Diurnal versus Nocturnal Exercise—Effect on the Gastrointestinal Tract

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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% VO2max (22.7°C, 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 (H2) 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 H2 responses (P = 0.019) showed that OCTT was significantly delayed on NIGHT (>84 min, with n = 3 showing no breath H2 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

Ultradistance 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
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% VO2max in ≥35.0°C ambient temperature (Tamb) or ≥3 h at 60% VO2max 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 anecdotaly 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%, VO2max = 52 ± 7 mL·kg⁻¹·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.
All participants provided written informed consent, which received approval from the local ethics committee (MUTHR 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 prettrial 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 accelerometer (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 accelerometer.

Participants were asked to void before nude body mass measurement, provide a breath sample into a 250-mL breath collection bag (Wagner Analyses 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 K₂EDTA (4 mL, 1.6 mg mL⁻¹ EDTA) vacutainers. Rectal temperature (Tₑₛ) 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% VO₂max in temperate ambient conditions (DAY, Tₑₛ = 23.1°C ± 1.2°C and 43.6% ± 5.5% relative humidity [RH]; NIGHT, Tₑₛ = 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 artefact 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 (H₂) 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 H₂ 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). Tₑₛ, 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 VO₂, carbon dioxide production (VCO₂), and respiratory exchange ratio for 5 min continuously every 30 min during exercise. Total nonprotein carbohydrate and fat.
Exercise-induced body mass loss did not differ between trials (overall mean = 1.1%, 95 CI = 0.9–1.3). Pre- and postexercise \( P_{\text{osmol}} \) 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). \( \Delta \) pre- to postexercise \( P_{\text{V}} \) (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 \( T_{\text{re}} \) (\( P < 0.001 \)), HR (\( P < 0.001 \)),
and RPE ($P = 0.001$) during exercise. No main effects were observed for thermal comfort ($P = 0.065$) (Fig. 2).

A trial–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
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 $\Delta = 0.44 \text{ nmol} \cdot \text{L}^{-1}$, 95 CI = 0.28–0.61, 41%) and noradrenaline (3.09 nmol·L$^{-1}$, 95 CI = 1.6–1.8), 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 $\Delta$ 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...
(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
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% V\(\dot{O}_2\)max, reporting a greater magnitude of peak \(\Delta\) 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).
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 H2 turning point was observed in all participants on DAY along the 3-h postexercise monitoring period, whereas three participant failed to present a breath H2 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·kg⁻¹·BM·h⁻¹ and females 0.8 g·kg⁻¹·BM·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·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 in the current study’s participant population, associated with feeding intolerance and gastrointestinal function 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 $T_{\text{amb}}$ 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
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 population.

The exercise-associated intestinal injury (i.e., Δ pre- to post-exercise plasma I-FABP concentration, 2269 pg·mL$^{-1}$; 143%) as a result of 3 h running at 60% VO$_{2\text{max}}$ 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% VO$_{2\text{max}}$ with (≥35.0°C) and without (~20.0°C) heat exposure (I-FABP, ≤1000 pg·mL$^{-1}$) (25,31–33) and 3 h running protocol with greater carbohydrate provision (90 g·h$^{-1}$: 2:1 glucose–fructose, 10% w/v) in the first 2 h (I-FABP, ≤1000 pg·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·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·mL$^{-1}$, 5.6%; and LBP: −1197 ng·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.
The systemic inflammatory cytokine profile as a result of 3 h running at 60% VO$_{2\text{max}}$ 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 synchronously 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 dependent.

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