International Journal of Sport Nutrition and Exercise Metabolism, 2023, 33, 305-315
https://doi.org/10.1123/ijsnem.2023-0009

Human Kinetics
ORIGINAL RESEARCH

The Impact of a Short-Term Ketogenic Low-Carbohydrate High-Fat Diet on Biomarkers of Intestinal Epithelial Integrity and Gastrointestinal Symptoms

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Endurance exercise can disturb intestinal epithelial integrity, leading to increased systemic indicators of cell injury, hyperpermeability, and pathogenic translocation. However, the interaction between exercise, diet, and gastrointestinal disturbance still warrants exploration. This study examined whether a 6-day dietary intervention influenced perturbations to intestinal epithelial disruption in response to a 25-km race walk. Twenty-eight male race walkers adhered to a high carbohydrate (CHO)/energy diet (65% CHO, energy availability = 40 kcal·kg FFM^{-1}·day^{-1}) for 6 days prior to a Baseline 25-km race walk. Athletes were then split into three subgroups: high CHO/energy diet ($n = 10$); low-CHO, high-fat diet (LCHF: $n = 8$; <50 g/day CHO, energy availability = 40 kcal·kg FFM^{-1}·day^{-1}); and low energy availability ($n = 10$; 65% CHO, energy availability = 15 kcal·kg FFM^{-1}·day^{-1}) for a further 6-day dietary intervention period prior to a second 25-km race walk (Adaptation). During both trials, venous blood was collected pre-, post-, and 1 hr postexercise and analyzed for markers of intestinal epithelial disruption. Intestinal fatty acid-binding protein concentration was significantly higher (twofold increase) in response to exercise during Adaptation compared to Baseline in the LCHF group ($p = .001$). Similar findings were observed for soluble CD14 ($p < .001$) and lipopolysaccharide-binding protein ($p = .003$), where postexercise concentrations were higher (53% and 36%, respectively) during Adaptation than Baseline in LCHF. No differences in high CHO/energy diet or low energy availability were apparent for any blood markers assessed ($p > .05$). A short-term LCHF diet increased intestinal epithelial cell injury in response to a 25-km race walk. No effect of low energy availability on gastrointestinal injury or symptoms was observed.

Keywords: LEA, I-FABP, bacterial endotoxin, FODMAP

Elite athletes place importance on nutrition practices that support their exercise energy expenditure (EEE) and muscle fuel needs (Thomas et al., 2016). Early sports nutrition guidelines promoted the universal and static application of a high carbohydrate (CHO) diet to achieve chronic restoration of muscle glycogen and exogenous CHO fuels for exercise (Coyle, 1991). However, contemporary recommendations recognize that the goals and fuel costs of individual training sessions and events vary between and within athletes (Burke et al., 2018; Thomas et al., 2016). Nevertheless, when training quality or competition performance is required, endurance athletes are guided to achieve "high CHO availability" (matching their finite body CHO stores to session fuel needs) by consuming CHO before, during, and between sessions (Burke et al., 2018).

An alternative approach to fueling endurance exercise is to maximize the rate of fat oxidation via adaptation to a ketogenic low-CHO, high-fat (LCHF) diet. Interest in this strategy originated in the early 1980s (Phinney et al., 1983) but has only been investigated more thoroughly recently (Burke et al., 2017, 2020; Shaw et al., 2019). Since even the leanest athlete has an abundance of endogenous lipid stores in comparison to their limited CHO reserves, there is theoretical merit in increasing the contribution of fat to muscle fuel by upregulating the various components that control fat storage, transport, and utilization (Burke, 2021; Volek et al., 2015). For athletes undertaking endurance-based exercise, particularly in ultraendurance events, a LCHF diet may facilitate extended durations of athletic performance without the need for frequent ingestion of CHO-based fuels (Volek et al., 2015).

Regardless of the prioritization of muscle fuel sources, the achievement of daily energy requirements is an important nutritional consideration underpinning athlete health and performance. Energy availability (EA) refers to the difference between dietary energy intake and EEE, relative to fat-free mass (FFM), and represents the amount of energy that can be partitioned to all other body functions (Loucks, 2004). Some scenarios of exposure to low EA (LEA) are associated with benign and reversible changes to

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body system biomarkers, representing adaptive energy partitioning and physiological plasticity in humans (Shirley et al., 2022). Indeed, short-term purposeful manipulations in EA, via reduced dietary intake or increased training volume, are not uncommon for athletes and can result in favorable alterations to body composition and performance (Stellingwerff, 2018). However, some exposure to LEA is problematic because it is associated with a variety of impairments of health and performance outcomes, collectively known as relative energy deficiency in sport (REDs; Mountjoy et al., 2023). The characteristics of problematic LEA are not completely clear or universal but are presumed to involve prolonged, severe mismatches between energy intake and EEE (Lieberman et al., 2018). The majority of controlled prospective investigations of short-term periods of LEA exposure have occurred in females (Loucks & Thuma, 2003; Papageorgiou et al., 2017, 2018), and studies in males have been fewer in number and less clear in showing negative outcomes (Koehler et al., 2016; Papageorgiou et al., 2017).

While dietary intake sets the maximal opportunity for fuel and energy intake, the gastrointestinal tract plays a critical role in determining circulatory nutrient availability via its absorptive functions. Furthermore, it provides a barrier to limit passage of luminal-originating pathogenic agents, namely bacteria (Camilleri et al., 2012). Endurance exercise is known to disturb the gastrointestinal protective barrier via direct (i.e., reduced blood flow to the splanchnic arena) or indirect mechanisms (i.e., increased cortisol and/or reduced stimulation of the enteric plexuses). This exercise-induced gastrointestinal syndrome (EIGS) may compromise the intestinal epithelium, with cell damage and increases in epithelial tight-junction space resulting in the paracellular translocation of luminal content, including bacteria and bacterial endotoxins (Costa et al., 2022). The consequences of local and systemic inflammatory effects include gastrointestinal symptoms (GIS) which may impair performance or require withdrawal from the exercise activity.

Investigations into the effects of macronutrient intake on epithelial tight-junction integrity and intestinal permeability have focused on the amount and type of CHO intake during exercise (King et al., 2022; Snipe et al., 2017). However, lipopolysaccharide concentrations have been observed to increase in humans and animals following high dietary lipid consumption, linking high-fat diets with increased lipopolysaccharide entry from the small intestine into the circulation (Cani et al., 2009; Erridge et al., 2007). Furthermore, gastrointestinal dysfunction has been associated with LEA, with increased GIS reported in female athletes with LEA, compared with those with adequate EA (Ackerman et al., 2019). Currently, some LEA screening tools are inclusive of gastrointestinal assessments (Melin et al., 2014), and gastrointestinal disturbances are recognized as a potentially negative health consequence of REDs (Mountjoy et al., 2018). Nevertheless, the gastrointestinal integrity status of athletes with LEA, or adhering to a LCHF diet, particularly in response to endurance exercise remains to be determined. Therefore, the purpose of this study was to investigate the effect of short-term (6-day) high CHO, LCHF, and LEA diets on blood markers of intestinal epithelial disruption following exercise.

Methods

Participants

Twenty-eight male race walkers were recruited for this study. The training status of participants according to McKay, Stellingwerff, et al. (2022) was Tier 5 (World Class; $n = 4$), Tier 4 (Elite; $n = 20$), and Tier 3 (Highly Trained; $n = 4$). This study conformed to the standards set by the Declaration of Helsinki and was approved by the ethics committee of the Australian Institute of Sport (20181203) and the Australian Catholic University (2020-238HC). The study protocol was explained verbally and in writing prior to athletes providing their written informed consent. Athletes were informed about the benefits and limitations of the dietary interventions and asked to nominate their preference for, or nonacceptance of, each intervention, as described previously (Burke et al., 2021). The research team allocated race walkers to a preferred dietary condition while achieving suitable matching between groups based on age, body mass, and aerobic capacity. No athlete allocated to the LCHF or LEA diets had tried this intervention before. Athlete characteristics are reported in Table 1.

Study Design

Using a parallel-group study design, this study took place during two separate, yet identical, 4-week training camps. One camp was held in Canberra, Australia, in 2019 ($n = 20$) and the other in Melbourne, Australia, in 2021 ($n = 8$). All athletes lived, trained, and ate meals together in a supervised training camp environment. Data were collected over the 4-week structured training block, which was divided into two phases (Baseline and Adaptation) and included two 25-km race walk trials (see Figure 1). The Baseline phase included a 6-day period of a standardized high energy/CHO (CON) diet to ensure adequate EA prior to the Baseline 25-km race walk trial. Athletes were then divided into three dietary groups: CON ($n = 10$), LCHF ($n = 8$), or LEA ($n = 10$). Athletes adhered to their allocated diet for 6 days before repeating the 25-km race walk trial. Data from this study were collected as a secondary outcome of previously published work (Burke et al., 2021, 2023).

Dietary Intervention

All food and fluids consumed during the study were provided and recorded by the research team. Meal plans were individually developed for each athlete as reported previously (Mirtschin et al., 2018) to integrate personal food preferences and nutrition requirements within the daily energy and macronutrient targets. The CON diet aimed to provide 65% CHO, 15% protein, and 20% fat and equated to an EA of 40 kcal·kg FFM^{-1}·day^{-1}. The ketogenic LCHF diet provided < 50 g of CHO daily and ~80% of energy provided in the form of fat. This diet was isocaloric to CON (40 kcal·kg FFM^{-1}·day^{-1}) and provided a similar protein content

Table 1 Athlete Characteristics at the Start of the Study

	CON ($n = 10$)	LCHF ($n = 8$)	LEA ($n = 10$)
Age (years)	26.7 ± 7.0	26.2 ± 3.9	30.0 ± 4.0
Body mass (kg)	66.3 ± 5.9	66.1 ± 7.5	67.7 ± 5.3
VO_2max (ml·kg^{-1}·min^{-1})	63.2 ± 3.6	67.5 ± 5.7	62.1 ± 6.1
10 km personal best (min:s)	41:56 ± 1:52	41:41 ± 2:10	40:22 ± 1:15

Note. Data are presented as mean ± *SD*. CON = high carbohydrate/high energy control diet; LCHF = low-carbohydrate, high-fat diet; LEA = low energy availability diet; VO_2max = maximal volume of oxygen consumed during exercise.

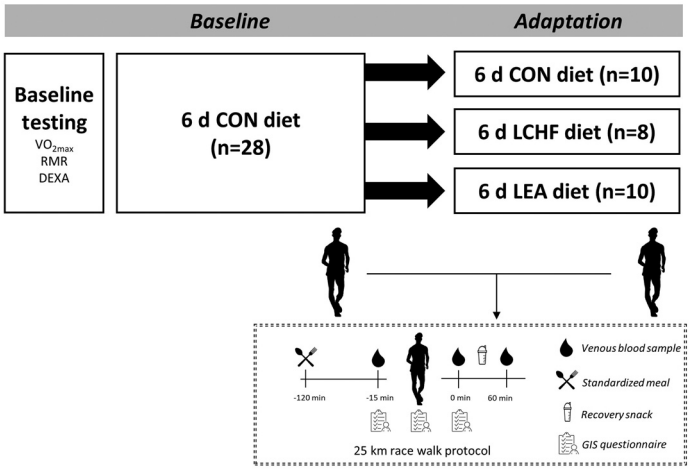

Figure 1 — Overview of study testing phases and dietary interventions. CON = high carbohydrate/high energy control diet; LCHF = low-carbohydrate, high-fat diet; LEA = low energy availability diet; GIS = gastrointestinal symptoms; RMR = resting metabolic rate; DEXA = dual-energy X-ray absorptiometry; VO_2max = maximal volume of oxygen consumed during exercise.

(15%). The LEA diet provided 60% CHO, 25% protein, and 15% fat, however restricted EA to just 15 kcal·kg FFM^{-1}·day^{-1}.

To achieve the desired EA, energy intake targets were derived using the following formula, where target EA was based on the study phase (40 and 15 kcal·kg FFM^{-1}·day^{-1} for CON/LCHF and LEA, respectively) with FFM determined via dual-energy X-ray absorptiometry:

$$\text{Target energy intake} = (\text{target EA} \times \text{FFM}) + \text{EEE (excluding resting metabolicrate [RMR])}.$$

EEE was prospectively calculated based on the athlete's training plan using the Weir equation (Weir, 1949). Here, respiratory gas data collected during the submaximal portion of each athlete's maximal volume of oxygen consumed during exercise (VO_2max) test were used to calculate EEE according to the following formula:

$$\text{EEE (kcal/km)} = (\text{[EEE (kcal/min)} \times 60\ \text{min]})/\text{speed [km/hr]}).$$

EEE (in kilocalories per kilometer) was multiplied by the daily training volume (in kilometers) to determine daily EEE. Checks of compliance to the training plan, dietary prescription, and reporting requirements were undertaken daily, with food choices and portion sizes being adjusted at lunch or dinner if actual training deviated from planned training by more than an EA equivalent of 2 kcal·kg FFM^{-1}·day^{-1}. All dietary plans were entered and analyzed using FoodWorks software (FoodWorks 9; Xyris Software). To avoid artifact outcomes and interpretation of results based on CHO intake type, the total and differential fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) content was assessed. A Monash University-designed FODMAP-specific database was used to assess the dietary plans for six athletes (Costa et al., 2022). Here, two participants from each dietary condition with a similar body mass (±1.6 kg) and dietary intake at Baseline (±66 kcal) were selected for analysis.

25-km Race Walking Trials

Two hours prior to the commencement of each trial, athletes consumed a standardized breakfast. During Baseline, and for CON during Adaptation, this meal consisted of 2 g/kg body mass (BM) CHO. During Adaptation, LCHF consumed an isocaloric meal in accordance with their dietary guidelines, while LEA consumed a meal providing 1 g/kg BM CHO. The 25-km race walking trials were then conducted under hybrid laboratory-field testing conditions, with Kilometers 0–1, 6–7, 12–13, 18–19, and 24–25 undertaken on a treadmill in the laboratory and the remainder on an outdoor loop of ~5 km, which included two water aid stations. One athlete was a junior (19 years old) and therefore only completed 3 laps, totaling 19 km. The speed at which athletes completed the treadmill portions of the walk was determined by a baseline VO_2max test. Athletes walked at either 12 or 13 km/hr, which approximated their 50-km race pace, or ~75% VO_2max (Burke et al., 2021). Heart rate (Polar Heart Rate Monitor, Polar Electro) and rating of perceived exertion (RPE) (Borg scale; 6–20) were collected during the final minute of exercise. This protocol was selected as significant GIS have been reported during a similar exercise bout associated with fueling strategies in race walkers (King et al., 2022). Air temperature and humidity were obtained retrospectively for each athlete's outdoor portion of the trial from the Bureau of Meteorology.

During the walk, fluids and food were provided to the athletes to mimic race feeding practices. Athletes were provided sports gels equating to ~60 g/hr CHO at Baseline (all athletes) and Adaptation (CON only). Within the LCHF Adaptation trial, athletes received fat-rich minimal-CHO cookies (44 g fat; 78%) to match the energy intake from their previous trial, while LEA athletes had their sports gel intake limited to provide 30 g/hr CHO.

Venous Blood

Venous blood samples were collected via cannulation pre-, post-, and 1 hr post each 25 km trial into 3 ml ethylenediaminetetraacetic acid and 3 ml lithium heparin tubes. Samples were immediately separated by centrifugation (1,500*g* for 10 min) into 200 µl aliquots and stored at −80 °C. Samples were later thawed, and plasma concentrations of intestinal fatty acid-binding protein (I-FABP), lipopolysaccharide-binding protein (LBP), and soluble CD14 (sCD14) were measured in duplicate using commercially available enzyme-linked immunosorbent assay kits (HK406, HK315, and HK320, respectively; Hycult Biotech). The intraassay coefficient of variation for each analyte was as follows: I-FABP = 4.2%, LBP = 5.3%, and sCD14 = 5.0%.

Gastrointestinal Symptoms

Using a validated, 10-point modified visual analog scale (Gaskell et al., 2019), symptoms were measured on six occasions throughout each 25-km race walk trial (preexercise, and after kilometers 1, 7, 13, 19, and 25). The assessment tool is subcategorized to provide information on gut discomfort, overall symptoms, and lower and upper abdominal symptoms. Responses of 1–4 were indicative of mild GIS, 5–9 of severe GIS, and 10 indicative of extremely severe GIS warranting exercise reduction or cessation. If no specific GIS was reported, this was reported as 0. For statistical analysis, exercise-associated GIS was calculated for each subcategory, by summing the five measured time points.

Statistical Analysis

Statistical analysis was performed with linear mixed modeling in R Studio (version 4.0.2). Models were estimated using restricted maximum likelihood. Normality was assessed via visual inspection of residual and quantile-quantile plots, which showed nonnormal distribution for all blood markers. Log transformation subsequently occurred. Homoscedasticity was tested with the Fligner–Killeen test. For each variable, fixed effects of diet (CON, LCHF, and LEA); trial (Baseline and Adaptation); and time (between three and six time points) were used. Athlete identification, camp, and environmental temperature were included as random intercepts. Models were then optimized by removing nonsignificant interactions and interpretation of Akaike information criterion statistics. Statistical significance of fixed effects occurred using Type II Wald tests with Kenward–Roger degrees of freedom. Where significant fixed effects were established, pairwise comparisons were identified using the Tukey post hoc adjustments. Significance was accepted at $p < .05$.

Results

Dietary Analysis

The implementation of dietary control was successful, with all athletes achieving their planned dietary targets (Table 2). No differences in energy or macronutrient intake were evident at Baseline ($p > .05$). As planned, LCHF had significantly lower CHO intake compared to both CON and LEA during the Adaptation trial ($p < .001$), with differences also noted between CON and LEA ($p < .001$). Energy intake was lowest in LEA ($p < .001$), with no differences detected between CON and LCHF ($p = .977$). Total daily FODMAP intake was significantly lower during Adaptation compared to Baseline in LCHF ($p < .001$) and LEA ($p = .044$). Specifically, excess fructose, lactose, and total oligosaccharides were significantly lower in LCHF during Adaptation, compared to Baseline ($p < .05$).

Physiological and Environmental Variables

Environmental temperature was significantly hotter at Baseline compared to Adaptation; however, no significant differences between diets were apparent ($p = .912$; Table 3). Exercise duration and intensity during the 25 km walk were similar for all dietary groups at Baseline; however, during the Adaptation trial, the LCHF group took an extra ~7.5 min to complete the protocol ($p < .001$), due to the self-chosen lower work intensity (–6% lower percentage of VO_2max; $p < .001$; Table 3). Despite the lower external workload, athletes adhering to the LCHF diet had an increased heart rate (HR) compared to Baseline (+10 beats per minute; $p = .035$). As previously reported elsewhere, all athletes adhering to the LCHF diet had ketone concentrations >0.5 mM on the morning of the Adaptation trial (range 0.6–2.6 mM) confirming ketosis (Burke et al., 2021).

Blood Markers of Gastrointestinal Disturbance

Plasma concentrations of markers of gastrointestinal integrity (I-FABP, LBP, and sCD14), measured pre-, post-, and 1 hr postexercise for Baseline and Adaptation trials are summarized in Figure 2. No significant differences in preexercise concentrations of plasma I-FABP, LBP, or sCD14 were detected between groups or trials ($p > .05$). A significant time effect showed that I-FABP concentrations increased postexercise and remained elevated at 1 hr postexercise ($p < .001$). A Diet × Trial interaction effect was also evident ($p = .002$), demonstrating that plasma I-FABP concentration was significantly higher during Adaptation compared to Baseline in the LCHF group ($p = .001$). No between-trial differences were evident for CON ($p = .991$) or LEA ($p = .998$). Finally, LCHF had significantly greater plasma I-FABP concentration when compared to both CON ($p < .001$) and LEA ($p = .001$) at Adaptation.

There was a Diet × Trial interaction ($p = .003$) for LBP, which showed concentrations of LBP were significantly higher during Adaptation compared to Baseline in LCHF ($p = .020$); however, no differences between Baseline and Adaptation were evident for either CON ($p = .970$) or LEA ($p = .977$). In addition, a significant Time × Trial ($p = .013$) interaction was evident, where the decrease from post- to 1 hr postexercise was larger at Baseline (–2.2 µg/ml; $p = .005$) compared to Adaptation (–0.2 µg/ml; $p = .998$). Plasma concentrations of sCD14 increased immediately after exercise ($p < .001$), before decreasing to preexercise levels 1 hr postexercise ($p = .263$). A two-way Diet × Trial interaction was seen for sCD14 ($p = .014$), where concentrations were significantly higher at

Table 2 Daily Dietary Intake for Athletes Adhering to the CON, LCHF, and LEA Diets at Baseline and Adaptation

	Baseline			Adaptation		
	CON	LCHF	LEA	CON	LCHF	LEA
Energy availability (kcal/kg FFM)	40 ± 3	41 ± 1	41 ± 3	41 ± 4	41 ± 2	15 ± 2*,#,^
Energy intake (kcal)	3,824 ± 623	3,843 ± 462	3,727 ± 335	3,970 ± 537	3,730 ± 411	2,335 ± 238*,#,^
Carbohydrate (g)	613 ± 102	616 ± 76	599 ± 53	639 ± 87	36 ± 6*,^	338 ± 33*,#,^
Protein (g)	144 ± 22	144 ± 17	141 ± 14	148 ± 21	145 ± 16	141 ± 14
Fat (g)	83 ± 15	84 ± 11	79 ± 9	84 ± 13	330 ± 39*,^	40 ± 7*,#,^
Total FODMAP (g)	43.5 ± 9.7	54.4 ± 22.9	47.5 ± 17.5	38.7 ± 17.2	8.2 ± 2.4*	31.2 ± 21.2*
Excess fructose (g)	16.8 ± 7.7	16.8 ± 5.0	14.5 ± 10.1	16.5 ± 9.0#	0.6 ± 0.5*	7.4 ± 8.3#
Lactose (g)	18.2 ± 11.8	29.5 ± 22.1	25.2 ± 13.2	12.6 ± 8.4	3.1 ± 2.1*	17.3 ± 12.8
Total polyols (g)	2.3 ± 1.5	2.4 ± 1.1	1.9 ± 1.4	2.7 ± 2.4	2.5 ± 1.4	2.1 ± 2.3
Total oligosaccharides (g)	6.2 ± 2.4	5.8 ± 2.1	5.8 ± 2.1	6.9 ± 2.9	1.9 ± 0.7*	4.5 ± 1.5

Note. Energy and macronutrient values are $n = 28$, with FODMAP analysis performed on $n = 6$. Data are presented as mean ± *SD*. FFM = fat-free mass; CON = high carbohydrate/high energy control diet; LCHF = low-carbohydrate, high-fat diet; LEA = low energy availability diet.
*A significant difference to Baseline. #A significant difference to LCHF. ^A significant difference to CON.

Table 3 Physiological and Environmental Characteristics During the 25 km-Long Walk Protocol at Baseline and Adaptation in Athletes Adhering to the CON, LCHF, and LEA Dietary Intervention

	Baseline			Adaptation		
	CON	LCHF	LEA	CON	LCHF	LEA
Temperature (°C)	28.2 ± 7.3	29.3 ± 4.4	27.4 ± 6.3	24.6 ± 6.0*	23.9 ± 5.4*	23.5 ± 6.1*
Humidity (%)	36.6 ± 14.9	35.8 ± 6.7	36.7 ± 7.4	45.9 ± 4.7*,#,^	59.3 ± 15.7*	54.5 ± 14.1*
Walk pace (min/km)	4:48 ± 0:07	4:59 ± 0:04	4:55 ± 0:10	4:49 ± 0:08	5:17 ± 0:22	4:59 ± 0:15
Intensity (%VO_2max)	74.8 ± 4.1	67.9 ± 5.1	71.6 ± 5.9	74.4 ± 4.3#	62.1 ± 3.9*,^	70.5 ± 5.6
Duration (hr:min:s)	2:00:17 ± 2:32	2:03:59 ± 5:20	2:02:24 ± 3:52	2:00:26 ± 3:07#	2:11:28 ± 8:46*	2:03:46 ± 6:09
Heart rate (bpm)	158 ± 9	160 ± 9	154 ± 7	156 ± 8#	170 ± 8*,^	152 ± 12
RPE (AU)	14 ± 2	14 ± 2	13 ± 1	14 ± 2*	17 ± 2*	15 ± 2*

Note. Data are presented as mean ± *SD*. Exercise intensity was calculated relative to the Baseline VO_2max test. bpm = beats per minute; CON = high carbohydrate/high energy control diet; LCHF = low-carbohydrate, high-fat diet; LEA = low energy availability diet; VO_2max = maximal volume of oxygen consumed during exercise; RPE = rating of perceived exertion.
*A significant difference to Baseline. #A significant difference to LCHF. ^A significant difference to LEA.

Adaptation, compared to Baseline in LCHF ($p < .001$). No between-trial differences were evident for either CON ($p = .961$) or LEA ($p = .069$).

Gastrointestinal Symptoms

The incidence and severity of GIS are presented in Table 4 (see additional data in Supplementary Table 1 [available online]). Incidence of exercise-associated GIS was generally mirrored between Baseline and Adaptation, respectively, in CON (60% and 50%), LCHF (88% and 88%), and LEA (90% and 90%), with large individual variation observed between trials. No Diet × Trial interactions were evident for gut discomfort ($p = .077$), overall GIS ($p = .169$), or lower GIS ($p = .460$). A significant interaction effect for upper GIS was evident ($p = .043$), which reflects a nonsignificant increase in upper GIS in LCHF ($p = .572$) and a decrease in LEA at Adaptation ($p = .308$).

Discussion

The results of the current study indicate that 6-day adherence to a ketogenic LCHF diet in combination with a high-fat preexercise meal may be associated with an increased severity of EIGS, as reflected by increased plasma concentrations of I-FABP, LPB, and sCD14 following a 2-hr exercise bout compared to Baseline. Despite the apparent increase in intestinal epithelial injury in our study, the impact on exercise-associated GIS was minimal. In addition, short-term exposure to LEA had no influence on intestinal epithelial perturbations or GIS when compared to a high CHO/high EA condition. From a translational perspective, athletes who undertake activities with a high risk of substantial perturbations to gastrointestinal integrity (e.g., endurance and ultraendurance events, and/or exertional heat stress) should be cautioned that short-term adherence to a LCHF diet may exacerbate EIGS.

Studies of different approaches to fueling prolonged exercise performance have traditionally focused on their effect on relative contributions and rates of oxidation of fat and CHO substrates, as well as performance outcomes (for reviews, see Burke, 2021; Stellingwerff & Cox, 2014). The impact of dietary changes on markers of gastrointestinal integrity in response to exercise has not been thoroughly investigated. Indeed, to our knowledge, this is the first human study to measure changes in biomarkers of EIGS, alongside GIS, following exercise after a short-term exposure to a LCHF diet.

Strenuous exercise can induce perturbations to the intestinal epithelial barrier, resulting in increased circulating concentrations of I-FABP, as well as markers of paracellular translocation of luminal pathogenic content due to increased tight-junction space and epithelial hyperpermeability (Costa et al., 2022). Data from the current study demonstrated significant increases in I-FABP, LBP, and sCD14 after a 6-day exposure to a LCHF diet in comparison to results seen in the CON group who consumed a high CHO diet. This suggests that exposure to a ketogenic LCHF diet may influence the integrity and permeability of the intestinal epithelium, leading to a greater influx of pathogenic agents into circulation. However, it is important to consider alternative explanations for these findings. Increases in luminal endotoxins and I-FABP are commonly reported in diets that increase lipid intake and oxidation (Akiba et al., 2020; Furuhashi & Hotamisligil, 2008). Indeed, I-FABP is a small intracellular protein present in mature enterocytes of the small intestine, and while used as a marker to represent intestinal epithelial injury, it may also represent an adaptive system that responds to the lipid status and stoichiometry of target cells (Furuhashi & Hotamisligil, 2008). The fatty acid-binding content in most cells is typically proportional to their rate of fatty acid metabolism (Furuhashi & Hotamisligil, 2008), with an increase in lipid uptake being associated with increased I-FABP concentrations at rest (Lau et al., 2016). In addition, the consumption of a high-fat meal has also been shown to acutely increase circulating lipopolysaccharide levels in healthy human volunteers, suggesting that dietary lipids may also play a role in facilitating lipopolysaccharide translocation into the circulation (Erridge et al., 2007). Since a companion paper from this investigation has previously reported a larger increase in serum free fatty acid concentrations during the 25 km walk after Adaptation to the LCHF diet (Burke et al., 2021), it is possible that the high-fat content of the LCHF diet and increase in free fatty acid turnover are responsible for the increased I-FABP concentrations in the current data set. In addition, it should be considered that longer periods of LCHF exposure and fat adaptation may alter this response.

Nevertheless, a strength of the current study is the combination of cellular and translocation biomarkers and capture of data at rest, and in association with exercise. Here, we note that the increase in I-FABP observed in the LCHF group occurred only in response to exercise and was accompanied by increased concentrations of

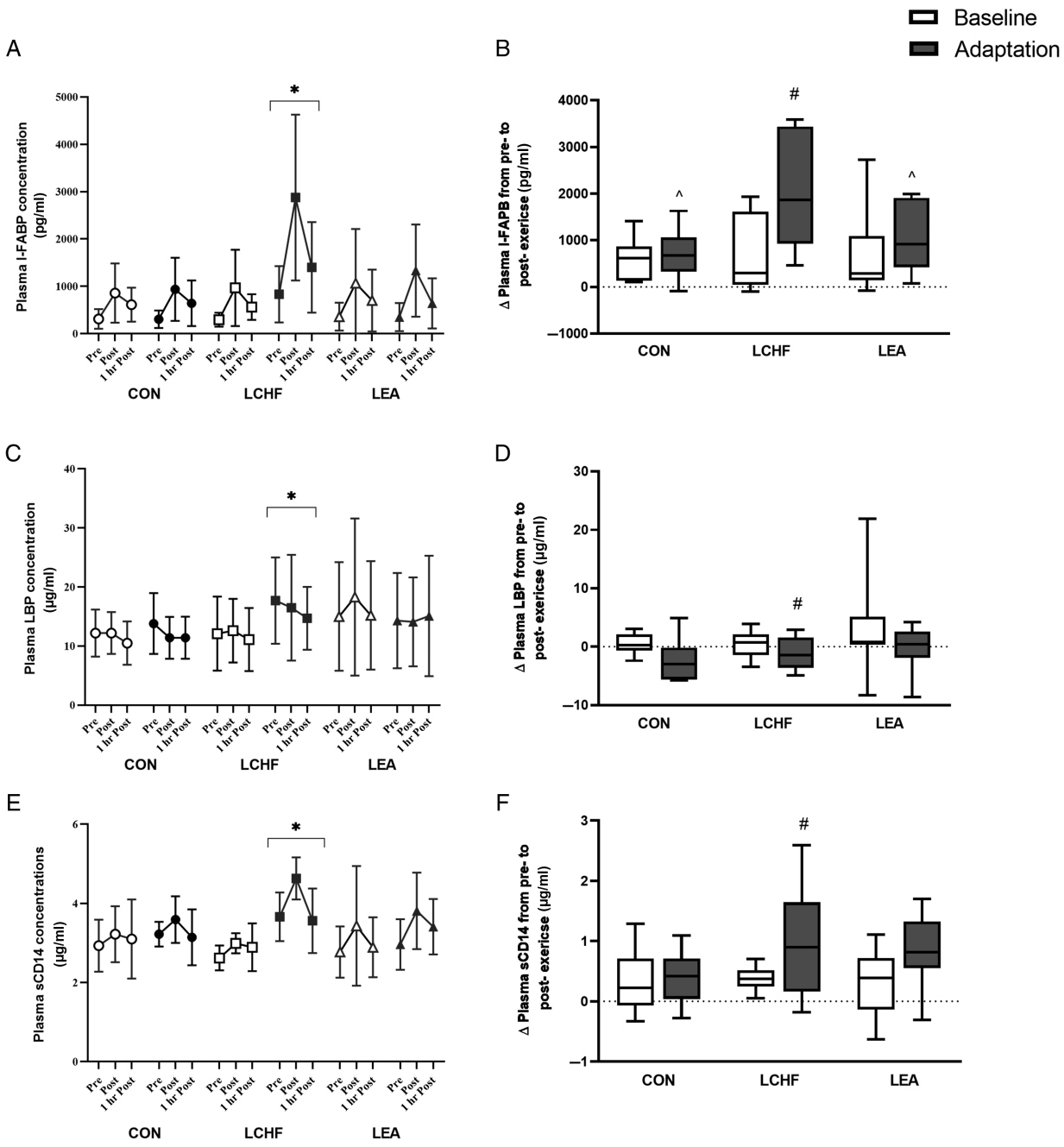

Figure 2 — Blood markers of gastrointestinal injury pre-, post-, and 1 hr postexercise trials in CON, LCHF, and LEA groups across the Baseline (light) and Adaptation (dark) trials. Absolute concentrations of I-FABP (A) LBP (C), and sCD14 (E) have been presented (mean ± *SD*), alongside delta change scores for pre- to postexercise (B, D, and F; data presented as 25th–75th percentile + min/max). *A significant Diet × Trial interaction effect. #The post hoc analysis, where differences compared to Baseline are evident. ^The post hoc analysis, where differences compared to LCHF are evident. CON = high carbohydrate/high energy control diet; LCHF = low-carbohydrate, high-fat diet; LEA = low energy availability diet; I-FABP = intestinal fatty acid-binding protein; LBP = lipopolysaccharide-binding protein; sCD14 = soluble CD14.

sCD14 and LBP. sCD14 is a human protein made by macrophages as a part of the innate immune system, which assists in the detection of harmful pathogens in circulation. When lipopolysaccharides translocate into the circulation, monocytes shed sCD14, binding in a complex with the acute-phase protein LBP (Paillaud et al., 2018). Therefore, the increase in sCD14 and LBP serves as a marker of lipopolysaccharide-induced monocyte or macrophage activation (Paillaud et al., 2018). Together, these findings suggest that the observed I-FABP response was indicative of exercise-associated gastrointestinal responses and that the LCHF diet was associated with an increase in intestinal epithelial injury and potentially compromised gastrointestinal integrity.

On this occasion, it was not possible to report on the systemic inflammatory response profile. However, previous investigations utilizing prolonged exercise bouts have shown increases in plasma I-FABP, LBP, and sCD14, and also reported a pronounced systemic inflammatory response (Gaskell et al., 2020; Snipe et al., 2018). Furthermore, a separate investigation examining the

Table 4 Incidence and Severity of GIS Reported During Baseline and Adaptation at Pre-, 1 km, 7 km, 13 km, 19 km, and Post the 25-km Race Walk Trial in Athletes Allocated to the CON, LCHF, and LEA Dietary Interventions

	Baseline								Adaptation							
	Pre	1 km	7 km	13 km	19 km	Post	Ex-GIS	%	Pre	1 km	7 km	13 km	19 km	Post	Ex-GIS	%
CON																
Gut discomfort	1 (0–3)	0 (0–2)	1 (0–6)	1 (0–3)	1 (0–2)	1 (0–7)	4 (0–13)	60	0 (0–2)	1 (0–3)	1 (0–6)	1 (0–6)	1 (0–3)	1 (0–4)	4 (0–19)	50
Total GIS	2 (0–9)	0 (0–3)	2 (0–10)	2 (0–9)	1 (0–4)	5 (0–31)	10 (0–45)	60	0 (0–2)	1 (0–8)	3 (0–17)	2 (0–13)	2 (0–15)	3 (0–18)	11 (0–71)	50
Upper GIS	1 (0–3)	0 (0–2)	1 (0–4)	1 (0–2)	0 (0–2)	2 (0–11)	4 (0–12)	50	0	0 (0–3)	4 (0–9)	1 (0–3)	1 (0–7)	1 (0–8)	4 (0–30)	30
Lower GIS	2 (0–7)	0 (0–1)	1 (0–6)	1 (0–9)	1 (0–3)	3 (0–20)	6 (0–33)	50	0 (0–2)	1 (0–5)	1 (0–8)	2 (0–10)	1 (0–8)	1 (0–10)	6 (0–41)	50
Nausea	0	0	0	0	0	0	0	0	0 (0–1)	0	0	0	0	0	0	0
LCHF																
Gut discomfort	2 (0–10)	0 (0–2)	2 (0–8)	2 (0–6)	1 (0–3)	1 (0–4)	7 (0–17)	88	1 (0–3)	2 (0–4)	4 (0–9)	3 (0–8)	2 (0–5)	4 (0–8)	14 (0–33)	88
Total GIS	6 (0–26)	1 (0–3)	3 (0–8)	4 (0–11)	2 (0–6)	2 (0–6)	11 (0–26)	88	2 (0–8)	2 (0–8)	8 (0–21)	7 (0–17)	3 (0–7)	7 (0–17)	27 (0–56)	88
Upper GIS	2 (0–12)	0 (0–1)	1 (0–3)	2 (0–7)	1 (0–3)	1 (0–6)	4 (0–19)	50	1 (0–2)	1 (0–4)	3 (0–10)	4 (0–15)	1 (0–4)	4 (0–11)	13 (0–36)	88
Lower GIS	4 (0–15)	1 (0–3)	2 (0–8)	2 (0–11)	1 (0–6)	0 (0–1)	6 (0–25)	75	1 (0–3)	2 (0–8)	5 (0–15)	3 (0–13)	2 (0–5)	4 (0–11)	14 (0–44)	75
Nausea	0	0	0	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–3)	13	0	0	0	0	0	0	0	0
LEA																
Gut discomfort	4 (0–10)	2 (0–6)	3 (0–8)	3 (0–11)	3 (0–8)	2 (0–6)	13 (0–29)	90	2 (0–8)	2 (0–8)	3 (0–8)	1 (0–3)	3 (0–8)	2 (0–8)	11 (0–35)	90
Total GIS	13 (0–58)	4 (0–17)	6 (0–28)	7 (0–27)	8 (0–28)	6 (0–20)	32 (0–105)	90	3 (0–14)	3 (0–14)	4 (0–14)	7 (0–54)	6 (0–20)	5 (0–19)	25 (0–121)	90
Upper GIS	4 (0–23)	3 (0–12)	4 (0–19)	4 (0–23)	4 (0–17)	3 (0–12)	18 (0–77)	90	1 (0–5)	1 (0–3)	1 (0–3)	3 (0–21)	2 (0–7)	2 (0–5)	8 (0–32)	70
Lower GIS	7 (0–29)	1 (0–5)	3 (0–9)	3 (0–9)	4 (0–20)	3 (0–14)	14 (0–55)	80	3 (0–14)	3 (0–14)	3 (0–14)	3 (0–24)	4 (0–14)	3 (0–14)	16 (0–80)	80
Nausea	0	0	0	0	0	0	0	0	0	0	0	0 (0–3)	0	0	0 (0–3)	10

Note. Data are presented as mean (range). No significant differences between trials or diets were evident. CON = high carbohydrate/high energy control diet; LCHF = low-carbohydrate, high-fat diet; LEA = low energy availability diet; GIS = gastrointestinal symptoms. Ex-GIS = exercise-associated gastrointestinal symptoms; % = incidence of Ex-GIS.

iron-regulatory and immune responses in this cohort showed that the interleukin-6 response to exercise was significantly elevated after 6-day adaptation to the LCHF diet (McKay, Peeling, et al., 2022). This suggests that the LCHF diet may pose a risk for exacerbation of exercise-associated systemic inflammatory responses and should be avoided in conjunction with other scenarios which also increase this risk, such as ultra-endurance events and events involving environmental heat exposure. Whether the influence of exercise intensity and/or duration has any influence on these results is difficult to determine. The LCHF group walked significantly slower during the Adaptation trial yet had a higher average HR and RPE. This response has been reported previously during the adaptation to a LCHF diet (McKay et al., 2023) and likely represents the shift toward fat as the primary fuel source (Burke et al., 2021). An increase in HR may reflect increased blood flow to the skin, rather than gastrointestinal tract, increasing EIGS. While the mechanisms that underpin these responses are still to be determined, this study replicates the daily training environment of elite athletes, and our findings should be considered before implementing this dietary approach with an athlete, particularly in individuals with a history of EIGS or in preparation for an important training block or competition.

Despite evidence of increased intestinal injury in the LCHF group, the changes in the incidence or severity of exercise-associated GIS associated with each diet were small and nonsignificant. One explanation for the lack of symptomology is that our cohort of highly trained athletes are accustomed to prolonged, steady-state exercise bouts and are experienced with gut-training strategies that increase tolerance to nutrient intake during exercise (King et al., 2022). Given the low incidence and severity of exercise-associated GIS during the Baseline exercise test, it is difficult to detect a meaningful reduction in symptomology due to adaptation to the dietary interventions. Whether these dietary interventions can have a more potent impact on GIS during exercise bouts where the likelihood of GIS is increased (i.e., prolonged training bouts in the heat) remains to be determined. Nevertheless, in the current study, there was a small difference in the severity of upper GIS between the LEA and LCHF groups, which may have become more pronounced with greater participant numbers.

Although the major focus of our dietary manipulations has been on changes to macronutrient and energy content, it is worth considering the gastrointestinal effects of individual components, particularly the FODMAP content. FODMAPs are fermentable short-chain CHOs which may be associated with increased gastrointestinal effects at rest and during exercise in susceptible individuals due to their malabsorption in the small intestine and subsequent fermentation in the colon (Lis, 2019). Several studies and case histories have been conducted in which an acute reduction in FODMAPs has been associated with a reduction in exercise-associated GIS (Lis, 2019; Gaskell et al., 2020). Furthermore, studies of GIS in response to exercise often implement 24 hr of standardized low-FODMAP diet to eliminate GIS otherwise associated with food and fluid intake; such dietary control is recommended within guidelines for undertaking such research (Costa et al., 2022). CHO-rich diets are typically higher in FODMAPs as was the case in the current study, with the LEA diet being associated with a small but significant decrease in FODMAPs due to the smaller food volume. Meanwhile, the LCHF diet contained only ~15% of the FODMAP content of the respective Baseline diet, mostly due to the near elimination of lactose and a reduction in excess fructose and total oligosaccharides. It is plausible that the low-FODMAP content of the LCHF diet was protective against symptom severity, which may have otherwise been apparent due to the increased intestinal injury and pathogenic translocation that occurred in this group. It is important to note that there was a highly variable response seen across all dietary conditions, likely making it difficult to detect significant differences in symptomology. However, this highlights the requirement for individualized nutrition strategies when it comes to minimizing exercise-associated GIS in athletic populations.

Gastrointestinal dysfunction has been reported in both male and female athletes in association with problematic exposure to LEA (Ackerman et al., 2019; Drew et al., 2017) to the extent that it is currently identified as an important variable within a well-known screening tool for the risk of LEA in females: the Questionnaire for Low EA in Females (Melin et al., 2014). However, the mechanism and etiology, including the time course and magnitude of severity of LEA associated with these gastrointestinal issues, are still unclear. In the current study, the LEA dietary intervention had no impact on any blood markers of gastrointestinal injury measured, nor did we see any effect on self-reported GIS at rest or during exercise. Therefore, our results suggest that short-term (6-day) adherence to a severe reduction (15 kcal·kg FFM $^{-1}$·day^{-1}) in EA does not exacerbate EIGS. With regard to symptomology during exercise, it has been shown that acute ingestion of CHO around the session can have a protective effect on the intestinal epithelium during exercise (Snipe et al., 2017). In the current study, athletes adhering to the LEA intervention received a moderate amount of CHO prior to (1 g/kg BM) and during (30 g/hr) exercise, which may have been sufficient to prevent large gastrointestinal disturbances from occurring. This protection may not be seen in sessions undertaken under fasting conditions. Furthermore, given the increased concentrations of indirect blood markers of gastrointestinal damage reported in the LCHF group, it appears that adequate CHO, rather than energy is of greater importance in limiting the exercise-induced gastrointestinal injury.

Previous research on extreme/chronic scenarios of LEA, such as anorexia nervosa, has reported a range of gastrointestinal concerns, including altered transit time, delayed gastric emptying, and irritable bowel syndrome (Kessler et al., 2020; Norris et al., 2016). However, since intestinal permeability does not appear to be affected by anorexia nervosa (Kleppe et al., 2022), these conditions appear to be independent of intestinal permeability. Then, results of the current study could also be explained by a similar scenario in athletic populations, whereby the underlying cause of gastrointestinal complaints associated with problematic LEA (Mountjoy et al., 2023) and the REDs syndrome is separate to the increased intestinal permeability. Future research should continue to examine the prevalence and mechanisms underpinning gastrointestinal issues in athletes with REDs, alongside other potential cases of gastrointestinal complaints.

Conclusions

The current study shows that a 6-day ketogenic LCHF dietary intervention combined with a high-fat preexercise meal resulted in greater exercise-associated perturbations to markers of intestinal integrity than a high CHO diet and even LEA. It appears that CHO restriction may provoke greater prevalence and severity of EIGS when compared with CHO-containing diets, even when there is short, severe energy restriction. Notably, the dietary interventions had minimal impact on the incidence and severity of GIS in this group of athletes; however, we acknowledge future work is required in this space where pre-existing GIS is of concern. Future

research should also further examine the impact of ketogenic LCHF diets to determine whether it is the restriction of CHO or excess of fat that is responsible for increased disruption of intestinal integrity. Finally, the mechanisms underpinning the high incidence of gastrointestinal complaints in athletes with LEA require further examination, as it may not always be associated with an impaired intestinal barrier during exercise.

Acknowledgments

This study was funded by a Grant from Australian Catholic University Research Fund (ACURF, 2017000034). The authors wish to thank all members of the broader research team for their assistance in data collection. The authors extend a special thank you to the athletes for their participation and would like to extend their gratitude to the Australian Institute of Sport for use of equipment and facilities, making this study possible. Part of sample analysis was conducted at Monash University, BASE Facility, Department of Nutrition Dietetics & Food, Melbourne, Australia. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. This study was conducted at the Australian Institute of Sport, Canberra, Australia, and at the Mary MacKillop Institute for Health Research at Australian Catholic University, Melbourne, Australia. **Author Contributions**: McKay and Wallett contributed equally to this work. *Conception and design of the experiments*: Burke, McKay, Whitfield, Tee, Heikura, Ross, Sharma. *Collection, assembly, analysis, and interpretation of data*: All authors. *Critical revision and drafting of the article for important intellectual content*: McKay, Wallett, Costa, Burke. *Approval of the final manuscript*: All authors.

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