports Med. 2025;55(5):1097–134. https://doi.org/10.1007/s40279-025-02186-6.
17. Desbrow B, McCormack J, Burke LM, et al. Sports Dietitians Australia position statement: sports nutrition for the adolescent athlete. Int J Sport Nutr Exerc Metab. 2014;24(5):570–84. https://doi.org/10.1123/ijsnem.2014-0031.
18. Wollmer E, Ungell AL, Nicolas JM, et al. Review of paediatric gastrointestinal physiology relevant to the absorption of orally administered medicines. Adv Drug Deliv Rev. 2022;181:114084. https://doi.org/10.1016/j.addr.2021.114084.
19. Merchant HA, Liu F, Orlu Gul M, Basit AW. Age-mediated changes in the gastrointestinal tract. Int J Pharm. 2016;512(2):382–95. https://doi.org/10.1016/j.ijpharm.2016.04.024.
20. Yuan X, Chen R, Zhang Y, Lin X, Yang X. Gut microbiota: effect of pubertal status. BMC Microbiol. 2020;20(1):334. https://doi.org/10.1186/s12866-020-02021-0.
21. Timmons BW, Tarnopolsky MA, Bar-Or O. Immune responses to strenuous exercise and carbohydrate intake in boys and men. Pediatr Res. 2004;56(2):227–34. https://doi.org/10.1203/01.PDR.0000132852.29770.C5.
22. Timmons BW, Tarnopolsky MA, Snider DP, et al. Immunological changes in response to exercise: influence of age, puberty, and gender. Med Sci Sports Exerc. 2006;38(2):293–304. https://doi.org/10.1249/01.mss.0000183479.90501.a0.
23. Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. Proc Biol Sci. 2015;282(1821):20143085. https://doi.org/10.1098/rspb.2014.3085.
24. Costa RJS, Young P, Gill SK, et al. Assessment of exercise-associated gastrointestinal perturbations in research and practical settings: methodological concerns and recommendations for best practice. Int J Sport Nutr Exerc Metab. 2022;32(5):387–418. https://doi.org/10.1123/ijsnem.2022-0048.
25. Young P, Russo I, Gill P, et al. Reliability of pathophysiological markers reflective of exercise-induced gastrointestinal syndrome (EIGS) in response to 2-h high-intensity interval exercise: a comprehensive methodological efficacy exploration. Front Physiol. 2023;14:1063335. https://doi.org/10.3389/fphys.2023.1063335.
26. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969;44(235):291–303. https://doi.org/10.1136/adc.44.235.291.
27. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970;45(239):13–23. https://doi.org/10.1136/adc.45.239.13.
28. Day K, Kwok A, Evans A, et al. Comparison of a bioelectrical impedance device against the reference method dual energy X-ray absorptiometry and anthropometry for the evaluation of body composition in adults. Nutrients. 2018;10(10):1469. https://doi.org/10.3390/nu10101469.
29. Gaskell SK, Snipe RMJ, Costa RJS. Test-retest reliability of a modified visual analog scale assessment tool for determining incidence and severity of gastrointestinal symptoms in response to exercise stress. Int J Sport Nutr Exerc Metab. 2019;29(4):411–9. https://doi.org/10.1123/ijsnem.2018-0215.
30. Bate JP, Irving PM, Barrett JS, et al. Benefits of breath hydrogen testing after lactulose administration in analysing carbohydrate malabsorption. Eur J Gastroenterol Hepatol. 2010;22(3):318–26. https://doi.org/10.1097/MEG.0b013e32832b20e8.
31. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol. 1974;37(2):247–8. https://doi.org/10.1152/jappl.1974.37.2.247.
32. Costa RJS, Camões-Costa V, Snipe RMJ, et al. Impact of exercise-induced hypohydration on gastrointestinal integrity, function, symptoms, and systemic endotoxin and inflammatory profile. J Appl Physiol. 1985;126(5):1281–91. https://doi.org/10.1152/japplphysiol.01032.2018.
33. Rehrer NJ, Goes E, DuGardeyn C, Reynaert H, DeMeirleir K. Effect of carbohydrate on portal vein blood flow during exercise. Int J Sports Med. 2005;26(3):171–6. https://doi.org/10.1055/s-2004-820957.
34. Matheson PJ, Wilson MA, Garrison RN. Regulation of intestinal blood flow. J Surg Res. 2000;93(1):182–96. https://doi.org/10.1006/jsre.2000.5862.
35. Snipe RMJ, Khoo A, Kitic CM, et al. Carbohydrate and protein intake during exertional heat stress ameliorates intestinal epithelial injury and small intestine permeability. Appl Physiol Nutr Metab. 2017;42(12):1283–92. https://doi.org/10.1139/apnm-2017-0361.
36. Timmons BW, Bar-Or O, Riddell MC. Influence of age and pubertal status on substrate utilization during exercise with and without carbohydrate intake in healthy boys. Appl Physiol Nutr Metab. 2007;32(3):416–25. https://doi.org/10.1139/H07-004.
37. Risøy BA, Raastad T, Hallén J, et al. Delayed leukocytosis after hard strength and endurance exercise: aspects of regulatory mechanisms. BMC Physiol. 2003;3:14. https://doi.org/10.1186/1472-6793-3-14.
38. Walsh NP, Gleeson M, Shephard RJ, et al. Position statement. Part one: Immune function and exercise. Exerc Immunol Rev. 2011;17:6–63.
39. Gill SK, Hankey J, Wright A, et al. The impact of a 24-h ultra-marathon on circulatory endotoxin and cytokine profile. Int J Sports Med. 2015;36(8):688–95. https://doi.org/10.1055/s-0034-1398535.
40. Gaskell SK, Rauch C, Parr A, et al. Diurnal versus nocturnal exercise—impact on the gastrointestinal tract. Med Sci Sport Exerc. 2021;53(5):1056–67.
41. Gaskell SK, Burgell R, Wiklendt L, et al. Impact of exercise duration on gastrointestinal function and symptoms. J Appl Physiol. 2023;134(1):160–71.
42. Falk B, Bar-Or O, MacDougall JD. Thermoregulatory responses of pre-, mid-, and late-pubertal boys to exercise in dry heat. Med Sci Sports Exerc. 1992;24(6):688–94.
43. Costa RJS, Miall A, Khoo A, et al. Gut-training: the impact of two weeks repetitive gut-challenge during exercise on gastrointestinal status, glucose availability, fuel kinetics, and running performance. Appl Physiol Nutr Metab. 2017;42(5):547–57. https://doi.org/10.1139/apnm-2016-0453.

Publisher's Note