# Diurnal versus Nocturnal Exercise—Effect on the Gastrointestinal Tract

STEPHANIE K. GASKELL, CHRISTOPHER E. RAUCH, ALEXANDRA PARR, and RICARDO J. S. COSTA

*Department of Nutrition, Dietetics and Food, Monash University, Notting Hill, Victoria, AUSTRALIA*

Downloaded from http://journals.lww.com/acsm-msse by GR9gVYMt5gmxEZ375+D21bOlVeMQJ8RQp1607haUmlEp42wkw2

**ABSTRACT**

GASKELL, S. K., .C. E. RAUCH, A. PARR, and R. J. S. COSTA Diurnal versus Nocturnal Exercise—Effect on the Gastrointestinal Tract. *Med. Sci. Sports Exerc.*, Vol. 53, No. 5, pp. 1056–1067, 2021. **Purpose:** The study aimed to determine the effect of diurnal versus nocturnal exercise on gastrointestinal integrity and functional responses, plasma lipopolysaccharide binding protein (LBP) and soluble CD14 (sCD14) concentrations (as indirect indicators of endotoxin responses), systemic inflammatory cytokine profile, gastrointestinal symptoms, and feeding tolerance. **Methods:** Endurance runners ($n$ = 16) completed 3 h of 60% $\dot{V}O_{2max}$ (22.7ºC, 45% relative humidity) running, on one occasion performed at 0900 h (400 lx; DAY) and on another occasion at 2100 h (2 lx; NIGHT). Blood samples were collected pre- and postexercise and during recovery to determine plasma concentrations of cortisol, catecholamines, claudin-3, I-FABP, LBP, and sCD14 and inflammatory cytokine profiles by ELISA. Orocecal transit time (OCTT) was determined by lactulose challenge test given at 150 min, with concomitant breath hydrogen ($H_2$) and gastrointestinal symptom determination. **Results:** Cortisol increased substantially pre- to postexercise on NIGHT (+182%) versus DAY (+4%) (trial–time, $P$ = 0.046), with no epinephrine (+41%) and norepinephrine (+102%) trial differences. I-FABP, but not claudin-3, increased pre- to postexercise on both trials (mean = 2269 pg·mL$^{-1}$, 95% confidence interval = 1351–3187, +143%) (main effect of time [MEOT], $P$ < 0.001). sCD14 increased pre- to postexercise (trial–time, $P$ = 0.045, +5.6%) and was greater on DAY, but LBP decreased (MEOT, $P$ = 0.019, −11.2%) on both trials. No trial difference was observed for systemic cytokine profile (MEOT, $P$ = 0.004). Breath $H_2$ responses ($P$ = 0.019) showed that OCTT was significantly delayed on NIGHT (>84 min, with $n$ = 3 showing no breath $H_2$ turning point by 180 min postexercise) compared with DAY (mean = 54 min, 95% confidence interval = 29–79). NIGHT resulted in greater total gastrointestinal symptoms ($P$ = 0.009) compared with DAY. No difference in feeding tolerance markers was observed between trials. **Conclusion:** Nocturnal exercise instigates greater gastrointestinal functional perturbations and symptoms compared with diurnal exercise. However, there are no circadian differences to gastrointestinal integrity and systemic perturbations in response to the same exertional stress and controlled procedures.

**Key Words:** CIRCADIAN, EPITHELIUM, ENDOTOXIN, CYTOKINE, SYMPTOMS, OROCECAL TRANSIT

APPLIED SCIENCES

Ultraendurance and endurance event numbers and participation, especially in running modality, have been increasing over several decades, with many events including a nocturnal segment (1). These include single-stage ultramarathon night events, ≥24-h ultramarathon competitions, night stage/s of multiday ultramarathon events, and even championship marathon competition—2019 World Athletics Championships. Considering the regulatory effects of the daily circadian cycle on human physiological processes (e.g., metabolic, circulatory, neuroendocrine, and immunological), it is plausible that strenuous exercise in the day (diurnal) and night (nocturnal) may result in differing physiological outcomes, namely, neuroendocrine factors (e.g., stress hormone responses), cardiometabolic strain, gastrointestinal integrity and functional perturbations, and immune activation (2–5). As an example, plasma cortisol concentration and immune responses (e.g., circulating leukocyte counts, systemic cytokine responses, granulocyte and lymphocyte cell functional response to *in vitro* bacterial or antigen challenge) have been reported to differ between diurnal and nocturnal timelines, irrespective of sleep deprivation status (2,3,6–8). These hormonal and immune outcomes aligned with exercise-induced gastrointestinal syndrome (EIGS) have the potential to negatively affect exercise performance through promoting greater gastrointestinal symptoms (GIS) (e.g., gut discomfort) and feeding intolerance (e.g., impaired fuel and fluid availability) during nocturnal compared with diurnal exercise (4). In addition, this may accentuate the systemic endotoxemia and cytokinemia consistently observed after endurance exercise (9,10), associated with a potentially higher starting concentration in nocturnal compared with diurnal exercise.

Gastrointestinal physiology and function follow regular daily fluctuations, including gastrointestinal motility, gastric acid and digestive enzyme secretion, small intestinal nutrient transport, mucosal immune responses, cell proliferation, and intestinal permeability (11,12). For example, a study assessing gastric emptying rate in healthy male volunteers showed a

Address for correspondence: Ricardo J. S. Costa, Ph.D., Department of Nutrition, Dietetics and Food, Monash University, Level 1, 264 Ferntree Gully Road, Notting Hill, VIC 3168, Australia; E-mail: ricardo.costa@monash.edu.
Submitted for publication June 2020.
Accepted for publication October 2020.

0195-9131/21/5305-1056/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®
Copyright © 2020 by the American College of Sports Medicine
DOI: 10.1249/MSS.0000000000002546



Copyright © 2021 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

longer gastric emptying half time of solids in the evening (2000 h) compared with morning (0800 h) (97.1 ± 11.5 vs 64.8 ± 6.4 min, respectively) (13). In addition, circadian variation in gastric motility has been demonstrated in a study involving healthy subjects, in which mean frequency of gastric electric activity varied from 2.92 ± 0.15 counts per minute at midday to 2.72 ± 0.13 counts per minute in the late night, assessed by electrogastrography (14). Moreover, daily circadian cycles regulate the expression of various hormones and enzymes involved in aerobic-based endogenous and exogenous energy substrate metabolism (e.g., insulin, glucagon, adiponectin, corticosterone, ghrelin, and leptin; and enzyme activity associated with glycogen and glucose metabolism such as glycogen synthase, glycogen phosphorylase, and phosphoenolpyruvate carboxykinase), which are potential mechanisms that play a role in regulating the neuroendocrine–gastrointestinal pathway of EIGS (9–11,15). Therefore, it is plausible that nocturnal activity may increase the risk of gastrointestinal perturbations, linked to health and performance implications (4,12,16). This is evident in nocturnal shift workers, who experience increased nocturnal activity, reporting an increased incidence of GIS, and potentially gastrointestinal disorders, compared with diurnal workers (17,18).

It is now well established that EIGS is a common feature of exercise, with substantial gastrointestinal integrity perturbations (e.g., epithelial injury) and gastrointestinal functional impairment (e.g., nutrient absorption) especially observed after endurance running (e.g., ≥2 h at 60% $\dot{V}O_{2max}$ in ≥35.0°C ambient temperature ($T_{amb}$) or ≥3 h at 60% $\dot{V}O_{2max}$ in temperate conditions) (9,10). EIGS-associated GIS, with incidence rates of >60% consistently observed after endurance running in laboratory controlled studies and field-based competitive events, have been reported as a major factor associated with limiting nutritional intake during and after events (e.g., ultramarathon and long course triathlon) and limiting competition outcomes (9,10,19,20). The causes of adverse GIS during and after exercise appear to be multifactorial in nature but seem related to EIGS primary causal mechanisms (i.e., splanchnic hypoperfusion and increased sympathetic drive) (9). The mode, intensity, duration, and ambient conditions of exercise; dietary intake before, during, and after exercise; and biological sex have all been identified as either extrinsic or intrinsic exacerbation factors influencing the magnitude of EIGS and GIS (10). However, despite the growing number of athletes anecdotally reporting greater GIS during nocturnal exercise activities, the magnitude to which circadian cycle disturbances affect gastrointestinal markers of EIGS and GIS remains unknown.

Considering increased sympathetic drive and immune activation are key pathophysiological mechanisms of EIGS, and are associated with clinical implications such as gastroparesis and/or paralytic ileus, and systemic inflammatory response outcomes, respectively (9,10), it is speculated and hypothesized that prolonged nocturnal exertion will result in greater exercise-associated gastrointestinal integrity and functional perturbations and promote greater incidence and severity of GIS and feeding intolerance compared with prolonged diurnal exertion (9,10,21–23). With this in mind, the current study aimed to determine the effect of diurnal versus nocturnal exertional stress on gastrointestinal integrity and functional responses, systemic lipopolysaccharide binding protein (LBP) and soluble CD14 (sCD14) concentration (as indirect indicators of endotoxin responses), systemic inflammatory cytokine profile, GIS, and feeding tolerance.

## METHODS

Sixteen ($n$ = 8 males and $n$ = 8 females) recreationally trained endurance runners (mean ± SD: age = 43 ± 9 yr, nude body mass = 69 ± 10 kg, height = 1.72 ± 0.10 m, % body fat mass = 21% ± 5%, $\dot{V}O_{2max}$ = 52 ± 7 mL·kg$^{-1}$ BM·min$^{-1}$) volunteered to participate in the study and experimental procedures (Fig. 1). Trials for female athletes were scheduled during the early–mid follicular phase of their menstrual cycle ($n$ = 5) or were postmenopause ($n$ = 3). According to the Morningness–Eveningness questionnaire, $n$ = 11 fell into the morning, $n$ = 2 evening, and $n$ = 2 intermediate-type categories (24).

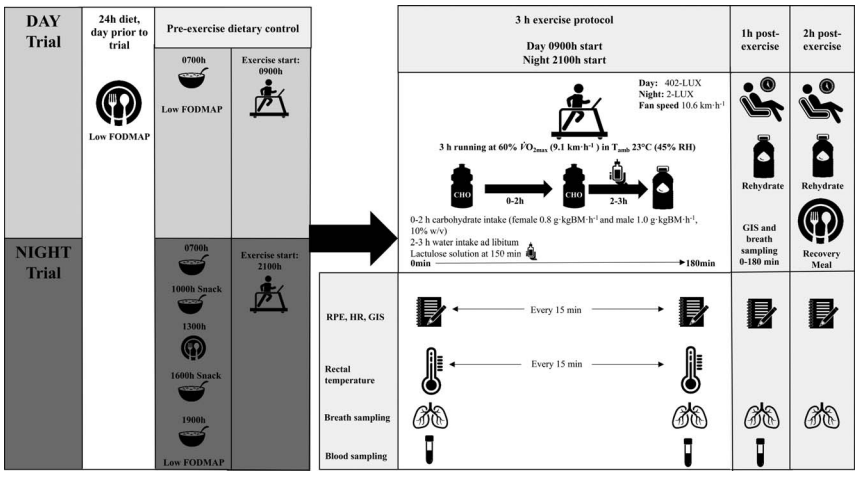


**FIGURE 1—Schematic illustration of the experimental design. FODMAP, fermentable oligo- di- mono-, and polyols; CHO, carbohydrate; *w/v*, water volume equivalent.**





Copyright © 2021 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

All participants provided written informed consent, which received approval from the local ethics committee (MUHREC ethics approval number 18587). The standardized exclusion criteria and the initial assessment were in accordance with Costa et al. (21). All participants reported experience of exercise-associated GIS during training and/or competition. Participants were not habitually night exercise trained.

Participants were provided with a low FODMAP diet (8.5 ± 0.2 MJ·d$^{-1}$, 322 ± 14 g·d$^{-1}$ carbohydrate, 78 ± 2 g·d$^{-1}$ protein, 49 ± 1 g·d$^{-1}$ fat, 36 ± 1 g·d$^{-1}$ fiber, and 2 ± 0 g·d$^{-1}$ total FODMAP) for the day before each experimental trial to reduce GIS confounded from the lead-in diet (25). In addition, on NIGHT, identically matched food (i.e., quantity and quality) and food intake timings along the trial day were provided. Participants reported to the laboratory at 0800 h (DAY) or 2000 h (NIGHT) after consuming the standardized pretrial low FODMAP meal (3.0 ± 0.3 MJ, 99 ± 11 g carbohydrate, 24 ± 3 g protein, 25 ± 5 g fat, 12 ± 2 g fiber, 1 ± 1 g total FODMAP), consumed at 0700 h (DAY) or 1900 h (NIGHT). The meal was consumed 2 h before the start of exercise on DAY and NIGHT, simulating real-life translational practice in the target population (19,20). A dietary log containing the prescriptive diet determined ingestion compliance and food waste. Participants refrained from strenuous exercise 48 h before trials. In addition, sleep duration (DAY, 8 h ± 48 min; NIGHT, 8 h ± 84 min) and activity metabolic equivalents (DAY, 1.6 ± 0.3 METs; NIGHT, 1.9 ± 0.3 METs) the full day (24 h) before each experimental trial were monitored by triaxial accelerometry (SenseWear 8.1; Bodymedia, Pittsburgh, PA), as previously reported (19,20). On NIGHT, participants also refrained from any form of exercise along the trial day before starting exercise at 2100 h, which was verified by triaxial accelerometry.

Participants were asked to void before nude body mass measurement, provide a breath sample into a 250-mL breath collection bag (Wagner Analysen Technick, Bremen, Germany), and complete a GIS assessment tool, as previously reported (26). In short, the GIS assessment tool applied was a modified visual analog scale (mVAS) for assessing GIS during exercise (10-point rating scale, each point indicative of 10 mm). Participants were educated and advised to complete the GIS rating scale as follows: 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. The maximum severity score possible for each individual GIS type for exercise was 120 (40 h$^{-1}$) and for trial total (exercise and recovery) was 240. Blood samples were then collected by venipuncture from an antecubital vein into lithium heparin (6 mL, 1.5 IU·mL$^{-1}$ heparin) and $K_3$EDTA (4 mL, 1.6 mg mL$^{-1}$ EDTA) vacutainers. Rectal temperature ($T_{re}$) was monitored during running with participants inserting a thermocouple 12 cm beyond the external anal sphincter (Alpha Technics Precision Temperature 4600 Thermometer, Oceanside, CA). In a counterbalanced randomized order, participants completed two experimental trials, with 1-wk washout, consisting of 3 h (initiated at 0900 h or 2100 h) running on a motorized treadmill at the previously determined speed (9.1 ± 0.7 km·h$^{-1}$) eliciting 60% $\dot{V}O_{2max}$ in temperate ambient conditions (DAY, $T_{amb}$ = 23.1°C ± 1.2°C and 43.6% ± 5.5% relative humidity [RH]; NIGHT, $T_{amb}$ = 22.3°C ± 0.8°C and 46.4% ± 5.9% RH, with dual fan wind speed 10.6 km·h$^{-1}$). Participants were provided with and instructed to consume an in-house formulated dextrose or dextrose–fructose solution (64 ± 15 g·h$^{-1}$, equivalent to males at 1.0 g·kg$^{-1}$ BM·h$^{-1}$ and females at 0.8 g·kg$^{-1}$ BM·h$^{-1}$, 10% *w/v*; beverage temperature: DAY, 23.4°C ± 1.4°C, and NIGHT, 22.1°C ± 1.1°C) for the first 2 h, with additional water allowed *ad libitum* (DAY, 640 ± 143 mL·h$^{-1}$; NIGHT, 642 ± 145 mL·h$^{-1}$). Fructose was only added if solution of >60 g·h$^{-1}$ of carbohydrate was required (mean ratio of participants requiring fructose addition, 4.6:1). The relative feeding quantity (e.g., volume) and quality (e.g., composition and temperature) are in accordance with individualized total carbohydrate oxidation rates during prolonged endurance exercise (i.e., ≥2 h), focused on reducing the risk of artifact GIS associated with feeding intolerance, and the neutral drink temperature applied to standardize effects on measured EIGS variables (21,27,28). Moreover, the carbohydrate feeding regime during exercise was applied to support participants completing the 3-h running exercise bout. Feeding was ceased at the 2-h time point to avoid interference with the orocecal transit time (OCTT) test procedures and to avoid gastrointestinal integrity protection commonly associated with consistent and frequent carbohydrate intake throughout exercise (29–33). Water was available *ad libitum* for the final hour (DAY, 276 ± 105 mL; NIGHT, 262 ± 113 mL). To determine OCTT, participants were provided with a 150-mL solution containing 20 g of lactulose (Actilax; alphapharm, QLD, Australia) 150 min into exercise. Breath samples were then collected immediately postexercise and every 15 min for 3 h postexercise. The time interval between ingestion of lactulose and rise in breath hydrogen ($H_2$) of 10 ppm, with two consecutive readings above basal, was used as a measure of OCTT (34), within the postexercise monitoring period (3 h). If participants failed to present a breath $H_2$ turning point before the end of the postexercise monitoring period, this was an indication of sub- or full-level gastroparesis and/or paralytic ileus from a clinical perspective (10,34). $T_{re}$, heart rate (HR), RPE, thermal comfort rating (TCR), body mass, and GIS were measured every 15 min during running. Breath-by-breath indirect calorimetry (Vmax Encore Metabolic Cart, CaseFusion-BD, Franklin Lakes, NJ) was used to determine $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), and respiratory exchange ratio for 5 min continuously every 30 min during exercise. Total nonprotein carbohydrate and fat





Copyright © 2021 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

oxidation was determined from the equations of Péronnet and Massicotte (35):

$$\text{total carbohydrate oxidation} = \left(4.585\ \dot{V}CO_2\right) - \left(3.226\ \dot{V}O_2\right)$$

$$\text{total fat oxidation} = \left(1.695\ \dot{V}O_2\right) - \left(1.701\ \dot{V}CO_2\right)$$

Immediately after exercise, a blood sample was collected, nude body mass measured, and GIS recorded. Blood samples were repeated 1 h postexercise. Participants remained seated during the recovery period and consumed water *ad libitum*. GIS were recorded every 15 min during the 3-h postexercise period. Participants were provided with a standard meal 2 h postexercise in accordance with ethical procedures.

Breath samples (20 mL) were analyzed in duplicate (CV, 3.0%) for hydrogen ($H_2$) content using a gas-sensitive analyzer in real time (Breathtracker Digital Microlyzer, Quintron, Milwaukee, WI). Whole blood hemoglobin was determined by a HemoCue system (Hb201; HemoCue, Ängelholm, Sweden), and hematocrit was determined by the capillary method with a microhematocrit reader (Thermo Fisher Scientific, Waltham, MA), both from heparin whole blood samples. Hemoglobin and hematocrit values were used to estimate changes in plasma volume ($P_V$) relative to baseline and used to correct plasma variables. Blood glucose concentration was measured before, every 30 min during, and after exercise (Accu-Chek Proforma; Roche Diagnostics, Indianapolis, IN) (CV, 3.0%). The remaining heparin and $K_3$EDTA whole blood samples were centrifuged at 4000 rpm (1500*g*) for 10 min within 15 min of sample collection. Plasma was aliquoted into 1.5 mL micro-storage tubes and frozen at −80°C until analysis, except for 2 × 50 µL heparin plasma that was used to determine plasma osmolality ($P_{Osmol}$) in duplicate (CV, 0.5%) by freeze point osmometry (Osmomat 030; Gonotec, Berlin, Germany). Plasma concentration of cortisol (DKO001; IBL International, Kiel, Germany), adrenaline and noradrenaline (KA1877; Abnova, Taipei City, Taiwan), intestinal fatty acid binding protein (I-FABP) (HK406-02; Hycult Biotech, Uden, Netherlands), claudin-3 (SEF293Hu, Cloud-Clone Corp., Katy, Texas), sCD14 (HK320-02, Hycult Biotech), and LBP (HK315, Hycult Biotech) were determined by ELISA. Plasma concentrations of interleukin (IL)-1β, tumor necrosis factor α (TNF-α), IL-6, IL-8, IL-10, and IL-1ra were determined by multiplex ELISA (HCYTOMAG-60K; EMD Millipore, Darmstadt, Germany). All variables were analyzed 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 plasma cortisol, catecholamines, I-FABP, claudin-3, sCD14, LBP, and cytokine concentrations were 10.4%, 5.5%, 3.9%, 7.8%, 6.8%, 4.6%, and 8.1%, respectively.

Based on the statistical test, mean, SD, and effect size (i.e., small 0.20, medium 0.50, and large 0.80) of previously established exercise stress models, with and without additional stress factors (e.g., heat) that induce gastrointestinal integrity (primary variables: plasma I-FABP, LBP and sCD14 concentration, and systemic inflammatory cytokine profile) perturbations, gastrointestinal function (primary variables: OCTT) impairment, and GIS (32,33,36–38), and applying a standard alpha (0.05) and beta value (0.80), the current participant sample size is estimated to provide adequate statistical power (power*, 0.80–0.99) for detecting significant trial differences (G*Power 3.1, Kiel, Germany). In addition to presenting the raw values for plasma cytokine concentrations, considering the habitually large individual variation in cytokine responses, the peak Δ pre- to postexercise for proinflammatory (IL-1β and TNF-α), response (IL-6 and IL-8), and anti-inflammatory (IL-10 and IL-1ra) cytokines were combined to establish an exercise-associated systemic inflammatory response profile, as previously reported (39). Data in the text and tables are presented as either mean ± SD or mean and 95% confidence interval (CI), as indicated, and accumulative score (total and corrected) and individual participant range for GIS, as previously reported (26). For clarity, data in figures are presented as mean ± SEM. Only participants with full data sets within each specific variable were used in data analysis. For example, $n = 3$ participants were not able to complete the exercise protocol due to GIS, and researchers had difficulty in obtaining full blood sample volumes at all time points in $n = 2$ participants. Therefore, $n = 13$ participant full data sets were used in the data analysis, except for plasma I-FABP, LBP, and cytokine concentrations ($n = 11$). Data set numbers are reported in Table 1 and Fig. 1. All data were checked for normal distribution by the Shapiro–Wilk test of normality, before applying appropriate parametric or nonparametric statistical tests. Variables with singular data points were examined using paired-sample *t*-test s or nonparametric Wilcoxon signed-rank test, where appropriate. Variables with multiple data points were examined using a two-way (trial × time) repeated-measures ANOVA (or nonparametric Friedman test, where appropriate). Assumptions of homogeneity and sphericity were checked and when appropriate adjustments to the degrees of freedom were made using the Greenhouse–Geisser correction method. Significant main effects were analyzed using a *post hoc* Tukey's HSD test. Statistics were analyzed using SPSS statistical software (version 26.0, IBM SPSS Statistics; IBM Corp., Armonk, NY) with significance accepted at $P < 0.05$. In addition, Cohen's standardized measurement of effect size between DAY and NIGHT for GIS was determined as $\delta = 0.20$, $\delta = 0.50$, and $\delta = 0.80$ for small, medium, and large effects, respectively.

## RESULTS

Exercise-induced body mass loss did not differ between trials (overall mean = 1.1%, 95 CI = 0.9–1.3). Pre- and postexercise $P_{Osmol}$ did not differ between trials (overall mean = 294 mOsmol·kg$^{-1}$, 95 CI = 290–298, and 294 mOsmol·kg$^{-1}$, 95 CI = 289–299, respectively). Δ pre- to postexercise $P_V$ (main effect of time (MEOT) $P < 0.001$) did not differ between trials (overall mean = −5.1%, 95 CI = −7.6 to 2.6), returning to baseline values 1 h postexercise. A MEOT was observed for $T_{re}$ ($P < 0.001$), HR ($P < 0.001$),





Copyright © 2021 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

TABLE 1. Incidence and severity of GIS and feeding tolerance variables in response to 3 h running at 60% $\dot{V}O_{2max}$ in ambient conditions ($T_{amb}$ 23°C, RH 45%) on nocturnal (NIGHT) and diurnal (DAY) trials.

| | Incidence during | First hour | Second hour | Third hour | Total Exercise | Trial Total (Exercise and Recovery) |
|---|---|---|---|---|---|---|
| **NIGHT** | | | | | | |
| Gut discomfort | NA | 8 (4–19) | 11 (1–26) | 13 (1–29) | 32 (5–53)* | 52 (5–84)* |
| Total GIS[a] | 100% | 19 (4–58) | 41 (2–137) | 36 (1–66) | 96 (9–218)** | 138 (9–265)** |
| Upper GIS[b] | 100% | 15 (4–58) | 23 (1–93) | 22 (1–54) | 59 (9–151)* | 79 (9–231)* |
| Belching | 100% | 5 (3–15) | 7 (2–20) | 5 (1–13) | 18 (3–45)* | 16 (3–45) |
| Heartburn | 46% | 0 (1–3) | 1 (2–4) | 0 (6) | 1 (1–6)** | 2 (1–7)*** |
| Upper abdominal bloating | 69% | 3 (3–19) | 6 (1–21) | 5 (3–18) | 11 (4–40)** | 19 (4–46)** |
| Upper abdominal pain | 46% | 0 (5) | 1 (2–4) | 2 (2–14) | 3 (2–14) | 5 (1–21)** |
| Urge to regurgitate | 39% | 2 (7–22) | 2 (14–15) | 3 (2–20) | 8 (2–51)* | 8 (2–51)* |
| Regurgitation | 23% | 2 (10–20) | 3 (10–30) | 2 (10–20) | 8 (20–40)* | 8 (20–40)* |
| Lower GIS[b] | 85% | 4 (3–25) | 17 (1–53) | 11 (1–33) | 33 (1–74)*** | 50 (1–126)* |
| Flatulence | 77% | 1 (1–9) | 4 (1–12) | 3 (3–13) | 8 (1–24)** | 14 (1–42)* |
| Lower abdominal bloating | 39% | 1 (16) | 3 (7–13) | 3 (13–16) | 8 (7–45)* | 14 (5–65)* |
| Urge to defecate | 54% | 2 (8–12) | 7 (7–21) | 4 (1–20) | 13 (10–35)** | 17 (10–72)** |
| Intestinal pain | 23% | 0 (2) | 0 (2) | 1 (1–5) | 1 (1–9)* | 2 (1–17) |
| Abnormal stools[c] | 15% | 0 (0) | 1 (10) | 0 (0) | 1 (10)* | 3 (10–20)* |
| Nausea | 23% | 0 (0) | 0 (0) | 2 (2–10) | 2 (2–10)* | 5 (10–32)** |
| Dizziness | 8% | 0 (0) | 0 (5) | 1 (19) | 1 (19)* | 2 (4–26) * |
| Abdominal stitch | 15% | 0 (6) | 1 (4–5) | 1 (2–6) | 2 (7–16) * | 2 (7–16) * |
| Feeding tolerance | | | | | | |
| Taste fatigue | NA | 3 (1–21) | 7 (3–34) | 8 (9–31) | 18 (6–86) | NA |
| Interest in food | NA | 2 (2–11) | 2 (2–11) | 5 (4–15) | 8 (9–32) | NA |
| Interest in fluid | NA | 9 (2–26) | 7 (3–19) | 7 (5–24) | 24 (2–60) | NA |
| Tolerance to food | NA | 18 (7–40) | 18 (4–40) | 20 (8–40) | 56 (34–120) | NA |
| Tolerance to fluid | NA | 31 (11–40) | 28 (12–40) | 28 (12–40) | 88 (35–120) | NA |
| Appetite | NA | 3 (2–13) | 2 (2–8) | 4 (7–14) | 9 (2–31) | NA |
| Thirst | NA | 10 (3–26) | 7 (2–19) | 8 (1–24) | 24 (1–56) | NA |
| **DAY** | | | | | | |
| Gut discomfort | NA | 5 (3–14) | 10 (1–22) | 10 (1–30) | 24 (3–57) | 37 (8–81) |
| Total GIS[a] | 92% | 6 (6–14) | 17 (1–50) | 20 (1–82) | 43 (3–144) | 75 (19–185) |
| Upper GIS[b] | 92% | 6 (3–14) | 12 (1–48) | 14 (1–77) | 31 (3–134) | 42 (3–149) |
| Belching | 92% | 4 (3–14) | 5 (1–17) | 5 (1–17) | 14 (2–40) | 15 (2–40) |
| Heartburn | 8% | 0 (0) | 0 (0) | 0 (3) | 0 (3) | 0 (3) |
| Upper abdominal bloating | 54% | 1 (4–10) | 2 (1–9) | 1 (2–10) | 5 (1–17) | 11 (3–65) |
| Upper abdominal pain | 39% | 0 (1) | 1 (3–9) | 2 (2–14) | 3 (1–23) | 8 (2–57) |
| Urge to regurgitate | 15% | 0 (0) | 2 (10–20) | 3 (8–30) | 5 (18–50) | 5 (18–50) |
| Regurgitation | 8% | 0 (0) | 2 (20) | 2 (30) | 4 (50) | 4 (50) |
| Lower GIS[b] | 69% | 0 (1–2) | 5 (2–26) | 6 (3–16) | 11 (3–39) | 31 (7–103) |
| Flatulence | 62% | 0 (1) | 1 (1–4) | 2 (1–9) | 3 (1–13) | 10 (2–64) |
| Lower abdominal bloating | 46% | 0 (0) | 1 (2–5) | 2 (1–12) | 3 (2–17) | 10 (3–49) |
| Urge to defecate | 31% | 0 (1) | 1 (2–12) | 1 (3–5) | 3 (5–16) | 7 (5–23) |
| Intestinal pain | 23% | 0 (1–2) | 1 (6) | 0 (0) | 1 (1–8) | 1 (1–8) |
| Abnormal stools[c] | 31% | 0 (0) | 2 (10–13) | 0 (0) | 2 (10–13) | 2 (10–13) |
| Nausea | 8% | 0 (0) | 0 (4) | 0 (2) | 0 (6) | 0 (6) |
| Dizziness | 15% | 0 (2) | 0 (1–3) | 0 (0) | 0 (1–3) | 1 (1–7) |
| Abdominal stitch | 15% | 0 (1) | 0 (0) | 0 (4) | 0 (1–4) | 1 (1–7) |
| Feeding tolerance | | | | | | |
| Taste fatigue | NA | 2 (4–15) | 7 (1–37) | 9 (1–40) | 19 (1–87) | NA |
| Interest in food | NA | 2 (1–13) | 3 (2–18) | 2 (3–9) | 7 (1–39) | NA |
| Interest in fluid | NA | 9 (1–32) | 7 (1–34) | 7 (5–40) | 23 (1–106) | NA |
| Tolerance to food | NA | 20 (7–40) | 17 (8–40) | 15 (5–40) | 52 (10–118) | NA |
| Tolerance to fluid | NA | 33 (13–40) | 29 (2–40) | 27 (1–40) | 90 (16–120) | NA |
| Appetite | NA | 3 (2–16) | 3 (5–16) | 3 (2–11) | 8 (4–21) | NA |
| Thirst | NA | 8 (2–29) | 8 (1–34) | 8 (1–39) | 24 (1–102) | NA |

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 (26). The maximum severity score possible for each individual GIS type for exercise was 120 (40 $h^{-1}$) and for trial total (exercise and recovery) was 240. Incidence during: Total number (%) of participants reporting GIS ≥ 1 on the mVAS for any GIS type during the 180 min of running exercise at 60% $\dot{V}O_{2max}$ in ambient conditions ($T_{amb}$ 23°C, RH 45%). First hour: GIS severity from 0 to 60 min of exercise; second hour: GIS severity from 60 to 120 min of exercise; third hour: GIS severity from 120 to 180 min of exercise; total exercise: GIS severity from 0 to 180 min of exercise; and trial total: GIS severity from 0 to 180 min plus 180 min of recovery. Overall participant reported summative accumulation of rating scale point score of measured periods and individual reported participant range ($n$ = 13).
Cohen's $\delta$: $^{*}\delta > 0.20$ small effect size, $^{**}\delta > 0.50$ moderate effect size, and $^{***}\delta > 0.80$ large effect size vs DAY.
[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, diarrhea, and/or fecal blood loss.

and RPE ($P = 0.001$) during exercise. No main effects were observed for thermal comfort ($P = 0.065$) (Fig. 2).

A trial–time interaction was observed for blood glucose responses ($P = 0.014$), whereby blood glucose concentration was significantly lower ($P < 0.05$) pre- and 1 h postexercise on DAY (4.4 mmol·$L^{-1}$, 95 CI = 4.1–4.7, and 5.5 mmol·$L^{-1}$, 95 CI = 5.2–5.9, respectively) compared with NIGHT (5.5 mmol·$L^{-1}$, 95 CI = 5.0–6.1, and 6.6 mmol·$L^{-1}$, 95 CI = 5.0–6.1, respectively), but no difference during exercise was observed (Fig. 2E). A MEOT ($P < 0.001$) was observed



Copyright © 2021 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

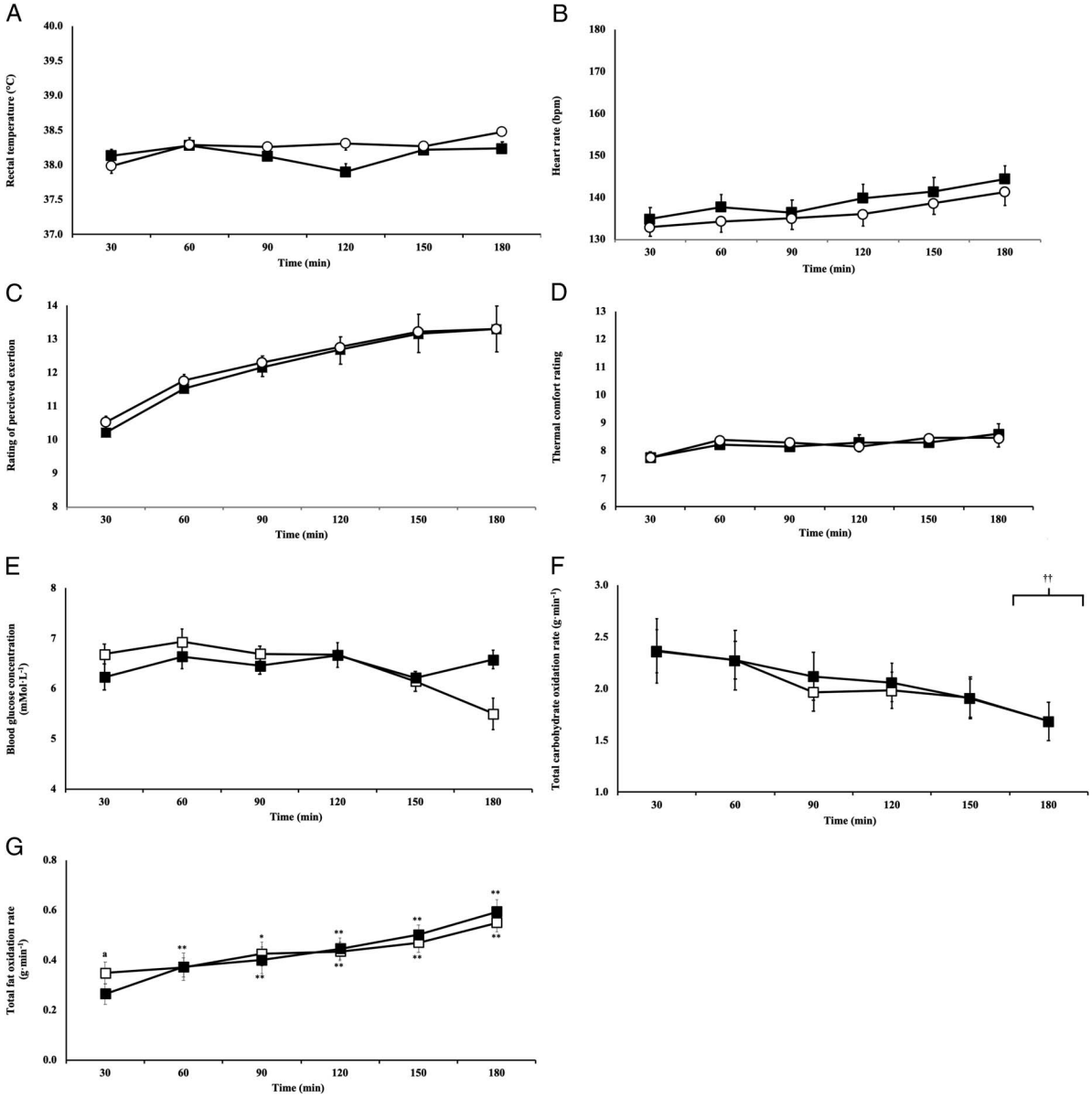


**FIGURE 2—$T_{re}$ (A), HR (B), RPE (C), thermal comfort rating (D), and blood glucose (E) responses, and total carbohydrate (F) and fat oxidation rates (G) during 3 h running at 60% $\dot{V}O_{2max}$ in ambient conditions ($T_{amb}$ 23°C, RH 45%) DAY (□) and NIGHT (■). Mean ± SEM ($n$ = 13): MEOT ††$P$ < 0.01 and †$P$ < 0.05 vs 30 min; **$P$ < 0.01 and *$P$ < 0.05 vs 30 min; and $^{a}P$ < 0.05 vs NIGHT.**

for total carbohydrate oxidation rate, which progressively decreased as exercise progressed on both trials (Fig. 2F). A trial–time interaction was observed for total fat oxidation rate ($P$ = 0.035), whereby oxidation rates increased as exercise progressed on both, but trial differences were only observed at 30 min (Fig. 2G). Plasma cortisol concentration significantly increased (trial–time, $P$ = 0.046) pre- to postexercise on NIGHT (182%) compared with DAY (4%) (Fig. 3Ai and Aii). No main effects were observed for the exercise-associated increase in plasma adrenaline (pre- to postexercise peak Δ = 0.44 nmol·L$^{-1}$, 95 CI = 0.28–0.61, 41%) and noradrenaline (3.09 nmol·L$^{-1}$, 95 CI = 1.50–4.67, 102%) concentrations.

Preexercise resting plasma I-FABP (overall mean = 1592 pg·mL$^{-1}$, 95 CI = 1221–1963), claudin-3 (18.0 ng mL$^{-1}$, 14.8–21.1), sCD14 (1.7 μg·mL$^{-1}$, 1.6–1.8), LBP (10.9 μg·mL$^{-1}$, 8.7–13.0), and cytokine concentrations (IL-1β: 2.0 pg·mL$^{-1}$, 1.0–3.0; TNF-α: 11.1 pg·mL$^{-1}$, 7.4–14.9; IL-6: 36.0 pg·mL$^{-1}$, 14.1–58.0; IL-8: 17.4 pg·mL$^{-1}$, 6.7–28.1; IL-10: 21.9 pg·mL$^{-1}$, 10.9–32.9; IL-1ra, 32.0 pg·mL$^{-1}$, 20.0–44.0) did not differ between trials. Plasma I-FABP concentration increased (MEOT, $P$ < 0.001) in response to the exercise stress (2269 pg·mL$^{-1}$, 1351–3187, 143%), but there was no trial difference (Fig. 3Bi and Bii). There were no main effects observed for plasma claudin-3 concentration (Fig. 3Ci and Cii). A trial–time interaction was observed for plasma sCD14 concentration ($P$ = 0.046), whereby peak Δ pre- to postexercise increase was greater on DAY (11.6%) compared with NIGHT (0.5%) (Fig. 3Di and Dii). A MEOT was observed for plasma LBP concentration





Copyright © 2021 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

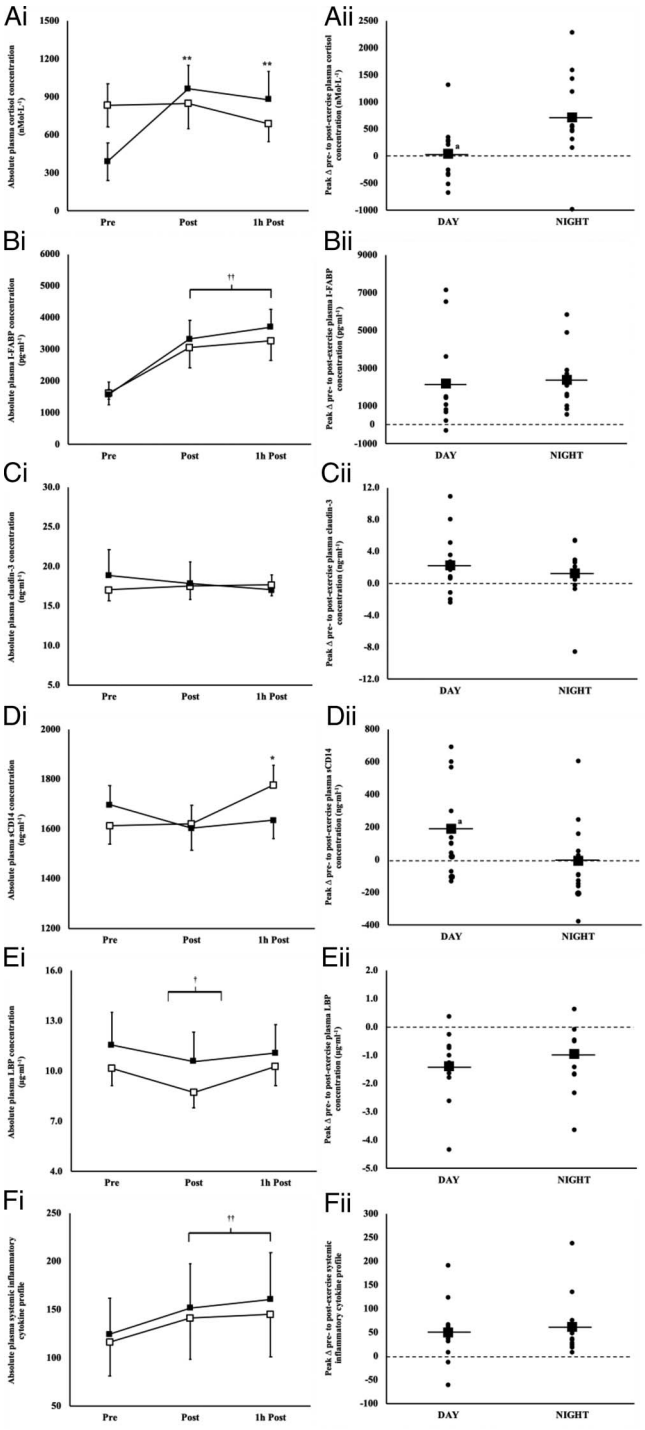


**FIGURE 3—Absolute (i) and peak magnitude of pre- to postexercise change (ii) in plasma cortisol (A), I-FABP (B), claudin-3 (C), sCD14 (D), and LBP (E) concentrations, and systemic inflammatory cytokine profile (F) in response to 3 h running at 60% $\dot{V}O_{2max}$ in ambient conditions ($T_{amb}$ 23°C, RH 45%) on DAY and NIGHT. Absolute values in mean ± SEM (DAY, □; NIGHT, ■), and magnitude response in mean (■) and individual responses (●) ($n$ = 13, except for I-FABP, LBP, and systemic inflammatory cytokine profile, $n$ = 11): MEOT ††$P$ < 0.01 and †$P$ < 0.05 vs preexercise; **$P$ < 0.01 and *$P$ < 0.05 vs preexercise; $^{a}P$ < 0.05 vs NIGHT.**

($P$ = 0.019) (Fig. 3Ei and Eii), indicative of a pre- to postexercise reduction (−11.2%) on both trials, which returned to similar baseline preexercise values by 1 h postexercise. A MEOT (pre- to postexercise increase) was observed for plasma IL-1β (34%, $P$ = 0.047), IL-8 (46%, $P$ = 0.034), and IL-1ra (40%, $P$ = 0.003) concentrations, but not for plasma TNF-α (10%, $P$ = 0.428), IL-6 (23%, $P$ = 0.069), and IL-10 (110%, $P$ = 0.061). Peak Δ pre- to postexercise for plasma IL-1β (0.7 pg mL$^{-1}$, − 0.1 to 1.4), TNF-α (1.1 pg mL$^{-1}$, −1.2 to 3.5), IL-6 (8.2 pg mL$^{-1}$, 0.9–15.5), IL-8 (7.9 pg mL$^{-1}$, 2.0–13.8), IL-10 (24.6 pg mL$^{-1}$, 3.0–46.3), and IL-1ra (12.8 pg mL$^{-1}$, 5.9–19.6) concentrations did not differ between trials. No trial difference was observed for overall pre- to postexercise (MEOT, $P$ = 0.004) systemic inflammatory cytokine profile (Fig. 3Fi and Fii).

A trial–time interaction was observed for breath $H_2$ concentration along the postexercise monitoring period ($P$ = 0.019). OCTT (breath $H_2$ turning point) was significantly delayed on NIGHT (>84 min) compared with DAY (54 (29–79) min) (Friedman test; $P$ < 0.001). Moreover, breath $H_2$ turning point was not detected in $n$ = 3 participants at NIGHT, whereas breath $H_2$ turning point was detected in all participants on DAY (Fig. 4A and B). Considering the absence of breath $H_2$ turning point in several participants by 180 min postexercise on NIGHT, the precise time quantification of OCTT was not possible for NIGHT. The incidence of GIS during running and in recovery was high in both trials; however, it was greater on NIGHT compared with DAY (Table 1). No significant difference in severity of gut discomfort and total GIS was observed between trials at rest. Severity of gut discomfort during exercise was not different between trials. Severity of total GIS ($P$ = 0.009), upper GIS ($P$ = 0.011), and lower GIS ($P$ = 0.019) were higher during running at NIGHT compared with DAY (Table 1). Two participants did not complete the NIGHT trial due to severe symptoms including nausea, projectile vomiting, and explosive bowel movements during exercise. Another participant on NIGHT experienced projectile vomiting immediately postexercise. One participant in the DAY trial did not complete the exercise protocol because of severe nausea as exercise progressed. There was no significant difference between trials for feeding tolerance markers (Table 1).

## DISCUSSION

The current study aimed to determine the effect of diurnal versus nocturnal exertional stress on gastrointestinal integrity and function, systemic LBP and sCD14 concentration (as indirect indicators of endotoxin responses), systemic inflammatory cytokine profile, GIS, and feeding tolerance. The exertional stress applied in the current study resulted in significant intestinal epithelial cell injury on both trials, but modest perturbations to plasma LBP and sCD14 concentrations and modest perturbations to the systemic inflammatory cytokine profile. In accordance with our hypothesis, nocturnal exercise (2100–0000 h) resulted in delayed OCTT and exacerbated exercise-associated GIS incidence and severity during exercise compared with diurnal exercise (0900–1200 h). The magnitude of cortisol response (i.e., indicative of stress response) on NIGHT was greater than on DAY. As such, the





Copyright © 2021 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

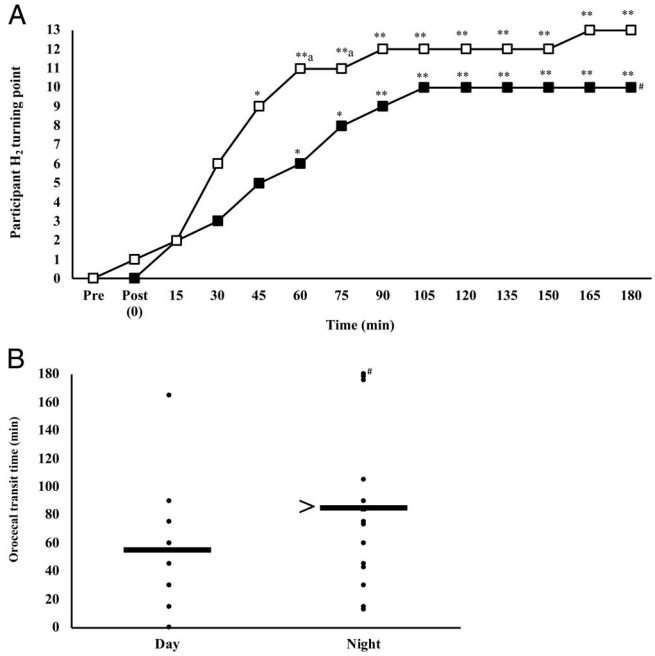


**FIGURE 4—Breath $H_2$ turning point (time interval between ingestion of lactulose and rise in breath hydrogen ($H_2$) 10 ppm, with two consecutive readings above basal) (A) and OCTT (B) in response to 3 h running at 60% $\dot{V}O_{2max}$ in ambient conditions ($T_{amb}$ 23°C, RH 45%) on DAY and NIGHT. Total participant numbers for $H_2$ turning point (DAY, □; NIGHT, ■) and individual responses (●) for OCTT ($n$ = 13): # $n$ = 3 participants failed to present a breath $H_2$ turning point within the 3-h postexercise monitoring period, > more than 84 min because three participants failed to present a breath $H_2$ turning point within the 3-h postexercise monitoring period, **$P$ < 0.01 and *$P$ < 0.05 vs 0 min; [a]$P$ < 0.05 vs NIGHT.**

substantial disturbance to the normal nocturnal circadian pattern in cortisol response may possibly account for the delayed OCTT and subsequent GIS. Contrary to our hypothesis, no substantial and consistent difference in exercise-associated perturbations to intestinal integrity (i.e., plasma I-FABP and LBP concentrations) and systemic inflammatory cytokine profile were observed between NIGHT and DAY. Collectively, these novel findings suggest that nocturnal exertion creates greater perturbations to some pathophysiological aspects of EIGS, namely, the neuroendocrine–gastrointestinal pathway, resulting in reduced gastrointestinal transit capacity and subsequent increased GIS incidence and severity. Nocturnal exertion does not appear to further exacerbate perturbations to gastrointestinal integrity and systemic responses (i.e., secondary outcomes of the circulatory–gastrointestinal pathway of EIGS).

There are numerous ultraendurance events that encompass a nocturnal segment, yet the magnitude to which circadian cycle disturbances affect the gastrointestinal markers of EIGS and GIS has largely been neglected. Considering the neuroendocrine–gastrointestinal pathway in the pathophysiology of EIGS, a key finding of the current study was a greater magnitude of rise in cortisol response (i.e., greater disturbance to normal circadian pattern in cortisol responses), but not catecholamine (i.e., adrenaline and noradrenaline) response, on NIGHT compared with DAY. With respect to the sample timelines in the current study, it is well established that resting plasma cortisol levels decrease from 2100 to 0000 h (~40%; trough at ~100 nmol·L$^{-1}$ at ~0300 h) and decrease from 0900 to 1200 h (~30%; peak at ~500 nmol·L$^{-1}$ between 0800 and 0900 h), with exercise duration and intensity dictating magnitude of responses supra ceding the daily circadian variation (21,36,40–42). This suggests that the greater HPA axis, but not SAM axis, activation with prolonged nocturnal exercise (e.g., 182% increase plasma cortisol concentration, at a point whereby circadian variation is inducing a decrease from 2100 to 0000 h) may account for the observed gastrointestinal function impairment and GIS, and also higher preexercise and recovery period blood glucose concentration and higher total fat oxidation rate on NIGHT compared with DAY circadian differences in metabolic profile in response to exercise. By contrast, the blunted plasma cortisol concentration on DAY appears to be associated with substantial reduction in resting plasma cortisol concentration observed from peak concentrations at 0800 to 0900 h, in conjunction with the prolonged steady-state exercise load along this time line (2,3,42). Nevertheless, when corrected for circadian variation previously reported in experimental models measuring resting plasma cortisol concentrations at the relative sample time points (2,3,42), NIGHT (Δ pre- to postexercise, ~780 nmol·L$^{-1}$) clearly resulted in greater HPA axis activation over DAY (~150 nmol·L$^{-1}$). Furthermore, the plasma cortisol responses from the current study is in accordance with a previous study of trained male participants exposed to 30 min of treadmill running at ~85% $\dot{V}O_{2max}$, reporting a greater magnitude of peak Δ in circulating cortisol levels with nocturnal exertion compared with different times of day (0000 h, 600%; 1900 h, 200%; and 0700 h, 150%) (43).

Abnormal and/or superimposed stress hormone response respective to the daily period of exercise may result in perturbed physiological functions (2,3). For example, the stress hormone





Copyright © 2021 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

activation of the pathological neuroendocrine–gastrointestinal pathway of EIGS likely explains the reduced gastrointestinal functional capacity observed (i.e., >30 min delayed OCTT on NIGHT vs DAY). Although there appears to exist a large individual variation in responses, breath $H_2$ turning point was observed in all participants on DAY along the 3-h postexercise monitoring period, whereas three participant failed to present a breath $H_2$ turning point on NIGHT. The reduced gastrointestinal transit capacity may be resultant of potential suppression of the myenteric and submucosal plexus activity through the HPA axis with or without SAM axis contribution, the exercise stress–associated reduction in gastrointestinal smooth muscle activating interstitial cells (e.g., cells of Cajal), and/or expression of other neurohormonal inhibitors of gastrointestinal smooth muscle contraction (e.g., glucagon-like peptide-1, γ-aminobutyric acid, and/or peptide YY) (44–46). The myenteric and submucosal plexus consists of numerous neuron clusters responsible for peristaltic activity and digestive secretion and nutrient absorption, respectively, whereas smooth muscle interstitial cell can independently regulate smooth muscle contractile properties (44). Considerations of the gastrointestinal tract myoelectrical activity composed of gastric slow waves and spike/second potentials, alterations in contraction frequencies, and antral area are other mechanisms involved in regulating gastric emptying and influencing intestinal transit (47). The role that these mechanisms have in determining the gastrointestinal transit observed in the current study was not determined and warrants future exploration, potentially by inclusion of electrogastrography techniques. In addition, potential strategies, previously established or novel approaches, to manage GIS through maintaining gastrointestinal functional patency during nocturnal exercise, such as gut and/or exercise training at night or combined dietary approach (i.e., manipulating residue, fiber, and FODMAP content of preexercise foods, e.g., 24 h prior), warrants further exploration.

High GIS incidence was reported on DAY and NIGHT, which is consistent with similar duration and intensity exercise protocols (21,23), suggesting exercise *per se* induces GIS incidence via EIGS pathways. However, other factors, such as circadian variation in HPA axis and/or differences in food provisions (i.e., quantity, quality, and timing) in the hours leading up to the diurnal or nocturnal exercise, may exacerbate GIS severity. Interestingly, GIS incidence and severity increase from the first to the second hour of exercise but plateaued in the third hour, likely associated with the *ad libitum* water intake in the third hour and subsequent voluntary reduced total intake volume (i.e., 276 mL on DAY and 262 mL on NIGHT) to support customization to gut comfort. These outcomes confirm previous findings from exertional and exertional-heat stress experimental protocols (32,33,36). However, an overall higher incidence and severity of total, upper (e.g., belching and upper abdominal bloating), and lower GIS (e.g., flatulence and urge to defecate) and nausea were exacerbated on NIGHT. On a concerning note, two participants experienced severe uncontrollable projectile vomiting and one experienced rapid onset explosive diarrhea on NIGHT, but not on DAY; thus, they were not included in the study's variable data analysis (i.e., noncompleters). Because of the observed delay in OCTT on NIGHT, the first 2 h of carbohydrate feeding (i.e., males 1.0 $g \cdot kg^{-1}$ $BM \cdot h^{-1}$ and females 0.8 $g \cdot kg^{-1}$ $BM \cdot h^{-1}$) may have exacerbated higher levels of postprandial intragastric and intraintestinal volume and pressure, promoting the ileal brake, and inducing both upper and lower GIS (21,23), compared with any exercise-associated function impairment on DAY. The during exercise feeding regime was used to support exercise completion, in accordance with guidelines and recommendations for the target population. In addition the feeding regime was aimed at avoiding artifact GIS from using previously proposed high concentration multiple transportable carbohydrate intakes during exercise up to 3 h (e.g., 90 $g \cdot h^{-1}$). These proposed high carbohydrate concentrations are notorious for inducing GIS in the current study's participant population, associated with feeding intolerance and ingested carbohydrate concentrations above total carbohydrate oxidation rates (21,23,48,49). It is important to highlight that the current study design focused on recreational level ultraendurance and endurance runners, and running speeds aligned with this population. Recreational runners make up the majority of ultraendurance and endurance running event participation and present high reported incidence of GIS associated with intolerance to feeding high carbohydrate doses during exercise (9,19,20,49,50). However, the study outcome may not be reflective of more well-trained and/or elite level ultraendurance and endurance runners, who present greater feeding tolerance during exercise and reports fewer GIS incidence during event participation, with aligned faster running speeds and sustained high exercise intensity (21,22,51). Moreover, the effect of the observed gastrointestinal functional outcomes, and the effect on GIS and feeding tolerance, in response to NIGHT versus DAY exercise has not been explored in other ultraendurance and endurance modalities that pertain a nocturnal element (e.g., ultraendurance cycling, adventure racing, motocross activities, open water kayaking, and/or rowing), which warrant further investigation and exploration.

From a practical perspective, compromised gastrointestinal function during nocturnal exercise presents a challenge to optimal food and fluid provisions (i.e., fuelling and hydration) during exercise, especially ultraendurance activities (50). First, it has the potential to limit and/or stop (i.e., gastroparesis with or without paralytic ileus) fuelling during prolonged nocturnal exercise requiring exogenous energy substrate provisions. Second, although $T_{amb}$ during nocturnal exertion cannot be considered exertional-heat stress (i.e., attainment of core body temperature ≥39.0°C), the duration of activity (e.g., ultraendurance) may be sufficient to compromise total body water if water provisions are insufficient. Such compromised gastrointestinal function may burden water bioavailability leading to substantial hypohydration, despite athletes' "interest and motivation to drink," and further compromise gastrointestinal functional responses and nutrient availability (e.g., greater malabsorption potentially associated with reduced nutrient digestion and absorption) as a result of hypohydration (36). It is also important to





Copyright © 2021 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

consider in the current study food and fluid provisions in the hours leading into diurnal and nocturnal exercise differed (Fig. 1). For example, overnight fast plus small breakfast 2 h before a 0900 h exercise start in the postprandial state in diurnal exercise, versus a full day of food and fluid provision before a 2100 h exercise start in the postprandial state in nocturnal exercise. Such differences in food–fluid intake behaviors and greater food–fluid volume intake before nocturnal exercise may inadvertently burden the gastrointestinal tract through greater content transit. This possibly provides another explanation for the greater GIS incidence and severity on NIGHT compared with DAY. However, the dietary control (i.e., provisions of food quantity, quality, and timing; Fig. 1) in the current experimental design attempted to limit any confounding factors associated with preexercise intake, highlighting the importance of preexercise dietary control to standardize experimental procedures during research, and/or preexercise dietary intervention to prevent or manage GIS incidence and severity during training and competition in susceptible athlete populations.

The exercise-associated intestinal injury (i.e., Δ pre- to postexercise plasma I-FABP concentration, 2269 $pg \cdot mL^{-1}$; 143%) as a result of 3 h running at 60% $\dot{V}O_{2max}$, even with carbohydrate feeding in the first 2 h, was greater than previous shorter exercise protocols (i.e., 2 h running at 60% or 70% $\dot{V}O_{2max}$) with (≥35.0°C) and without (~ 20.0°C) heat exposure (I-FABP, ≤1000 $pg \cdot mL^{-1}$) (25,31–33) and 3 h running protocol with greater carbohydrate provision (90 $g \cdot h^{-1}$ 2:1 glucose–fructose, 10% *w/v*) in the first 2 h (I-FABP, ≤1000 $pg \cdot mL^{-1}$) (21). The mechanistic explanation for the exacerbated intestinal injury with exercise duration is likely linked to the prolonged splanchnic hypoperfusion exposure as part of the circulatory–gastrointestinal pathway of EIGS (10,11) and removal of carbohydrate provision in the third hour of exercise (21). However, the exercise stress model used did not result in any substantial change (Δ pre- to postexercise, 1.7 $ng \cdot mL^{-1}$; +10%) in plasma claudin-3 concentration, a surrogate marker of epithelial tight-junction protein complex damage and/or dysregulation of intestinal permeability (e.g., intestinal hyperpermeability). These outcomes suggest that 1) prolonged steady-state endurance exercise in temperate ambient conditions can induce substantial intestinal epithelial cell injury, 2) nocturnal exercise does not further exacerbate intestinal epithelial injury, and 3) tight-junction protein complexes appear more resistant to perturbation than epithelial cell membranes to exercise-associated splanchnic hypoperfusion and sympathetic drive—primary causal mechanisms of EIGS. These findings support previous exertional and exertional-heat stress experimental protocols that observed substantial increases in intestinal epithelial cell injury (i.e., plasma I-FABP concentration), without changes in intestinal permeability determined by lactulose–rhamnose dual sugars challenge (32,33,52). Thus, the modest disturbance to plasma sCD14 and LBP concentrations (i.e., Δ pre- to postexercise for sCD14: 89.7 $ng \cdot mL^{-1}$, 5.6%; and LBP: −1197 $ng \cdot mL^{-1}$, −11.0%) and systemic inflammatory cytokine profile (55.3 arbitrary units, 31.4%) observed in both trials is likely due to both epithelial cell injury resulting in barrier breakage and hyperpermeability-associated translocation due to epithelial tight-junction functional dysregulation (e.g., gap width size and hyperpermeability duration) rather than epithelial tight-junction protein complex structural breakage or damage linked dysregulation.

The application of surrogate markers plasma LBP and sCD14 responses as an indirect indicator for assessing the magnitude of bacterial endotoxin luminal to systemic translocation in response to exercise is a generally novel approach within exercise gastroenterology research. A small number of recent studies have used such markers in replacement of LAL gram-negative plasma endotoxin detection kits (25,36). This analytical transition is due to 1) limitations associated with narrow scope of direct endotoxin detection with currently available measurement kits, 2) environmental and contact contamination factors during sample handling and analysis procedures, 3) need for antiendotoxin antibodies (e.g., IgA, IgM, and/or IgG) adjunct analysis and interpretation, and 4) consistent immune and hepatic surveillance and clearance confounding factors (9,10). Considering that sCD14 acts more broadly than plasma LBP in identifying pathogen-associated molecular patterns, it is not surprising that there is a consistent increase in sCD14 (e.g., expressed in response to general bacterial ligand detection) in proportion to the exertional stress model, peaking immediately to 1 h postexercise and returning to baseline thereafter (9,25,36). Although postexercise decreases in plasma LBP have also been observed, potentially associated with baseline concentration at the onset of exercise and the utilization rate (i.e., lipopolysaccharide binding and TLR-4 presentation) in response during exercise surpasses *de novo* synthesis rates until the postexercise recovery period (9,25,36). Similarly, exercise-induced changes in plasma LBP appear to reach peak change immediately to 1 h postexercise and returning to baseline thereafter (9,25). In the current study, it is not clear why DAY resulted in a significantly higher sCD14 response to the exertional stress but suggests a difference in broad-spectrum bacterial pathogenic endotoxin epithelial translocation, above solely lipopolysaccharide endotoxin epithelial translocation. Nevertheless, these results show that direct (e.g., LPS) and indirect (e.g., sCD14, LBP, and antiendotoxin antibodies) systemic endotoxin biomarkers respond differently to exertional stress, with inclusive exacerbation factors (e.g., circadian variation). This highlights the importance and need to assess a cluster of endotoxin profile biomarkers (e.g., LAL end point gram-negative endotoxin, antiendotoxin antibodies [IgA, IgG, and/or IgM], sCD14, and LBP), and not merely selecting one option, to avoid results misinterpretation and misinformed translation in professional practice. In addition, despite no significant differences being observed in preexercise plasma LBP and sCD14 response biomarkers between DAY and NIGHT, the outcomes reported highlight the need to assess the 24-h circadian variation in resting values of these markers, which present a substantial gap in the current exercise gastroenterology literature.





Copyright © 2021 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

The systemic inflammatory cytokine profile as a result of 3 h running at 60% $\dot{V}O_{2max}$ and plasma LBP and sCD14 absolute values and response magnitude (Fig. 3) are indicative and represent plasma endotoxin concentration <10 pg·mL$^{-1}$ as a result of exercise-associated bacterial translocation previously reported (31–33,37). These changes are modest in nature, of no clinical relevance, and unlikely to have contributed to the GIS because the circulatory–gastrointestinal pathway of EIGS is synonymously asymptomatic in the acute phase of exertional stress (11). The current study observed a classic exercise-associated profile with proinflammatory cytokines IL-1β and TNF-α responding minimally (Δ pre- to postexercise overall mean < 2 pg·mL$^{-1}$), response cytokines IL-6 and IL-8 responding modestly (<10 pg·mL$^{-1}$), and anti-inflammatory cytokines showing the greatest response in the postexercise recovery period (<30 pg·mL$^{-1}$), which is consistent with previous research (25,31–33,36,37,39). These outcomes highlight two factors: 1) it is erroneous to measure a selected few inflammatory cytokine markers, especially proinflammatory and response cytokines, which is notorious in sport and exercise research; and 2) in respect to exercise load, only in extreme exercise situations (e.g., ultraendurance activities) will systemic endotoxemia and cytokinemia reach clinical significance warranting medical attention (38). It is however acknowledged that such proposed systemic septic and inflammatory status is well reported in the clinical, public, and occupational health literature, for example, in the case of heat stroke pathophysiology (53–56). Nevertheless, future exercise gastroenterology research needs to be mindful on the selected exercise load for the experimental design, ensuring sufficient exertional stress is used to reach a minimal gastrointestinal perturbation criteria.

In conclusion, time of day appears to play a role in the magnitude of gastrointestinal disturbance in response to exertional stress. Nocturnal exercise has the potential to result in greater gastrointestinal transit disturbance compared with diurnal exercise, most likely due to exacerbated neuroendocrine–gastrointestinal pathway of EIGS. Consequently, nocturnal exercise appears to present a greater risk for GIS incidence and severity, which potentially have performance and clinical implications, over diurnal exercise. Moreover, the time of day that prolonged strenuous exercise is performed does not further exacerbate exercise-associated perturbations to gastrointestinal integrity and systemic responses, such issues appear to be exercise load dependant.

The study was supported by a 2019 Ultra Sports Science Foundation grant.
The authors thank all the participants that volunteered to take part in this study. The authors' contributions are as follows: R. C. was the chief investigator of this research. R. C. and S. G. contributed toward the original research idea and development of the experimental design. R. C., S. G., C. R., and A. P. contributed to the various aspects of data collection, sample collection, and analysis. R. C. and S. G. contributed to the analysis of the raw data. S. G. and R. C. contributed to the draft preparation of the manuscript. All authors reviewed and approved the final manuscript. The study was supported by Monash University, Faculty of Medicine Nursing and Health Sciences, Department of Nutrition, Dietetics and Food, Be Active Sleep Exercise (BASE) Facility. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, and/or inappropriate data manipulation. The authors also declare that the results of the present study do not constitute endorsement by the American College of Sports Medicine. All authors declare no other conflicts of interest.



## REFERENCES

1. Knoth C, Knechtle B, Rüst CA, Rosemann T, Lepers R. Participation and performance trends in multi-stage ultra-marathon—the 'Marathon des sables' 2003–2012. *Extrem Physiol Med*. 2012;1:1–11.
2. Costa RJ, Smith AH, Oliver SJ, et al. The effects of two nights of sleep deprivation with or without energy restriction on immune indices at rest and in response to cold exposure. *Eur J Appl Physiol*. 2010; 109:417–28.
3. Costa RJS, Cartner L, Oliver SJ, et al. No effect of a 30 h period of sleep deprivation on selected immune responses to exercise. *Eur J Appl Physiol*. 2008;105:499–504.
4. Oliver SJ, Costa RJS, Laing SJ, Bilzon JLJ, Walsh NP. One night of sleep deprivation decreases treadmill endurance performance. *Eur J Appl Physiol*. 2009;107:155–61.
5. Oliver SJ, Harper Smith AD, Costa RJS, et al. Two nights of sleep deprivation with or without energy restriction does not impair the thermal response to cold. *Eur J Appl Physiol*. 2015;115:2059–68.
6. Born J, Lange T, Hansen K, Mölle M, Fehm HL. Effects of sleep and circadian rhythm on human circulating immune cells. *J Immunol*. 1997;158:4454–64.
7. Banks S, Dinges DF. Behavioral and physiological consequences of sleep restriction. *J Clin Sleep Med*. 2007;3:519–28.
8. Dinges DF, Douglas SD, Zaugg L, et al. Leukocytosis and natural killer cell function parallel neurobehavioral fatigue induced by 64 hours of sleep deprivation. *J Clin Invest*. 1994;93:1930–9.
9. Costa RJS, Gaskell SK, McCubbin AJ, Snipe RMJ. Exertional-heat stress-associated gastrointestinal perturbations during Olympic sports: management strategies for athletes preparing and competing in the 2020 Tokyo Olympic Games. *Temperature (Austin)*. 2019;7: 58–88.
10. Costa RJS, Snipe RMJ, Kitic CM, Gibson PR. Systematic review: exercise-induced gastrointestinal syndrome—implications for health and intestinal disease. *Aliment Pharmacol Ther*. 2017;46:246–65.
11. Hoogerwerf WA. Role of clock genes in gastrointestinal motility. *Am J Physiol Gastrointest Liver Physiol*. 2010;299:G549–55.
12. Konturek PC, Brzozowski T, Konturek SJ. Gut clock: implication of circadian rhythms in the gastrointestinal tract. *J Physiol Pharmacol*. 2011;62:139–50.
13. Goo RH, Moore JG, Greenberg E, Alazraki NP. Circadian variation in gastric emptying of meals in humans. *Gastroenterology*. 1987;93:515–8.
14. Lindberg G, Iwarzon M, Hammarlund B. 24-hour ambulatory electrogastrography in healthy volunteers. *Scand J Gastroenterol*. 1996;31:658–64.
15. Bailey SM, Udoh US, Young ME. Circadian regulation of metabolism. *J Endocrinol*. 2014;222:R75–96.
16. Bron R, Furness JB. Rhythm of digestion: keeping time in the gastrointestinal tract. *Clin Exp Pharmacol Physiol*. 2009;36:1041–8.
17. Knutsson A, Boggild H. Gastrointestinal disorders among shift workers. *Scand J Work Environ Health*. 2010;36:85–95.
18. Zhen Lu W, Ann Gwee K, Yu Ho K. Functional bowel disorders in rotating shift nurses may be related to sleep disturbances. *Eur J Gastroenterol Hepatol*. 2006;18:623–7.
19. Costa RJS, Gill SK, Hankey J, Wright A, Marczak S. Perturbed energy balance and hydration status in ultra-endurance runners during a 24 h ultra-marathon. *Br J Nutr*. 2014;112:428–37.
20. Costa RJS, Swancott AJM, Gill S, et al. Compromised energy and macronutrient intake of ultra-endurance runners during a multi-stage ultra-marathon conducted in a hot ambient environment. *Int J Sports Sci*. 2013;3:51–62.



Copyright © 2021 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

21. 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;42:547–57.
22. 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.
23. Miall A, Khoo A, Rauch C, et al. Two weeks of repetitive gut-challenge reduce exercise-associated gastrointestinal symptoms and malabsorption. *Scand J Med Sci Sports*. 2018;28:630–40.
24. Terman M, Terman JS. Light therapy for seasonal and nonseasonal depression: efficacy, protocol, safety, and side effects. *CNS Spectr*. 2005;10:647–63.
25. Gaskell SK, Taylor B, Muir J, Costa RJS. 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.
26. 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;29:411–9.
27. Alcock R, McCubbin A, Camões-Costa V, Costa RJS. Case study: providing nutritional support to an ultraendurance runner in preparation for a self-sufficient multistage ultramarathon: rationed versus full energy provisions. *Wilderness Environ Med*. 2018;29:508–20.
28. Snipe RMJ, Costa RJS. Does the temperature of water ingested during exertional-heat stress influence gastrointestinal injury, symptoms, and systemic inflammatory profile? *J Sci Med Sport*. 2018;21:771–6.
29. Flood TR, Montanari S, Wicks M, et al. Addition of pectin-alginate to a carbohydrate beverage does not maintain gastrointestinal barrier function during exercise in hot-humid conditions better than carbohydrate ingestion alone. *Appl Physiol Nutr Metab*. 2020;45(10):1145–55.
30. Jonvik KL, Lenaerts K, Smeets JSJ, Kolkman JJ, van Loon LJC, Verdijk LB. Sucrose but not nitrate ingestion reduced strenuous cycling-induced intestinal injury. *Med Sci Sport Exerc*. 2019;51:436–44.
31. Snipe RMJ, Khoo A, Kitic CM, Gibson PR, Costa RJS. Carbohydrate and protein intake during exertional heat stress ameliorates intestinal epithelial injury and small intestine permeability. *Appl Physiol Nutr Metab*. 2017;42:1283–92.
32. Snipe R, Khoo A, Kitic C, Gibson P, Costa RJS. Heat stress during prolonged running results in exacerbated intestinal epithelial injury and gastrointestinal symptoms. *Euro J Appl Physiol*. 2018;118:389–400.
33. Snipe R, Khoo A, Kitic C, Gibson P, Costa RJS. Mild heat stress during prolonged running results in exacerbated intestinal epithelial injury and gastrointestinal symptoms. *Int J Sports Med*. 2018;39:255–63.
34. Bate JP, Irving PM, Barrett JS, Gibson PR. Benefits of breath hydrogen testing after lactulose administration in analysing carbohydrate malabsorption. *Eur J Gastroenterol Hepatol*. 2010;22:318–26.
35. Péronnet F, Massicotte D. Table of nonprotein respiratory quotient: an update. *Can J Sport Sci*. 1991;16:23–9.
36. Costa RJS, Camões-Costa V, Snipe RMJ, Dixon D, Russo I, Huschtscha Z. Impact of exercise-induced hypohydration on gastrointestinal integrity, function, symptoms, and systemic endotoxin and inflammatory profile. *J Appl Physiol (1985)*. 2019;126:1281–91.
37. Gill SK, Allerton DM, Ansley-Robson P, Hemming K, Cox M, Costa RJS. Does acute high dose probiotic supplementation containing *lactobacillus casei* attenuate exertional-heat stress induced endotoxaemia and cytokinaemia? *Int J Sports Nutr Exerc Metab*. 2016;26:268–75.
38. Gill SK, Teixeira A, Rama L, et al. Circulatory endotoxin concentration and cytokine profile in response to exertional-heat stress during a multi-stage ultra-marathon competition. *Exerc Immunol Rev*. 2015; 21:114–28.
39. Bennett C, Henry R, Snipe SMJ, Costa RJS. Is the gut microbiota bacterial abundance and composition influence the magnitude of intestinal integrity perturbations, systemic cytokinaemia, and gastrointestinal symptoms in response to exertional-heat stress? *J Sci Med Sport*. 2020;S1440-2440(20):30662–9.
40. Costa RJ, Oliver SJ, Laing SJ, et al. Influence of timing of postexercise carbohydrate-protein ingestion on selected immune indices. *Int J Sport Nutr Exerc Metab*. 2009;19:366–284.
41. Costa RJS, Walters R, Bilzon JLJ, Walsh NP. Effects of immediate post-exercise carbohydrate ingestion with and without protein on neutrophil degranulation. *Int J Sport Nutr Exerc Metab*. 2011;21:205–13.
42. Wright KP Jr, Drake AL, Frey DJ, et al. Influence of sleep deprivation and circadian misalignment on cortisol, inflammatory markers, and cytokine balance. *Brain Behav Immun*. 2015;47:24–34.
43. Kanaley JA, Weltman JY, Pieper KS, Weltman A, Hartman ML. Cortisol and growth hormone responses to exercise at different times of day. *J Clin Endocrinol Metab*. 2001;86:2881–9.
44. Hirst GD, Ward SM. Interstitial cells: involvement in rhythmicity and neural control of gut smooth muscle. *J Physiol*. 2003;550(Pt 2):337–46.
45. Layer P, Peschel S, Schlesinger T, Goebell H. Human pancreatic secretion and intestinal motility: effects of ileal nutrient perfusion. *Am J Physiol Gastrointest Liver Physiol*. 1990;258:G196–201.
46. Shin HS, Ingram JR, McGill AT, Poppitt SD. Lipids, CHOs, proteins: can all macronutrients put a 'brake' on eating? *Physiol Behav*. 2013;120:114–23.
47. Lu CL, Shidler N, Chen JD. Enhanced postprandial gastric myoelectrical activity after moderate-intensity exercise. *Am J Gastroenterol*. 2000;95:425–31.
48. Burke LM, Castell LM, Casa DJ, et al. International Association of Athletics Federations Consensus statement 2019: nutrition for athletics. *Int J Sport Nutr Exerc Metab*. 2019;29:73–84.
49. Costa RJS, Knechtle B, Tarnopolsky M, Hoffman MD. Nutrition for ultramarathon running: trail, track, and road. *Int J Sport Nutr Exerc Metab*. 2019;29:130–40.
50. Costa RJS, Hoffman MD, Stellingwerff T. Considerations for ultraendurance activities. Part 1: nutrition. *Res Sports Med*. 2019;27:166–81.
51. Pfeiffer B, Stellingwerff T, Hodgson AB, et al. Nutritional intake and gastrointestinal problems during competitive endurance events. *Med Sci Sports Exerc*. 2012;44:344–51.
52. March DS, Marchbank T, Playford RJ, Jones AW, Thatcher R, Davison G. Intestinal fatty acid-binding protein and gut permeability responses to exercise. *Eur J Appl Physiol*. 2017;117:931–41.
53. Caradonna L, Amati L, Magrone T, et al. Enteric bacteria, lipopolysaccharides and related cytokines in inflammatory bowel disease: biological and clinical significance. *J Endotoxin Res*. 2000;6:205–14.
54. Hodgin KE, Moss M. The epidemiology of sepsis. *Curr Pharm Des*. 2008;14:1833–9.
55. Lim CL. Heat sepsis precedes heat toxicity in the pathophysiology of heat stroke—a new paradigm on an ancient disease. *Antioxidants (Basel)*. 2018;7:149.
56. Opal SM. Endotoxins and other sepsis triggers. *Contrib Nephrol*. 2010;167:14–24.





Copyright © 2021 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.