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RESEARCH ARTICLE

^{13}C-labelled glucose-fructose show greater exogenous and whole-body CHO oxidation and lower O_2 cost of running at 120 versus 60 and 90 g·h^{-1} in elite male marathoners

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Abstract

We evaluated the effects of carbohydrate (CHO) ingestion at rates of 60 (maltodextrin:fructose ratio 1:0), 90 (2:1), and 120 (1:1) g·h^{-1} on whole body substrate metabolism, exogenous CHO oxidation (via U-^{13}C enriched glucose-fructose drinks) and gastrointestinal (GI) symptoms in elite male marathon runners [n = 8; marathon personal best (PB), 02:22:54 ± 00:05:37]. After 24 h of a high-CHO (8 g·kg^{-1}) diet and pre-exercise meal (2 g·kg^{-1}), participants completed 120-min running trials comprising 15 min at 95% lactate threshold (LT), 90 min at 94% lactate turnpoint, and a final 15 min at 95% LT. Mean whole-body CHO oxidation (120 g·h^{-1}, 3.07 ± 0.54; 90 g·h^{-1}, 2.46 ± 0.34; 60 g·h^{-1}, 2.09 ± 0.09 g·min^{-1}) and *hour 2* mean exogenous CHO oxidation (120 g·h^{-1}, 1.68 ± 0.16; 90 g·h^{-1}, 1.31 ± 0.18; 60 g·h^{-1}, 0.89 ± 0.11 g·min^{-1}) were different between all trials ($P < 0.01$ for all pairwise comparisons), such that 120 g·h^{-1} > 90 g·h^{-1} > 60 g·h^{-1}. Running economy was improved in the 120 g·h^{-1} condition, with a 8.1 O_2·kg^{-1}·km^{-1} lower O_2 cost compared with 60 g·h^{-1} ($P = 0.039$). The incidence of moderate or severe (≥4) GI symptoms was high in all trials, though peak symptoms of nausea, stomach fullness, and abdominal cramps were greatest for 120 g·h^{-1}. We report for the first time that CHO ingestion at 120 g·h^{-1} confers a metabolic advantage to male marathoners by better maintaining whole body rates of CHO oxidation, increasing exogenous CHO oxidation, and improving running economy. However, gut training strategies, preceding practical application, are warranted.

NEW & NOTEWORTHY We used stable isotope methodology to evaluate exogenous rates of CHO oxidation in elite male marathoners. We report that 120 (maltodextrin:fructose ratio 1:1) g·h^{-1} CHO induces greater whole body and exogenous CHO oxidation compared with 60 (1:0)–90 (2:1) g·h^{-1} and reduces the O_2 cost of running. However, the performance implications of such doses remain to be determined. The prevalence of GI symptoms across the doses suggests targeted fueling practice and gut training is warranted.

fructose; maltodextrin; marathon; stable isotopes

INTRODUCTION

The classical model of endurance performance suggests that key physiological determinants of marathon performance include a runner's $\dot{V}O_{2max}$, the percentage of $\dot{V}O_{2max}$ that can be sustained throughout the race (commonly associated with lactate threshold or critical speed), and the oxygen cost of submaximal running, referred to as running economy (mL·O_2·kg^{-1}·km^{-1}) (1–3). External factors (course profile, environment, pacing, drafting, nutrition, and footwear) also significantly influence marathon performance (3). Although the biomechanical (4–6), environmental (7), and physiological aspects (3, 8) of a sub-2-h marathon have recently received increased research focus, research on nutritional demands remains underexplored.

One viewpoint emphasized that in addition to possessing a superior $\dot{V}O_{2max}$, lactate threshold (LT), and running economy, achieving optimal performance will require an individualized and meticulous fueling strategy, a high aptitude for exogenous carbohydrate (CHO) oxidation, and an absence of gastrointestinal (GI) distress (9). Indeed, the depletion of finite glycogen stores within the muscle leads to a critical reduction in the rate at which adenosine triphosphate (ATP) can be resynthesized within muscle cells, thereby compromising the energy supply required to sustain muscle contraction and force output, ultimately resulting in fatigue (10, 11). Therefore, in addition to glycogen supercompensation (12, 13), the consumption of CHO during exercise has consistently been shown to enhance capacity for exercise lasting >1 h (14–16). In events lasting >2.5 h, that would otherwise

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be limited by glycogen depletion (12), the improvement in exercise capacity with CHO feeding is primarily achieved by maintaining plasma glucose, whole body CHO oxidation rates (17), and sparing liver glycogen (18), playing a critical role for performance and endurance capacity (19). In addition, increasing evidence suggests that hepatic overflow of exogenous CHO ingestion can also contribute directly to skeletal muscle metabolism and reduce endogenous CHO utilization, particularly at low to moderate exercise intensities, when hepatic glycogen stores are sufficiently replete (20, 21). As a result, CHO intake during exercise, using a combination of CHO monosaccharides, is recommended for endurance athletes. It has been suggested that the use of a single source CHO (e.g. glucose), particularly at high rates, can saturate the SGLT-1 transporter that aids in its absorption, after which no increase in exogenous CHO oxidation is observed (22). However, the addition of another monosaccharide, such as fructose, which uses a different transporter (GLUT5) results in higher exogenous CHO oxidation rates (23). This can be attributed to the recruitment of a different transporter that can maximize exogenous CHO oxidation. Therefore, multiple-transportable CHO formulations constitute a key strategy to enhance carbohydrate delivery and availability, mitigate liver glycogen depletion, and thereby sustain performance (24).

A recent modeling study (25) assessed the exogenous CHO intake required to run a sub-2-h marathon across sexes and calculated that male runners would require 93 ± 26 g·h^{-1} of exogenous CHO to run a sub-2-h marathon, suggesting that the current ≤90 g·h^{-1} recommendations are insufficient for 65% of modeled athletes (25). Though based on anecdotal practitioner experience and field observations, some reviews already recommend CHO intakes exceeding 100 g·h^{-1} if GI tolerance allows (14). However, the current American College of Sports Medicine (ACSM) recommendations for CHO intake are 30–60 g·h^{-1} for exercise lasting 1–2.5 h and up to 90 g·h^{-1} of multiple transportable CHO for exercise lasting >2.5 h (19). Although modeling data suggests that higher CHO intakes may be necessary to meet the metabolic demands of a sub-2-h marathon, direct evidence assessing exogenous CHO oxidation rates at these higher intakes in elite endurance runners does not exist. Therefore, further research is needed to empirically evaluate whether CHO intake exceeding 90 g·h^{-1} can be effectively oxidized and tolerated in this population.

Recent data from our laboratory demonstrated that trained male cyclists can tolerate 120 g·h^{-1} of multiple transportable CHO blends with minimal GI discomfort, reaching peak exogenous CHO oxidation rates of 1.87 g·min^{-1} (26). This demonstrates that exogenous CHO bioavailability may surpass the currently recommended upper limit of 90 g·h^{-1} (27). Nonetheless, the feasibility of such doses in runners, particularly *tier 3* (i.e. highly trained/national level) and *4* runners (elite/international level), exercising at an intensity within the heavy intensity domain, is unclear with limited research supporting such claims. This discrepancy may arise from the higher incidence of GI complaints in runners compared with cyclists (28), potentially stemming from the repetitive high-impact mechanics of running that can cause damage to the intestinal lining and gastric jostling (29). Among the studies that have implemented running as an exercise modality, the majority have used lesser trained subjects and/or lower relative exercise intensities (30–33). As a result, the absolute exercise intensities and metabolic requirements are lower than what would be observed in well-trained or elite marathon runners running at, or close to, marathon pace. Furthermore, CHO intakes >1.5 g·min^{-1} during running exercise have also been associated with GI symptoms, which can further hinder performance (30, 34–36).

The aim of the present study was, therefore, to evaluate the effects of CHO ingestion at rates of 60, 90, and 120 g·h^{-1} on whole body substrate metabolism, exogenous CHO oxidation, and GI symptoms. We hypothesized that CHO feeding would support greater rates of whole body CHO oxidation and exogenous CHO oxidation in a dose-dependent manner. To this end, we recruited eight male highly trained/elite runners from the England Athletics Endurance Program, all whom had marathon personal bests that were faster than 2 h 30 min. According to the participant classification framework (37), these runners are representative of both *tier 3* (highly trained/national level athletes) and *tier 4* (elite/international level athletes). Runners consumed CHO at rates of 60, 90, and 120 g·h^{-1} during 2 h of running, wherein 90 min corresponded to a running pace just below projected marathon pace [i.e. 94% of lactate turn point (LT2)] (3, 38). To assess rates of exogenous CHO oxidation, CHO was ingested in fluid form, and all drinks were uniformly enriched with both ^{13}C-glucose and ^{13}C-fructose.

METHODS

Participants

Eight male elite marathon runners participated in the study. Participant characteristics are presented in Table 1. Participants were defined as highly trained and elite (*tier 3* and *4*) in accordance with the classification of Mckay et al. (37) in which *tier 3* denotes highly trained/national-level athletes and *tier 4* denotes elite/international-level athletes. Participants were required to have completed a certified race within the 12 mo before the study, with a qualifying time of 2:30:00 or faster for the marathon, or 1:13:00 or faster for the half-marathon. All provided written informed consent after receiving a comprehensive explanation of all experimental procedures and risks. Sample size was determined according to our primary outcome variable (i.e. exogenous CHO oxidation), assuming an estimated mean exogenous CHO oxidation of 1.5 ± 0.3 g·min^{-1} and 1.0 ± 0.3 g·min^{-1} in the 120 and 60 g·h^{-1} CHO trials, respectively [estimated rates are taken from previous CHO dose studies from both running and cycling (26, 31, 39)]. These data give an effect size of dz = 1.2, where a sample size of eight would provide an alpha value of 0.05 and

Table 1. *Participant characteristics*

Participant Characteristics	*n* = 8
Age	33 ± 6
Height, cm	177 ± 7
BM, kg	67.4 ± 3
Marathon PB	2:22:54 ± 05:37
Speed at 95 % LT, km·h^{-1}	15.3 ± 1
Speed at 94 % LTP, km·h^{-1}	16.4 ± 1

BM, body mass; LT, lactate threshold; LTP, Lactate turn point.

a power of 0.80 (GPower, version 3.1.9.6). Participants presenting with musculoskeletal injuries, metabolic disease, gastrointestinal infections, diseases, and/or disorders, asthma, cardiovascular, and cerebrovascular disease were excluded from the study. Furthermore, those on specific medication (e.g., baclofen, methotrexate, tacrolimus and voriconazole, β-blockers, or diuretics) were also excluded from the study.

Ethical Approval

The study received approval from the Ethics committee of Liverpool John Moores University (23/SPS/055) and adhered to the standards outlined in the latest revision of the Declaration of Helsinki for Human Research Ethics.

Experimental Overview

A schematic illustration describing the experimental procedures is shown in Fig. 1. Utilizing a double-blind randomized crossover design, each participant completed three experimental trials during which they were provided with either 60, 90, or 120 g·h^{-1} of a CHO drink in a random order. Each trial consisted of a 120-min run, and the intensities prescribed are presented in Fig. 1. Participants were given a standardized diet for the 24 h preceding each trial, consisting of 8.0 g·kg^{-1} CHO; 2.0 g·kg^{-1} protein and 1.0 g·kg^{-1} fat. This was repeated for each subsequent visit.

Preliminary Testing

At least 1 wk before experimental trials, participants completed an incremental test on a motorized treadmill (Pulsar H/p, Cosmos, Germany) to determine their first and second lactate thresholds. The initial incremental step test began with the slope set at 1% (40) and an initial speed of 12–14 km·h^{-1}, depending on each subject's fastest marathon time or equivalent. At the end of each 3-min stage, a fingertip blood sample was collected and analyzed immediately for blood lactate concentration (Biosen C-Line analyzer by EKF Diagnostics, Cardiff, UK). Sampling and analysis occurred while the athlete commenced the subsequent stage to minimize interruption. The second lactate turn point (LT2) was identified as the running speed eliciting a sudden and sustained increase in [La^{-}] above preceding values. Once this rise was observed, participants completed the stage in progress, and the blood sample obtained at its conclusion was used to confirm LT2. Blood lactate concentration was plotted against running speed, with LT defined as the initial elevation in blood lactate above the baseline value. Lactate turn point (LTP) was characterized by a subsequent and sudden increase in blood lactate concentration. Both were determined through visual inspection after being reviewed by two independent researchers (3). Heart rate (HR) (Polar H10; Polar, Kempele, Finland) and expired gas (Moxus modular metabolic system; AEI Technologies Inc., PA) were monitored throughout.

Following a 15–30-min rest period, subjects underwent a 60-min familiarization protocol, during which they ran for 60 min at the prescribed speeds (corresponding to the first hour of the 120-min experimental trial; see Fig. 1). Participants received 125 mL of fluid at the start of exercise and every 15 min thereafter to replicate the CHO drink protocol used during the experimental trials. HR and ratings of perceived exertion (RPE) were monitored throughout, while expired gas was collected for 3 min at 15-min intervals to calculate whole-body substrate utilization. Blood lactate and body mass were measured at 0, 15, 45, and 60 min. The session was also used to evaluate the prescribed running speeds and, if necessary, adjust them, based on participant feedback and lactate trajectory to ensure the target exercise intensities were achieved.

Experimental Trials

On the morning of the experimental trial, participants reported to the laboratory at ~0900 h after consuming a standardized high CHO breakfast (2 g·kg^{-1} CHO; 0.25 g·kg^{-1} protein and 0.1 g·kg^{-1} fat). Before exercise, participants provided a resting finger prick blood sample to measure resting blood lactate concentration and hematocrit (Hirschmann Hematocrit Tubes, Germany). The same measures were collected postexercise. A resting breath sample was also collected into a 10 mL exetainer (Labco, High Wycombe, UK). Participants then completed a modified visual analogue scale for GI symptom assessment (41). Subjects were educated and advised to complete the 10-point GI symptom rating scale as follows: 1 to 4 indicated mild GI symptoms (i.e. sensation of GI symptoms, but not substantial enough to interfere with exercise), 5 to 9 indicated severe GI symptoms (i.e. GI symptoms substantial enough to interfere with

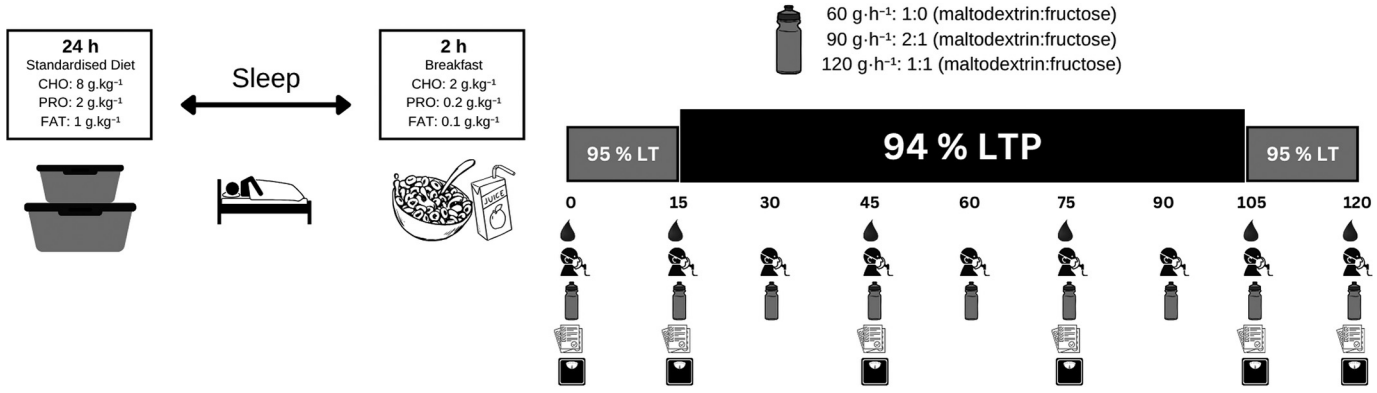

Figure 1. Schematic overview of the experimental protocol used in each trial. Following 24 h of a high CHO diet, subjects consumed a high CHO pre-exercise meal before undertaking 120 min of running at 94% lactate turn point (LTP) with the first and final 15 min at 95% lactate threshold (LT) during, which they consumed 60, 90, and 120 g·h^{-1} CHO drinks.

exercise), and 10 indicated extreme GI symptoms warranting exercise cessation. If no specific GI symptom was experienced, participants reported zero. Perceived satiety, drink sweetness, and desire to drink were assessed using a modified visual analogue scale (42). Body mass (BM) was measured at standardized 1-min pauses at prespecified time points (0, 15, 45, 75, 105, 120 min) that were identical across all three trials. This was used to adjust running economy values for changes in body mass over time within each trial. Running economy was expressed as the oxygen cost of running (mL·O_2·kg^{-1}·km^{-1}), calculated from steady-state $\dot{V}o_2$, adjusted for the speed required to cover 1 km, and corrected for body mass at 30-min intervals. This was then converted to energetic cost (kJ·kg^{-1}·km^{-1}) (43). A calibrated floor scale (Seca, Germany) was positioned adjacent to the treadmill, and conscious efforts were made to minimize any additional time off the treadmill. This data was incorporated into running economy calculations to account for within-trial changes in body mass.

Participants completed 120 min running on a motorized treadmill with the incline set at 1% (40) at speeds corresponding to 95% LT for the first and final 15 min intervals and 94% LTP for 90 min. Expired gas was collected for a 3-min period every 15 min to calculate whole-body substrate utilization. The final minute of this period was used to collect expired gas into the evacuated exetainer tubes to determine the ^{13}C-to-^{12}C ratio in CO_2. Perceived satiety, drink sweetness and desire to drink, along with HR and RPE were also assessed every 15 min. A finger prick blood sample was collected, and GI symptoms and BM were recorded every 30 min.

CHO Drink

CHO drinks were formulated using maltodextrin and fructose (Science in Sport PLC, Blackburn, UK) in the following ratios for each condition: 60 g·h^{-1} (120 g maltodextrin, 0 g fructose, 0.08 g U-^{13}C glucose tracer), 90 g·h^{-1} (120 g maltodextrin, 60 g fructose, 0.08 g U-^{13}C glucose tracer, 0.04 g U-^{13}C fructose tracer), and 120 g·h^{-1} (120 g maltodextrin, 120 g fructose, 0.08 g U-^{13}C glucose tracer, 0.08 g U-^{13}C fructose tracer). Each formulation was made into a 1 L solution (total over 2 h), and 125 mL was consumed every 15 min (i.e. 500 mL·h^{-1}). The maltodextrin dose was held constant across all conditions (120 g over 2 h); fructose was varied (0, 60, 120 g over 2 h) to achieve the target intakes and blend ratios. The resulting carbohydrate concentrations were 12% (60 g·h^{-1}), 18% (90 g·h^{-1}), and 24% (120 g·h^{-1}) (wt/vol). A fixed fluid volume was used across conditions for ecological validity; therefore, dose and concentration varied jointly by design.

Indirect Calorimetry

Respiratory gas exchange variables were measured using a mixing chamber (Moxus modular metabolic system; AEI Technologies Inc, PA). Oxygen uptake ($\dot{V}o_2$), carbon dioxide production ($\dot{V}co_2$), and respiratory exchange ratio (RER) were measured for 3 min at 15-min intervals, before exercise and for the final 3 min every 15 min during exercise, with mean values calculated for each 30 s. Breath samples were extracted in duplicate directly from the mixing chamber during the final minute of each 3-min gas analysis period, during which the sample line was briefly disconnected. Fat oxidation and CHO oxidation were calculated indirectly using previously established calculations of oxidation rates during moderate to high intensity exercise (44). Urinary nitrogen was not measured, and values, therefore, reflect nonprotein substrate partitioning. As a result, the following equations were used.

$$\text{CHO oxidation}\,(\text{g}\cdot\text{min}^{-1}) = 4.21 \times \dot{V}co_2 - 2.962 \times \dot{V}o_2$$

$$\text{Fat oxidation}\,(\text{g}\cdot\text{min}^{-1}) = 1.695 \times \dot{V}o_2 - 1.701 \times \dot{V}co_2.$$

^{13}C/^{12}C analysis of CHO drink.

An elemental analyzer-isotope ratio mass spectrometer (Europa Scientific 20-20; Iso-Analytical Ltd., Crewe, UK) was utilized to quantify the ^{13}C enrichment of freeze-dried (U-^{13}C glucose/U-^{13}C fructose) drink samples and the natural ^{13}C background of the glucose-fructose drink, and this was expressed as δ^{13}C ‰ versus Pee Dee belemnite (PDB).

^{13}C/^{12}C analysis of breath CO_2.

A Sercon 20-20 isotope ratio mass spectrometer (IRMS), linked to a Sercon ANCA NT GC system and a Gilson 222 autosampler, was used to analyze breath samples. Breath samples were continuously transferred through a Valco sampling port in a helium flow, and carbon dioxide was separated from other gases using a capillary column (PoraPLOTQ; Agilent JW columns) with dimensions of 27.5 m × 0.32 mm × 10 μm. The oven temperature was maintained at 68°C. A magnesium perchlorate trap was used to remove water from the sample. Samples were analyzed in multiples, with the contents of the sample loop switched to the gas chromatography (GC) column every 50 s, initiating a restart of the GC separation process. Ions with mass-to-charge ratios (m/z) of 44 and 45 were monitored for CO_2 and $^{13}CO_2$, respectively. Results for ^{13}C enrichment in breath samples were expressed as δ^{13}C ‰ versus PDB.

Exogenous CHO oxidation was calculated using the following formula:

$$\text{Exogenous CHO oxidation}(\text{g}\cdot\text{min}^{-1}) = \dot{V}co_2 \times (\delta\text{Exp} - \delta\text{Expbkg})/(\delta\text{Ing} - \delta\text{Expbkg})/k.$$

In which δExp represents the ^{13}C enrichment observed in the expired air at various exercise time points, δIng signifies the [U-^{13}C] enrichment present in the ingested maltodextrin drink, δExpbkg denotes the ^{13}C enrichment in the expired air before exercise (background), and k is the quantity of CO_2 in liters (L·CO_2) generated by the oxidation of 1 g of glucose ($k = 0.7467$ L·CO_2·g^{-1} glucose).

Statistical Analysis

All statistical analyses were performed using R (The R Project for Statistical Computing, Version 4.3.0). Differences in mean exogenous CHO oxidation, whole body CHO and fat oxidation, HR, RPE, energy expenditure, running economy, and blood glucose and blood lactate concentrations were all analyzed by two-way repeated-measures ANOVA. Peak exogenous CHO oxidation was analyzed by one-way repeated measures ANOVA. Mauchly's test for sphericity was used, and in cases where this assumption was violated, the Greenhouse–

Geisser correction was applied. Where a significant main effect was found, pairwise comparisons were conducted using the Holm post hoc test, and Cohen's *d* was calculated (with 0.2, 0.5, and >0.8 representing a small, moderate, and large effect, respectively), and 95% confidence intervals (CI) for paired differences are also presented. Non-normally distributed data were analyzed using Friedman's ANOVA, the nonparametric equivalent of a one-way repeated-measures ANOVA. Where a significant main effect was found, pairwise comparisons were conducted using a Wilcoxon's signed ranks test. Differences in symptom prevalence across conditions were analyzed using Cochran's *Q* test followed by pairwise McNemar tests with Holm adjustment. All data in text, figures, and tables are presented as means ± SD, with *P* values ≤ 0.05 indicating statistical significance.

RESULTS

Effects of Dose of CHO Ingestion on Physiological, Metabolic, and Perceptual Responses to Exercise

In regard to running velocity (i.e. differences between time points where participants ran at LT and 94% of LTP), there were significant differences between physiological measures collected at 15 and 120 min (i.e. running speed at 95% LT) compared with those during the 90-min higher intensity portion of the protocol i.e., running at 94% of LTP (Fig. 2). When considering the 90 min completed at 94% of LTP, there were progressive increases in heart rate (Fig. 2*A*) and RPE (Fig. 2*B*), a deterioration of running economy (Fig. 2*D*) but no difference in blood lactate (Fig. 2*C*) or caloric cost of running (Fig. 2*E*). Across the whole 2 h period, exercise significantly increased heart rate, blood lactate concentration, RPE, and impaired running economy (all $P < 0.001$) (Fig. 2). No differences were observed between CHO trials for these variables ($P = 0.73$, 0.11, 0.79, 0.15) with the exception of running economy ($P = 0.024$), where there were significant differences in oxygen cost between the 60 and 120 g·h^{-1} trial ($P = 0.04$, mean difference 8.11, 95% CI [2.4, 13.9] O_2·kg^{-1}·km^{-1}, Cohen's $d = 1.18$) but no differences between the 60 and 90 g·h^{-1} trials ($P = 0.097$, mean difference = 4.42, 95% CI [−2.85, 10.23] O_2·kg^{-1}·km^{-1}, Cohen's $d = 0.47$) or 90 and 120 g·h^{-1} trials ($P = 0.259$, mean difference = 4.42, 95% CI [0.48, 8.36] O_2·kg^{-1}·km^{-1}, Cohen's $d = 0.94$) (Fig. 2*D*). The O_2 cost was 3.6% lower in the 120 g·h^{-1} trial and 1.5% lower in the 90 g·h^{-1} trial compared with the 60 g·h^{-1} trial, respectively.

Effects of Dose of CHO Ingestion on Blood Glucose Concentration and Whole Body Substrate Metabolism

In accordance with CHO provision during exercise, blood glucose significantly increased ($P < 0.001$) during exercise (Fig. 3*A*) and was also significantly different between trials ($P = 0.028$). In relation to trial specific comparisons, blood glucose was greater in the 120 g·h^{-1} trial compared with 60 g·h^{-1} ($P = 0.036$; mean difference = 0.81, 95% CI [0.27, 1.35] mmol·L^{-1}, Cohen's $d = 1.24$, mean concentrations of 5.94 ± 0.70 vs. 5.13 ± 0.24 mmol·L^{-1}, respectively). However, no differences were apparent between 120 and 90 g·h^{-1} ($P = 0.254$; mean difference = 0.36, 95% CI [−0.37, 1.08] mmol·L^{-1}, Cohen's $d = 0.41$, mean concentration 5.59 ± 0.52 mmol·L^{-1}, respectively) or between 90 and 60 g·h^{-1} ($P = 0.127$; mean difference = 0.45, 95% CI [0.04, 0.87] mmol·L^{-1}, Cohen's $d = 0.932$).

In relation to the effects of exercise, there was a progressive decline in both RER ($P < 0.001$) and whole body CHO oxidation ($P < 0.001$) and an accompanying progressive increase in fat oxidation ($P < 0.001$) (Fig. 3). However, with increasing dose of CHO ingestion, RER ($P < 0.001$), rates of whole body CHO ($P < 0.001$) and fat oxidation ($P < 0.001$), and total CHO ($P < 0.001$) and fat oxidation ($P < 0.001$) during exercise were all significantly different between conditions (Fig. 3, *B–F*, respectively). Specifically, RER (mean ± SD: 0.83 ± 0.01, 0.86 ± 0.02, 0.89 ± 0.03), rate of CHO oxidation (mean ± SD: 2.09 ± 0.09, 2.46 ± 0.34, 3.07 ± 0.54 g·min^{-1}), rate of fat oxidation (mean ± SD: 1.07 ± 0.22, 0.90 ± 0.27, 0.67 ± 0.20 g·min^{-1}), total CHO (mean ± SD: 250 ± 11, 295 ± 41, 368 ± 65 g), and total fat oxidation (mean ± SD: 128 ± 26, 110 ± 29, 81 ± 24 g) all displayed significant pairwise differences (mean values reported for 60, 90, and 120 g·h^{-1}, respectively) between trials (all $P < 0.01$), such that 120 g·h^{-1} > 90 g·h^{-1} > 60 g·h^{-1} (RER: mean difference 0.03, 95% CI [0.02, 0.05], Cohen's $d = 2.00$; mean difference 0.03, 95% CI [0.01, 0.04], Cohen's $d = 1.48$; CHO oxidation: mean difference 0.61, 95% CI [0.38, 0.83] g·min^{-1}, Cohen's $d = 2.25$; mean difference 0.37, 95% CI [0.09, 0.66] g·min^{-1}, Cohen's $d = 1.11$; fat oxidation: mean difference 0.23, 95% CI [0.10, 0.36] g·min^{-1}, Cohen's $d = 1.45$; mean difference 0.17, 95% CI [0.05, 0.29] g·min^{-1}, Cohen's $d = 1.21$; total CHO: mean difference 73, 95% CI [46, 99] g, Cohen's $d = 2.26$; mean difference 45, 95% CI [11, 79] g, Cohen's $d = 1.12$; and total fat: mean difference 30, 95% CI [17, 43] g, Cohen's $d = 1.90$; mean difference 18, 95% CI [5, 31] g, Cohen's $d = 1.20$). All previous comparisons are reported for 120 versus 90 g·h^{-1}, and 90 versus 60 g·h^{-1}, respectively.

No differences were apparent between trials ($P = 0.413$) in rate of total energy expenditure during exercise (Fig. 4*A*), though energy expenditure was significantly greater ($P < 0.001$) during the 90 min of exercise undertaken at 94% of LTP compared with the 2 × 15 min periods of exercise completed at 95% LT. Of note, the metabolic crossover point (i.e., the time-point during exercise at which fat provides the greater contribution toward total energy expenditure) was delayed by ∼40 min when participants consumed 90 g·h^{-1} compared with consuming 60 g·h^{-1} (Fig. 4, *C* and *B*, respectively), whereas 120 g·h^{-1} prevented the occurrence of a crossover point. When taken together, the results demonstrate that a dose of 120 g·h^{-1} maintained whole body CHO oxidation to a greater extent than both the 90 and 60 g·h^{-1} doses (Fig. 4*D*).

Effects of Dose of CHO Ingestion on Exogenous CHO Oxidation and Efficiency

Exogenous rates of CHO oxidation during exercise are presented in Fig. 5*B*. Exogenous CHO oxidation was significantly different between trials ($P < 0.001$) such that mean exogenous CHO oxidation during *hour 2* was greater with an ingestion rate of 120 g·h^{-1} (1.68 ± 0.16 g·min^{-1}) compared with both 90 g·h^{-1} (1.31 ± 0.18 g·min^{-1}; $P = 0.0025$, mean difference 0.37, 95% CI [0.23, 0.51] g·min^{-1}, Cohen's $d = 2.22$) and 60 g·h^{-1} (0.89 ± 0.11 g·min^{-1}; $P < 0.0001$ mean difference 0.79, 95% CI [0.65, 0.92] g·min^{-1}, Cohen's $d = 5.01$). In

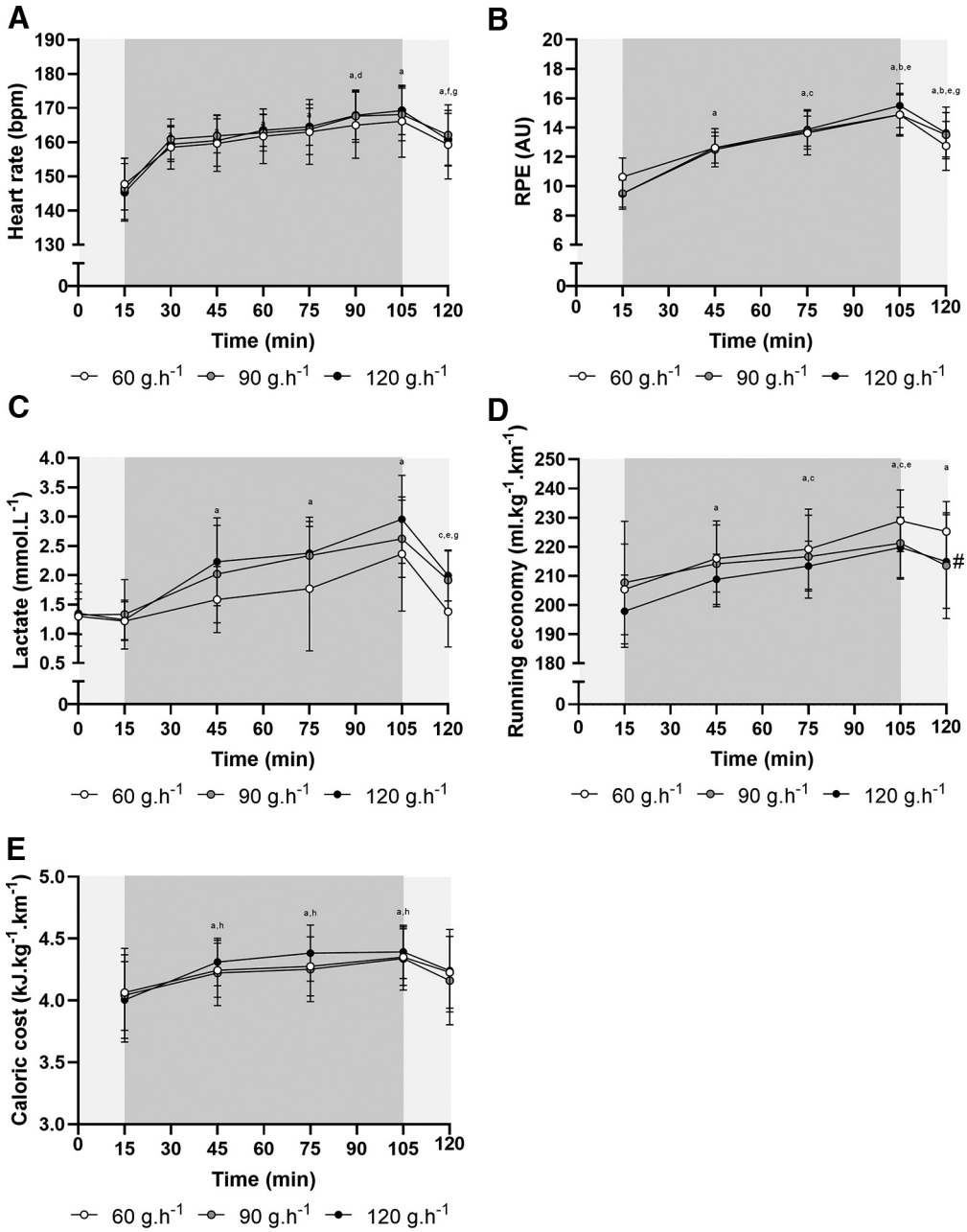

Figure 2. Heart rate (*A*), RPE (*B*), blood lactate (*C*), running economy (*D*) and caloric cost of running (*E*), during prolonged treadmill running with CHO ingestion at 60, 90, and 120 g·h^{-1}. Data are means ± SD for $n = 8$ elite male marathon runners. Running economy was calculated as the oxygen cost of submaximal exercise. Statistical differences were assessed by repeated-measures ANOVA with Holm–Bonferroni correction. a–h denotes $P < 0.05$ vs. [a]15, [b]30, [c]45, [d]60, [e]75, [f]90, [g]105, and [h]120 min, respectively; #$P < 0.05$ vs. 60 g·h^{-1} trial.

addition, exogenous CHO oxidation during *hour 2* was also significantly greater with an ingestion rate of 90 g·h^{-1} compared with 60 g·h^{-1} ($P = 0.0034$, mean difference 0.41, 95% CI [0.24, 0.59] g·min^{-1}, Cohen's $d = 1.94$) (Fig. 5*C*). Similarly, peak exogenous CHO oxidation rates (Fig. 5*D*) also exhibited significant differences between conditions ($P < 0.001$) between all pairwise comparisons (all $P < 0.001$) such that 120 g·h^{-1} (1.77 ± 0.13 g·min^{-1}) > 90 g·h^{-1} (1.41 ± 0.12 g·min^{-1}) > 60 g·h^{-1} (1.00 ± 0.10 g·min^{-1}) (mean difference 0.41, 95% CI [0.29, 0.53] g·min^{-1}, Cohen's $d = 2.87$; mean difference 0.41, 95% CI [0.29, 0.54] g·min^{-1}, Cohen's $d = 2.74$ for 120 vs. 90 g·h^{-1}, and 90 vs. 60 g·h^{-1}, respectively).

There was no significant difference in oxidation efficiency (Fig. 5*E*) between trials during *hour 2* (120 g·h^{-1}: 84 ± 8%; 90 g·h^{-1}: 87 ± 12%; 60 g·h^{-1}: 89 ± 11%, $P = 0.554$) (Fig. 5*D*). The contribution of endogenous CHO oxidation, exogenous CHO oxidation, and fat oxidation toward energy expenditure during the second hour of exercise is also presented in Fig. 5*F*. The contribution of endogenous CHO oxidation was not different between trials ($P = 0.312$). However, in accordance with the dose-response effect of CHO ingestion on exogenous CHO oxidation, the contribution of exogenous CHO oxidation toward total energy expenditure was also significantly different between trials ($P < 0.001$), where pairwise comparisons (all $P < 0.001$) again confirmed that ingestion rates of 120 g·h^{-1} (39 ± 5%) > 90 g·h^{-1} (30 ± 4%) > 60 g·h^{-1} (21 ± 4%) (mean difference 9, 95% CI [5, 13] %, Cohen's $d = 1.73$; mean difference 10, 95% CI [6, 14] %, Cohen's $d = 1.97$ for 120 vs. 90 g·h^{-1}, and 90 vs. 60 g·h^{-1}, respectively). In contrast, the contribution of fat toward total energy expenditure was significantly lower in the 120 g·h^{-1} trial (35 ± 8%) compared with both the 90 g·h^{-1} (49 ± 8%) (mean difference 14%

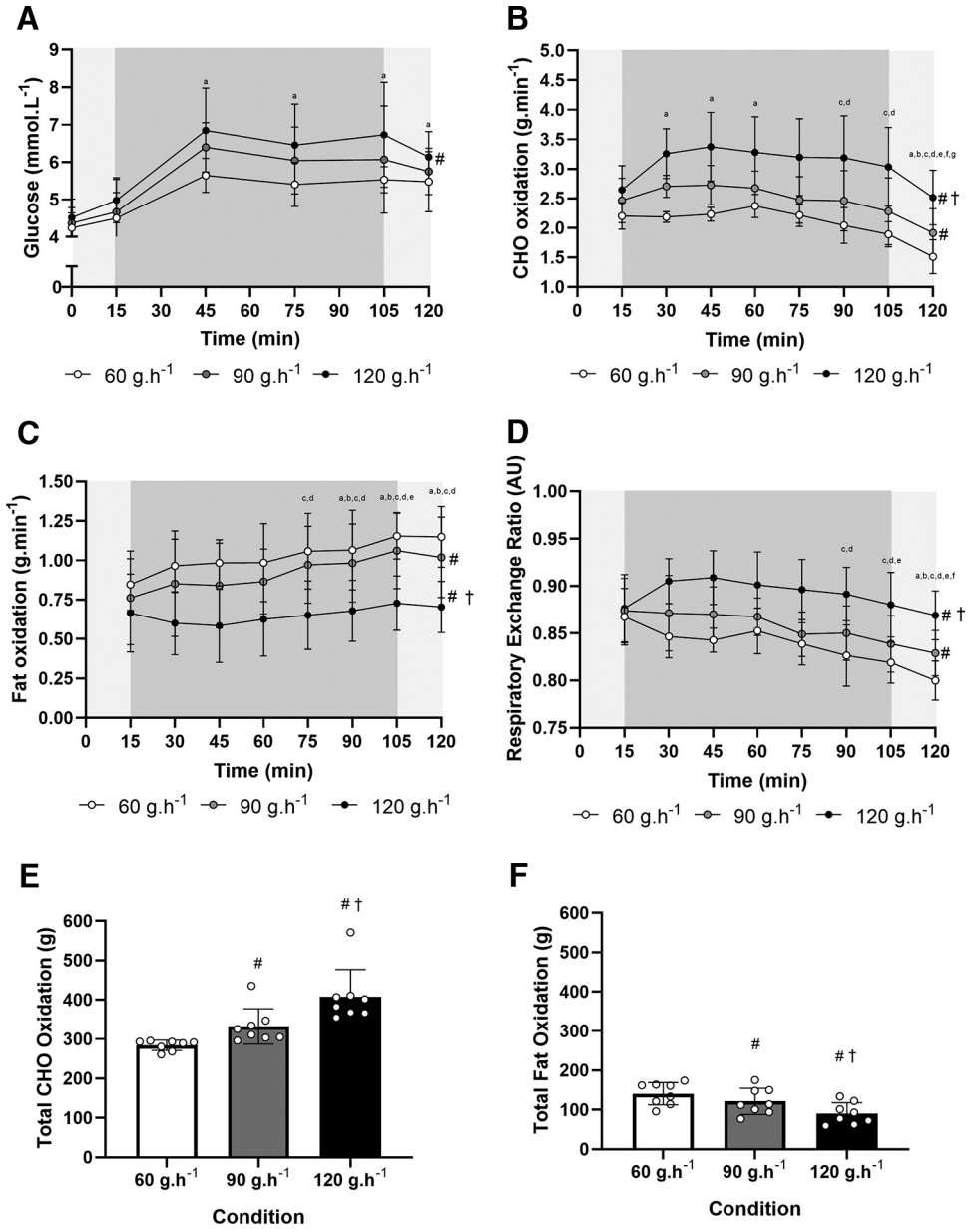

Figure 3. Blood glucose concentration (*A*), whole body CHO oxidation rate (*B*), fat oxidation rate (*C*), respiratory exchange ratio (RER) (*D*), total CHO use (*E*), and total fat (*F*) use during prolonged treadmill running with CHO ingestion at 60, 90, and 120 g·h^{-1}. Data are means ± SD for $n = 8$ elite male marathon runners. Statistical differences were analyzed using two-way repeated-measures ANOVA with Holm–Bonferroni correction; total carbohydrate and fat use were analyzed using one-way repeated measures ANOVA. a–g denotes $P < 0.05$ vs. [a]15, [b]30, [c]45, [d]60, [e]75, [f]90, and [g]105, respectively; #$P < 0.05$ vs. 60 g·h^{-1} trial; †$P < 0.05$ vs. 90 g·h^{-1} trial.

CI [11, 18] %, Cohen's $d = 3.57$) and 60 g·h^{-1} trials (57 ± 6%) (mean difference 22% CI [13, 31] %, Cohen's $d = 2.11$) (both $P < 0.001$). In addition, the contribution of fat was significantly greater in the 60 g·h^{-1} trial compared with the 90 g·h^{-1} trial ($P < 0.001$) (mean difference 9% CI [0.04, 16] %, Cohen's $d = 0.83$). Such data collectively demonstrate that CHO dependency is only maintained with CHO ingestion rates of 120 g·h^{-1} (i.e. 66% CHO contribution), whereas ingestion rates of 90 and 60 g·h^{-1} result in a transition toward fat dependence during the second hour of exercise (Fig. 5*F*).

Effects of Dose of CHO Ingestion on Gastrointestinal Discomfort and Drink Palatability

The incidence of moderate or severe (≥4) GI symptoms was high across all conditions (see Fig. 6). All runners reported experiencing one or more moderate or severe symptoms. Cumulative gastrointestinal (GI) symptom scores did not differ significantly [$P = 0.206$; between CHO doses 52 (30–126); 59 (30–153); 85 (40–127) for 60, 90, and 120 g·h^{-1}, respectively]. However, peak scores for nausea ($P = 0.01$), stomach fullness ($P = 0.026$), and abdominal cramps ($P = 0.012$) varied between conditions. Nausea scores were significantly higher at 120 g·h^{-1} compared with both 60 g·h^{-1} ($P = 0.021$, mean difference 3, 95% CI [2, 5] AU, $r = 1$) and 90 g·h^{-1} ($P = 0.041$, mean difference 3, 95% CI [2, 6] AU, $r = 1$). Stomach fullness was also reported to be greater at 120 g·h^{-1} than at 60 g·h^{-1} ($P = 0.004$, mean difference 2, 95% CI [1, 3] AU, $r = 0.89$) and 90 g·h^{-1} ($P = 0.014$, mean difference 2, 95% CI [1, 2] AU, $r = 1$). In addition, abdominal cramps were significantly different between conditions, with higher scores at 120 g·h^{-1} compared with 60 g·h^{-1} ($P = 0.005$, mean difference 2, 95% CI [1, 5] AU, $r = 1$) and at 90 g·h^{-1} ($P = 0.050$, mean difference 1, 95% CI [−2, 3] AU, $r = 0.42$). Cochran's Q test revealed a significant difference in symptom prevalence across conditions ($Q = 6.4$; $P = 0.040$) for nausea. Post hoc McNemar comparisons (Holm-adjusted)

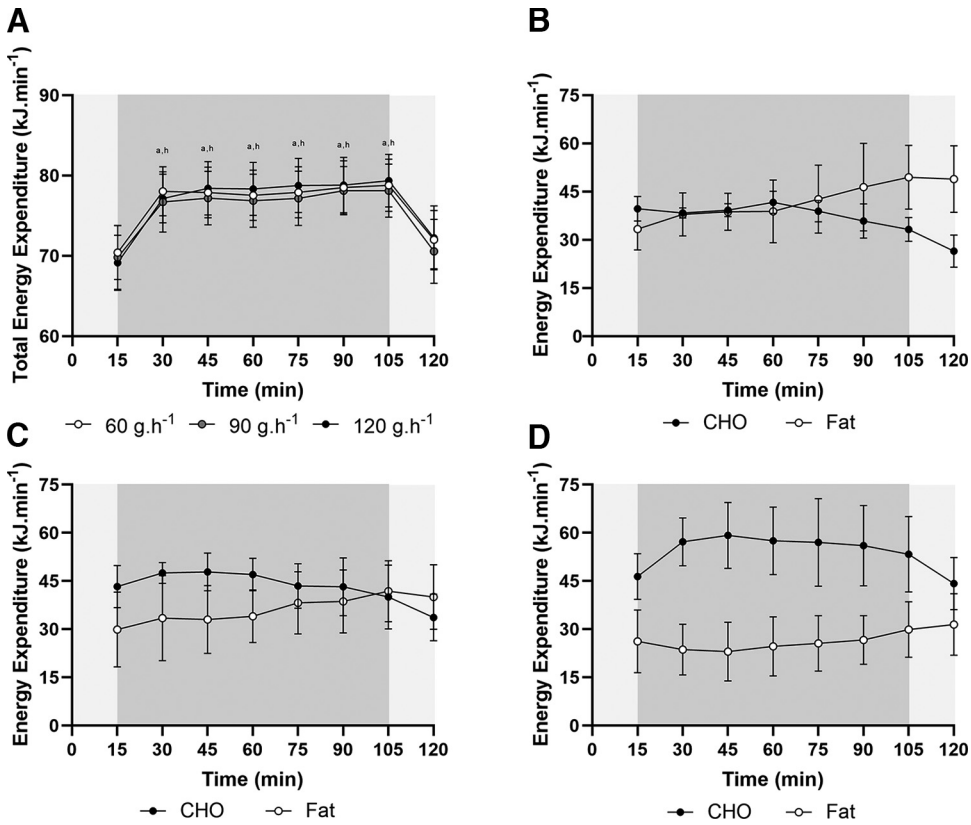

Figure 4. *A*: total energy expenditure during prolonged treadmill running. Energy expenditure derived from CHO and fat for 60 g·h^{-1} (*B*), 90 g·h^{-1} (*C*), and 120 g·h^{-1} (*D*). Data are means ± SD for $n = 8$ elite male marathon runners. For total energy expenditure, statistical differences were analyzed using two-way repeated-measures ANOVA with Holm–Bonferroni correction; a–h denotes $P < 0.05$ vs. [a]15, [b]30, [c]45, [d]60, [e]75, [f]90, [g]105, and [h]120 min, respectively.

were not significant: 60 versus 90 ($P = 1.00$; matched OR = 1.00, [0.03, 38.49]); 60 vs. 120 ($P = 0.133$; matched OR = ∞, [0.90, ∞]); 90 vs. 120 ($P = 0.133$; matched OR = ∞, [0.90, ∞]). A greater proportion of participants reported nausea in the 120 g·h^{-1} trial (75%) compared with 90 g·h^{-1} (25%) and 60 g·h^{-1} (25%). Similarly, a significant difference was observed for abdominal cramps ($Q = 6.33$; $P = 0.042$), with no differences in Post hoc McNemar comparisons (Holm-adjusted) 60 vs. 90 ($P = 0.479$; matched OR = ∞, [0.29, ∞]); 60 vs. 120 ($P = 0.073$; matched OR = ∞, [1.22, ∞]); 90 vs. 120 ($P = 0.371$; matched OR = 4.00, [0.52, 96.98]). Abdominal cramps were reported by 37.5% of participants in the 120 g·h^{-1} trial, compared with 12.5% in the 90 g·h^{-1} and 12.5% 60 g·h^{-1} trials. Notably, all eight participants reported symptoms of stomach fullness above four for all conditions, resulting in 100% prevalence of moderate to severe symptoms across the 120 g·h^{-1}, 90 g·h^{-1}, and 60 g·h^{-1} trials for stomach fullness. Perception of drink sweetness was significantly greater at 120 g·h^{-1} 8 (5–9) compared with 60 g·h^{-1} 3 (1–6), ($P = 0.006$, mean difference 5, 95% CI [3, 6] AU, $r = 1$) and 90 g·h^{-1} 5 (4–7), ($P = 0.020$, mean difference 2, 95% CI [1, 3] AU, $r = 1$). The urge to drink and drink pleasantness were low across all conditions with no difference between conditions (Fig. 7).

DISCUSSION

Confirming our hypothesis, we report for the first time that CHO ingestion during exercise increases whole body and exogenous rates of CHO oxidation in elite male marathon runners in a dose-dependent manner. In using an exercise duration (i.e., 2 h) and intensity that is somewhat representative of marathon pace (i.e., the majority of the exercise stimulus was completed at 94% of LTP), we observed that whole body rates of CHO oxidation and CHO dependency are only maintained with CHO ingestion rates of 120 g·h^{-1}. In contrast, CHO ingestion rates aligned to the current CHO guidelines of 60–90 g·h^{-1} are associated with a reduction in whole body CHO utilization and an accompanying transition toward fat dependence during the second hour of exercise (though we acknowledge the absence of isotopic or nitrogen balance markers does not account for protein oxidation). In using U-^{13}C glucose stable isotope tracers for glucose and fructose, we also report some of the highest rates of exogenous CHO oxidation observed to date in runners, with individual values ranging from 1.64 to 1.99 g·min^{-1} in the 120 g·h^{-1} trial. Of note, such changes in exercise metabolism were accompanied by differences in running economy such that higher CHO ingestion rates led to lower oxygen consumption rates. Taken together, this suggests a metabolic advantage of higher CHO doses. However, the high prevalence of GI symptoms across the doses of 60–120 g·h^{-1} (with symptoms of nausea, stomach fullness, and abdominal cramps greatest with 120 g·h^{-1}), along with low urge to drink, suggests that further investigations of practical strategies for athlete fueling are warranted.

The physiological requirements to run a sub-2-h marathon (3) has led to increased academic interest on the endogenous and exogenous carbohydrate requirements to sustain the required absolute intensity. Indeed, recent modeling has suggested that exogenous CHO requirements for males and females would be 93 ± 26 and 108 ± 22 g·h^{-1}, respectively (25).

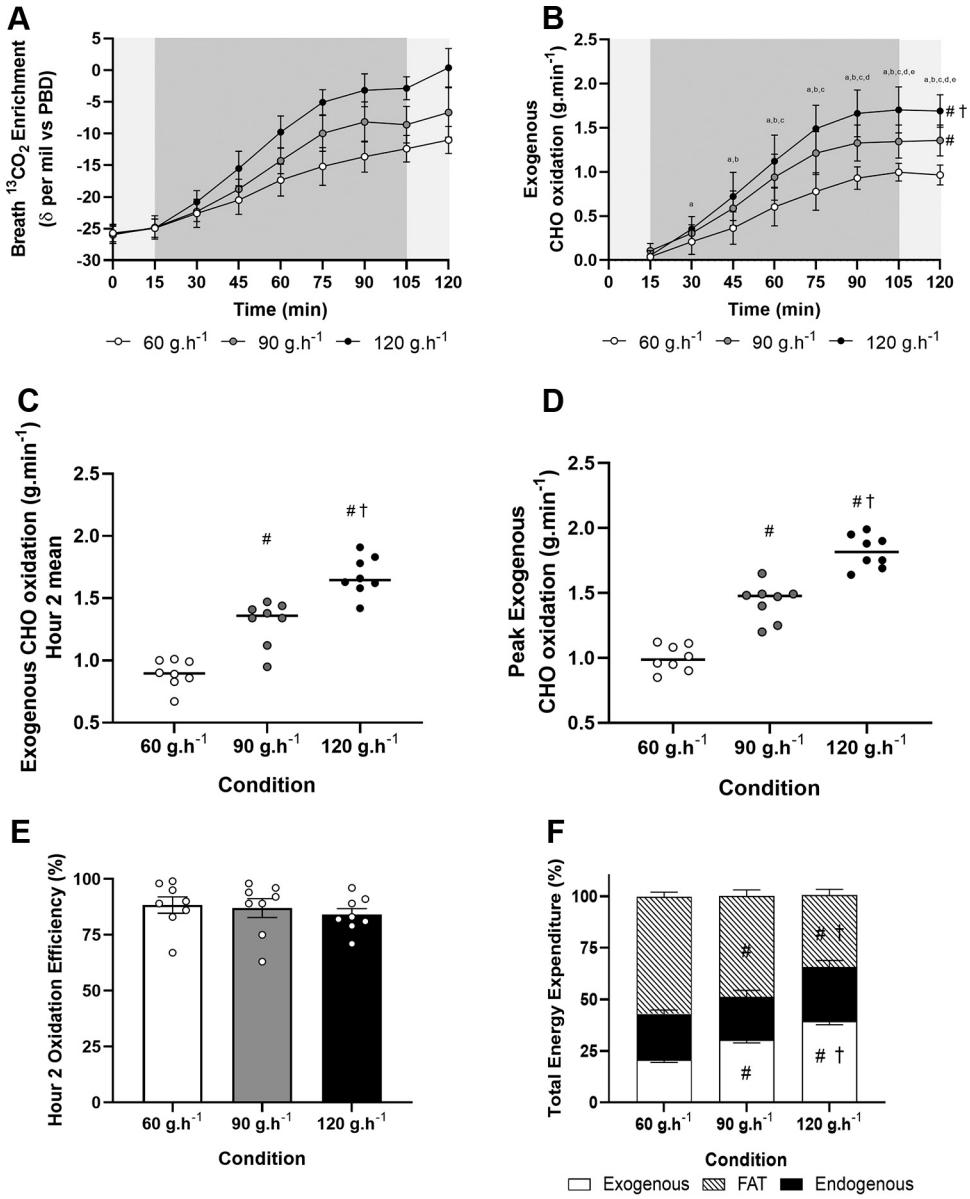

Figure 5. Breath $^{13}CO_2$ enrichment (*A*) and exogenous CHO oxidation (*B*) during prolonged treadmill running in the 60, 90, and 120 g·h^{-1} trials. *C*: individual participants' mean exogenous CHO during *hour 2* (*C*) and peak exogenous CHO oxidation (*D*) during prolonged treadmill running oxidation efficiency (*E*) and substrate contributions to total energy expenditure (*F*) during *hour 2* of exercise. Data are means ± SD for $n = 8$ elite male marathon runners. Statistical differences in exogenous CHO oxidation during exercise were assessed by two-way repeated-measures ANOVA; *hour 2* mean and peak exogenous CHO oxidation, oxidation efficiency, and substrate contribution were analyzed using one-way repeated measures ANOVA. with Holm–Bonferroni correction; a–h denotes $P < 0.05$ vs. [a]15, [b]30, [c]45, [d]60, [e]75, [f]90, [g]105, and [h]120 min, respectively. #$P <$ 0.05 vs. 60 g·h^{-1} trial; †$P < 0.05$ vs. 90 g·h^{-1} trial.

In that study, 65% of modeled runners (regardless of sex, body mass, and/or running economy) were suggested to need more than the current CHO recommendations of 90 g·h^{-1}. Nonetheless, the practicality and feasibility of consuming such doses are limited by the lack of direct observations on elite athletes. Accordingly, we collaborated with the England Athletics Endurance Program to recruit a cohort of male runners with personal best marathon times all faster than 2 h 30 min. Although we acknowledge that our chosen exercise protocol did not replicate the absolute running speeds required to run a sub-2 h marathon, our study holds ecological validity considering we clamped the majority of the exercise stimulus close to each participant's estimated race pace, i.e., 94% of LTP. Furthermore, participants completed each 2 h running protocol in conditions of high CHO availability, that is currently recognized as best practice, as achieved by 24 h of a standardized high CHO diet (8 g·kg^{-1}) and prerace meal (2 g·kg^{-1}). Our chosen CHO intervention utilized incremental doses of CHO ingestion such that absolute doses of 60 g·h^{-1} (single source, maltodextrin), 90 g·h^{-1} (dual source blend of maltodextrin and fructose in a 2:1 ratio), and 120 g·h^{-1} (dual source blend of maltodextrin and fructose in a 1:1 ratio) were ingested. Importantly, our form of CHO delivery was in fluid format so as to replicate the predominant method and frequency of CHO delivery that elite runners typically utilize during racing (125 mL every 15 min). The enrichment of drinks with both ^{13}C-glucose and ^{13}C-fructose tracers also allowed us to directly assess exogenous rates of CHO oxidation and efficiency. Though our CHO solutions were formulated using maltodextrin, it is considered that the use of [U-^{13}C] glucose as a tracer remains appropriate in this context. In contrast to the utilization of an insoluble starch tracee (45), the utilization of maltodextrin (as a soluble α-1,4 glucose polymer) is rapidly hydrolyzed by intestinal enzymes to free glucose before absorption. Once hydrolyzed, maltodextrin-derived glucose and the ingested ^{13}C-glucose tracer enter the same systemic pool via SGLT1/GLUT2 and follow identical oxidative pathways (46). Indeed, previous data from our

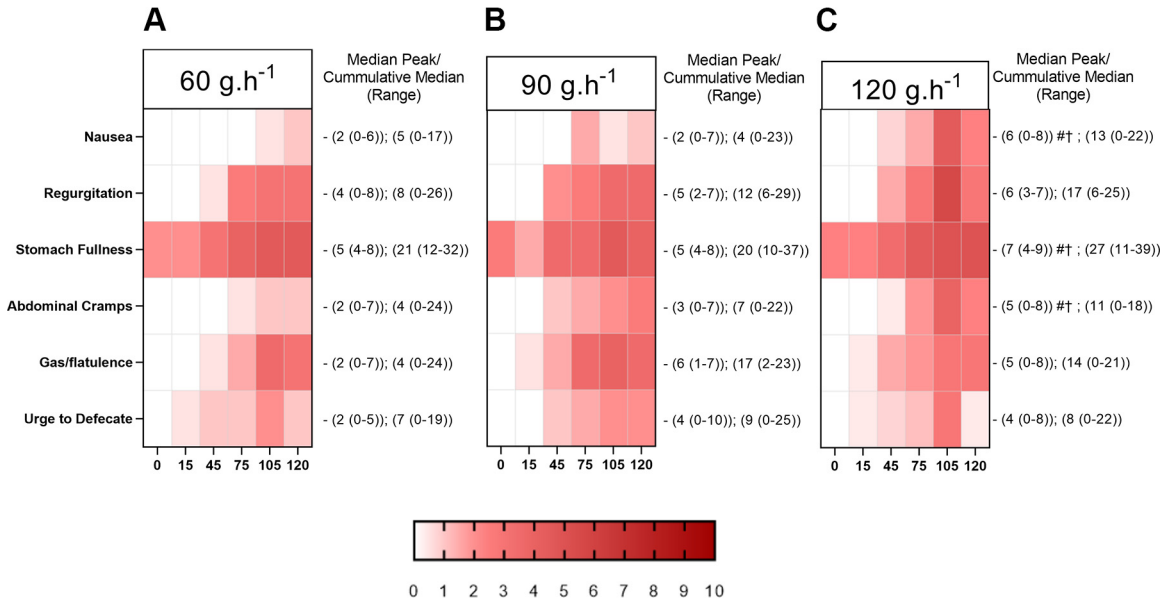

Figure 6. Gastrointestinal symptom progression during exercise in the 60 (*A*), 90 (*B*), and 120 g·h^{-1} (*C*) trials during prolonged treadmill running. Data are means ± SD for $n = 8$ elite male marathon runners. Statistical differences in symptom progression across time were analyzed using Friedman's ANOVA.

laboratory demonstrated that plasma U-^{13}C glucose enrichment reached a plateau within 30–120 min of exercise when participants ingested 60 g·h^{-1} of a maltodextrin solution, thereby excluding differences in appearance kinetics between the maltodextrin solution and the U-^{13}C tracer (47). Such data would appear to validate the use of a mixture of naturally enriched maltodextrins with U-^{13}C glucose tracer to estimate the oxidation of maltodextrin during exercise, an approach utilized in multiple studies evaluating exogenous CHO oxidation (26, 45, 48). Furthermore, although total CHO intake reached 120 g·h^{-1} in the present study, the absolute maltodextrin dose studied here (i.e., 60 g·h^{-1}) was constant across trials and matched the dose previously used by Pugh et al. (47). Moreover, we also observed that *1*) breath ^{13}C-glucose enrichment reached and maintained a plateau during exercise across trials (Fig. 5*B*), *2*) breath $^{13}CO_2$ exhibited the expected time course and dose-response (Fig. 5*A*), and *3*) exogenous CHO oxidation increased with intake in a dose-response manner (Fig. 5*B*). Nevertheless, this remains a methodological limitation, and for definitive validation, it is acknowledged that future studies should directly compare U-^{13}C glucose with a labelled maltodextrin tracer under identical ingestion rates (i.e., 60 g·h^{-1}).

To our knowledge, the present study represents the first direct assessment of exogenous CHO oxidation in elite male marathon runners. Our results clearly demonstrate a dose-response effect whereby peak exogenous CHO oxidation rates increased in accordance with absolute CHO ingestion (see Fig. 5*B*). Our study extends previous evaluations of exogenous CHO oxidation in runners (31, 33, 39, 49) and we report some of the highest exogenous oxidation rates observed in runners to date, with peak rates of 1.00 ± 0.10 g·min^{-1}, 1.48 ± 0.13 g·min^{-1}, and 1.77 ± 0.13 g·min^{-1} with CHO ingestion rates of 60, 90, and 120 g·h^{-1}, respectively. Although peak values provide useful context, we acknowledge that single time points can overestimate utilizable carbohydrate if considered in isolation. Accordingly, we also report mean values during *hour 2* of exercise (0.89 ± 0.11 g·min^{-1}, 1.31 ± 0.18 g·min^{-1}, and 1.68 ± 0.16 g·min^{-1} for 60, 90, and 120 g·h^{-1}), which in our data set closely aligned with the peak responses. In relation to the latter, these values are comparable to recent observations from our laboratory in trained male cyclists, where we observed peak exogenous CHO oxidation rates of ∼1.6 g·min^{-1} when 120 g·h^{-1} (1:0.8 ratio of maltodextrin to fructose) was administered in fluid format (26). High rates of exogenous CHO oxidation in cyclists (i.e., 1.5–2.0 g·min^{-1} with ingestion rates 90–120 g·h^{-1}) are now well documented within the literature (26, 50, 51).

An evaluation of existing studies clearly demonstrates that exogenous CHO oxidation rates do not equate to ingestion rates, with oxidation efficiencies typically reported in the range of 70%–90% (52, 53). Recently, Podlogar et al. (51) reported higher oxidation efficiencies with 90 g·h^{-1} (86 ± 10%) using a 2:1 maltodextrin:fructose ratio compared with 120 g·h^{-1} of CHO (76 ± 11%) with a 1:0.8 ratio, with higher exercise intensities. In the present study, we observed high oxidation efficiencies across all conditions (89 ± 4% at 60 g·h^{-1}, 87 ± 4% at 90 g·h^{-1}, and 84 ± 3% at 120 g·h^{-1}), with no significant difference between doses. It is possible that during exercise above LT associated with increased (albeit stable) metabolic acidosis, the respiratory exchange ratio (RER) may provide an inaccurate estimation of substrate utilization, primarily due to the increased release of nonrespiratory CO_2 and depletion of the labile bicarbonate pool (54). However, stoichiometric calculations remain valid under these conditions, as $\dot{V}co_2$ continues to reflect tissue-level CO_2 production with reasonable accuracy up to intensities of 80%–85% $\dot{V}o_{2max}$ (55). The high CHO oxidation efficiencies observed here may then be due to the participants' elite training status and associated metabolic adaptations, together with the running modality and high exercise intensity. In addition, the use of a 1:1 ratio of maltodextrin and

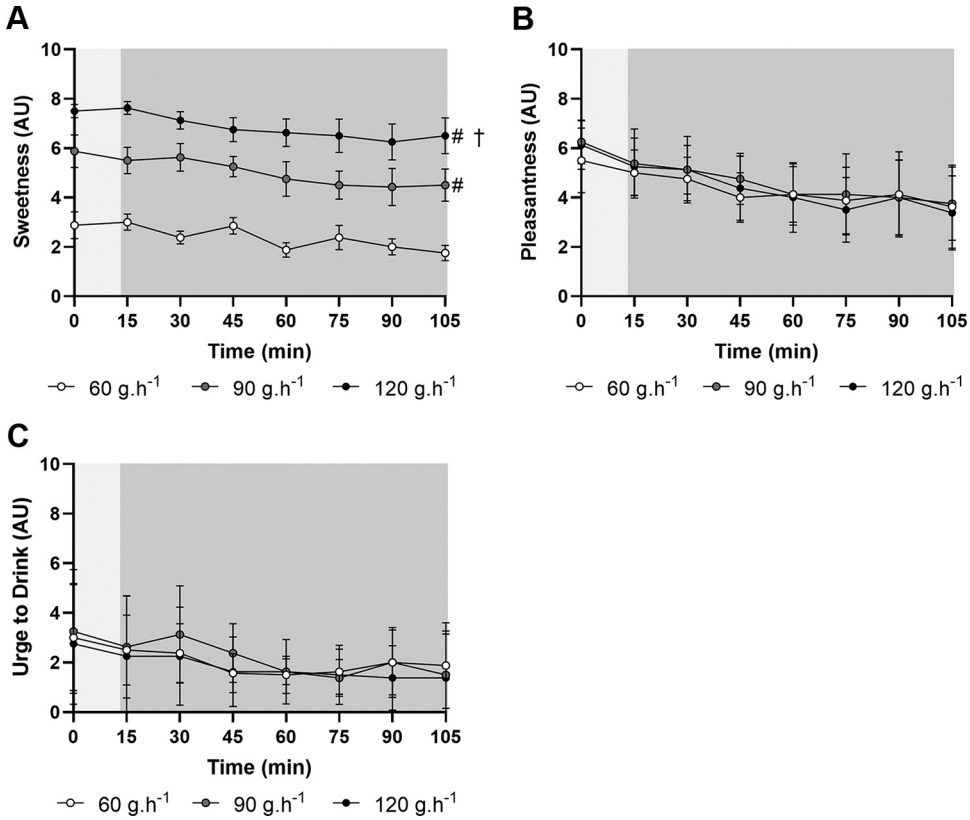

Figure 7. Drink sweetness (*A*), drink pleasantness (*B*), and urge to drink (*C*) during the 60, 90, and 120 g·h^{-1} trials during prolonged treadmill running. Data are means ± SD for $n = 8$ elite male marathon runners. Statistical differences in symptom progression across time were analyzed using Friedman's ANOVA. #Significance from the 60 g·h^{-1} trial and †significance from the 90 g·h^{-1}.

fructose (as opposed to 2:1 or 1:0.8 ratio) may have also contributed to the high oxidation efficiencies observed here (53).

The effects of increasing the dose of CHO ingestion during exercise had profound effects on whole body substrate metabolism. Indeed, although it has long been posed that both half-marathon (56) and marathon running (57) are CHO dependent, the present study demonstrates that rates of whole body CHO oxidation and CHO dependency (i.e., 65% CHO contribution during the 2nd hour of "simulated" marathon running) are only maintained with the higher CHO ingestion rate of 120 g·h^{-1}. Indeed, 120 g·h^{-1} prevented the occurrence of a metabolic crossover point (i.e., the timepoint during exercise at which fat provides the greater contribution toward total energy expenditure). In contrast, ingestion rates of 90 and 60 g·h^{-1} resulted in an apparent transition toward fat dependence whereby fat provided 49% and 54% of the energy contribution during the second hour of exercise, versus 40% and 46% of the energy contribution in the first hour, respectively. Collectively, these results demonstrate that fat does not provide a negligible contribution to energy production at intensities close to marathon race pace but, rather, provides an obligatory role in sustaining ATP production. Nevertheless, it must be noted that the reliance solely on indirect calorimetry to assess substrate oxidation does not account for potential protein oxidation, as the RER for protein (∼0.80) lies closer to fat (∼0.75) than carbohydrate (1) (57). Accordingly, when nitrogen excretion is not quantified and nonprotein stoichiometric equations are applied, any resultant lowering of RER may be partly attributable to protein oxidation (potentially biasing estimates toward greater fat oxidation rates, particularly with low glycogen availability), giving rise to greater leucine oxidation and greater negative net balance (57, 58). However, in nitrogen-corrected protocols during 120 min of prolonged treadmill running, protein contributed ∼4%–5% of total energy and was not affected by carbohydrate feeding during exercise (2 g·min^{-1}), indicating that under well-fed conditions, the likely misclassification is small (59). Therefore, the capacity for high rates of fat oxidation (i.e., ≥1 g·min^{-1}) at these high relative intensities and absolute running speeds could also be due to the elite training status and extensive training history of the participants and, in the context of the present study, also reflects limited CHO availability during the second hour of exercise.

It is noteworthy that even with ingestion rates of 120 g·h^{-1}, CHO dependency slightly decreased from *hour 1* (69%) to *hour 2* (65%). In relation to running a sub-2 h marathon (i.e., necessitating higher absolute running speeds and associated CHO requirement), this provides further support that CHO ingestion rates of 90–120 g·h^{-1} are likely to confer a metabolic advantage compared with traditional recommendations of 60–90 g·h^{-1}. Furthermore, a significant metabolic advantage was also observed as the O_2 cost of running was lower in the 120 g·h^{-1}. This preferential reliance on CHO is logical, as the energy yield from CHO oxidation is more efficient than that of fat, providing greater energy per liter of O_2 consumed (60). Minimizing the decline in running efficiency over time would likely enhance physiological resilience and support the preservation of critical speed during prolonged efforts (61). Nonetheless, given the limited capacity for both muscle and liver glycogen storage in elite marathoners (and that is accessible to active muscle), the present results also suggest that elite runners must possess a high capacity for fat oxidation even at race pace. Indeed, unpublished observations from our

laboratory on a male Ethiopian distance runner (body mass 53.2 kg) during incremental exercise testing demonstrated whole body CHO and fat oxidation rates of 3.79 (75% energy contribution) and 0.57 g·min^{-1} (25% energy contribution) with an RER of 0.96 when running at 21 km·h^{-1}, respectively. Clearly, further research is needed to directly quantify the energetic requirements and associated CHO cost to readily meet the physiological demands of running a marathon at the elite level. In this regard, the interaction of training and nutritional strategies (i.e., models of CHO periodization aligned to the principle of fueling for the work required) may permit the training adaptations that are likely necessary to simultaneously oxidize both carbohydrate and fat at such high intensities (62).

Despite the potential metabolic advantage associated with higher rates of CHO ingestion, there remain questions over the feasibility and practical application of such doses, largely due to issues regarding GI tolerability. Indeed, although we observed a high incidence of moderate or severe GI symptoms across the range of 60–120 g·h^{-1}, peak symptoms of nausea, stomach fullness, and abdominal cramps were reported in the 120 g·h^{-1} trial. Nonetheless, in considering that cumulative GI scores were not different between trials alongside previous observations of minimal GI symptoms when runners ingest up to 90 g·h^{-1} during lower intensity running protocols (31, 33, 49), it is possible that the GI disturbances frequently reported are perhaps more driven by exercise intensity, as opposed to CHO intake per se (63, 64). However, the CHO concentration could also play a role in symptom progression (52). In this regard, perturbations to GI homeostasis, driven by splanchnic hypoperfusion (65), altered gastric myoelectrical rhythms, and cumulative exercise stress (66) likely underpin symptom escalation during prolonged high-intensity exercise, thus suggesting that exercise-induced GI dysfunction, rather than CHO load alone, is a key modulator of nutrient tolerance and ingestion behavior.

Notwithstanding a lack of knowledge and awareness of CHO guidelines (67), such physiological mechanisms may explain, in part, the consistently low CHO intake that is self-reported during marathon running (68). For example, marathon runners reportedly consume relatively modest amounts of CHO (35 ± 26 g·h^{-1}), though individual intakes vary widely from as little as 6 g·h^{-1} to 136 g·h^{-1}. This variability suggests that only a small proportion of runners (<15%) consume CHO at levels exceeding the upper limits of current guidelines (68). However, despite the runners in the present study having experience of CHO intakes 100–120 g·h^{-1}, the decline in drink pleasantness over time and the consistently low urge to drink reported here (as evident in all trials) further suggest that GI issues may also limit habitual in-race fueling practices. To address potential gastrointestinal limitations, gut training (35, 69) and personalized CHO intakes (70) have been proposed to enhance CHO absorption, improve tolerance, and reduce symptoms. Notably, given that previous research from our laboratory has shown that CHO feeding forms do not affect exogenous CHO oxidation (26), behavioral strategies that promote an individualized approach to CHO intake (e.g., altering drink palatability through taste, temperature, fluid volume, and inclusion of alternative feeding forms such as gels) may help to practically achieve higher CHO ingestion rates and potentially increase GI tolerance.

Despite the novelty and practical relevance of our data, we acknowledge that this study had several limitations. Indirect calorimetry, which does not explicitly quantify protein oxidation, can bias estimates toward fat since the RER of protein (~0.80) is closer to fat (~0.75) than carbohydrate (1). As such, future studies should incorporate tracer-based methodology to directly quantify whole body protein oxidation. In addition, though we acknowledge an apparent upward trend in blood lactate during exercise (which may be interpreted as non-steady state conditions) it is noteworthy that this response was driven by *participants 2* and *6* (Supplemental Fig. S1) who lost steady-state during exercise. This is physiologically expected, as critical speed/LT2 declines with fatigue (71), but the majority remained in steady state, and inclusion or exclusion of these cases did not alter statistical inferences for either mean exogenous CHO oxidation during *hour 2* or peak exogenous CHO oxidation (Supplementary Tables S1 and S2). Therefore, stable blood lactate further supports the validity of indirect calorimetry-based substrate partitioning in this context (55). Because our study was primarily powered to detect changes in exogenous CHO oxidation, we also acknowledge our inability to identify smaller differences in other measures, such as GI symptoms, between trials. It is noteworthy that CHO concentration varied across drinks (24%, 18%, 12% at a fixed 500 mL·h^{-1}), hence differences between trials (i.e. exogenous CHO oxidation and GI symptoms) cannot be attributed solely to dose given that concentration-dependent differences in gastric emptying may also have contributed to the findings reported here. Future studies should therefore further evaluate the effects of dose, ratio, and concentration (52, 72) in an elite running cohort. Although we maintained a double-blind design, we also acknowledge that the choice to keep the flavor neutral meant that differences in sweetness and concentration likely made the CHO dose easily identifiable to participants. Furthermore, CHO was provided only in drink form, whereas athletes typically use a mix of drinks, gels, and other sources during competition. We also did not include a water-only trial, which would have served as a true placebo condition for comparison. The absence of performance-based measures also means that we were unable to directly assess the impact of CHO dose on exercise performance, and this clearly warrants future investigation.

In summary, the present data demonstrate for the first time a clear dose-response effect of CHO ingestion in trained male runners on both whole body and exogenous CHO oxidation during simulated marathon running. Importantly, our results suggest that an ingestion rate of 120 g·h^{-1} may confer a metabolic advantage (as also evidenced by improved running economy) compared with currently recommended doses of 60 and 90 g·h^{-1}. We also observed the highest rates of exogenous CHO oxidation yet reported in runners in the literature, with peak individual oxidation rates ranging from 1.64 to 1.99 g·min^{-1}. Nonetheless, the potential performance implications of such higher CHO doses remain to be evaluated, and the high prevalence of GI symptoms across all conditions suggests that marathon runners would likely benefit from strategies that enhance CHO tolerance and mitigate GI distress.

DATA AVAILABILITY

Data will be made available upon reasonable request.

SUPPLEMENTAL MATERIAL

Supplemental Fig. S1 and Supplemental Tables S1 and S2: https://doi.org/10.6084/m9.figshare.30432313.v3.

GRANTS

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

S.R., K.O.J., A.M.J., J.P.M., and J.N.P. conceived and designed research; S.R., K.G.S., and H.J.M. performed experiments; S.R. and T.M.B. analyzed data; S.R., T.M.B., D.J.O., J.P.M., and J.N.P. interpreted results of experiments; S.R. prepared figures; S.R., D.J.O., J.P.M., and J.N.P. drafted manuscript; S.R., J.B.L., T.M.B., D.J.O., A.M.J., J.P.M., and J.N.P. edited and revised manuscript; S.R., K.G.S., H.J.M., J.B.L., T.M.B., D.J.O., A.M.J., J.P.M., and J.N.P. approved final version of manuscript.

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