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Human Kinetics
ORIGINAL RESEARCH

Frequent Carbohydrate Ingestion Reduces Muscle Glycogen Depletion and Postpones Fatigue Relative to a Single Bolus

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The timing of carbohydrate ingestion and how this influences net muscle glycogen utilization and fatigue has only been investigated in prolonged cycling. Past findings may not translate to running because each exercise mode is distinct both in the metabolic response to carbohydrate ingestion and in the practicalities of carbohydrate ingestion. To this end, a randomized, cross-over design was employed to contrast ingestion of the same sucrose dose either at frequent intervals (15 × 5 g every 5 min) or at a late bolus (1 × 75 g after 75 min) during prolonged treadmill running to exhaustion in six well-trained runners ($\dot{V}O_2$max 61 ± 4 ml·kg^{-1}·min^{-1}). The muscle glycogen utilization rate was lower in every participant over the first 75 min of running (Δ 0.51 mmol·kg dm^{-1}·min^{-1}; 95% confidence interval [–0.02, 1.04] mmol·kg dm^{-1}·min^{-1}) and, subsequently, all were able to run for longer when carbohydrate had been ingested frequently from the start of exercise compared with when carbohydrate was ingested as a single bolus toward the end of exercise (105.6 ± 3.0 vs. 96.4 ± 5.0 min, respectively; Δ 9.3 min, 95% confidence interval [2.8, 15.8] min). A moderate positive correlation was apparent between the magnitude of glycogen sparing over the first 75 min and the improvement in running capacity (r = .58), with no significant difference in muscle glycogen concentrations at the point of exhaustion. This study indicates that failure to ingest carbohydrates from the outset of prolonged running increases reliance on limited endogenous muscle glycogen stores—the ergolytic effects of which cannot be rectified by subsequent carbohydrate ingestion late in exercise.

Keywords: metabolism, sucrose, timing, treadmill running

Energy metabolism during moderate- to high-intensity exercise is predominantly supported by carbohydrate oxidation (Hawley & Leckey, 2015). Consequently, finite endogenous stores (i.e., glycogen) are progressively depleted and are implicated in the initiation of fatigue (Bergström et al., 1967; Ørtenblad & Nielsen, 2015). Supplementing endogenous energy stores through exogenous carbohydrate ingestion during exercise can postpone fatigue and improve performance (Stellingwerff & Cox, 2014). Despite decades of research culminating in duration-specific guidelines for the amount and type of carbohydrate to ingest during exercise (Jeukendrup, 2014), there is a limited evidence base regarding *when* carbohydrate should be ingested (i.e., the timing/pattern of nutrient delivery).

The timing of carbohydrate ingestion during exercise can mediate various physiological responses that impact performance; for instance, the timecourse of gastric emptying (Jeukendrup & Jentjens, 2000), and thus temporal variance in the availability of systemic metabolites, including glucose and nonesterified fatty acids (NEFA; Fielding et al., 1985; McConell et al., 1996; Schweitzer et al., 2009), which can subsequently regulate the delivery of the substrate to metabolically active tissues (Heesch et al., 2014). This process can provide a supplementary energy supply to exercising skeletal muscles and thus reduce the primary reliance on muscle glycogen as a fuel, although this response may differ between different exercise modalities. Specifically, the ingestion of mixed carbohydrate solutions throughout prolonged *running* results in lower rates of muscle glycogen degradation than a noncaloric placebo (Tsintzas et al., 1995; 1996a, 2001). In contrast, during prolonged exercise *cycling* carbohydrate ingestion does not result in muscle glycogen sparing; rather, the ergogenic effects operate via reduced hepatic glycogenolysis and maintenance of euglycemia and carbohydrate oxidation (Claassen et al., 2005; Coyle et al., 1986; 1991; Gonzalez et al., 2015). Prolonged running and cycling may therefore differ subtly in the mechanisms through which fatigue is initiated and/or the mechanisms through which carbohydrate ingestion can offset fatigue. This raises interesting questions about whether the timing of carbohydrate ingestion elicits similar metabolic and ergogenic effects across different modes of exercise.

The current state of understanding about when carbohydrate should be ingested during prolonged cycling rests predominantly on a series of experiments conducted by a single research group. This group investigated exogenous carbohydrate provision, during cycling, either via frequent ingestion (Coyle et al., 1986), via a single bolus ingested late in exercise (Coggan & Coyle, 1989), or via glucose infusion following fatigue (Coggan & Coyle, 1987). This systematic approach revealed that prolonged cycling capacity (70–75% $\dot{V}O_2$max) is extended by a similar magnitude (~45 min)

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when compared with water, regardless of the timing of exogenous carbohydrate provision. Therefore, Coggan and Coyle (1991) concluded that there is no practical advantage of any feeding schedule over another. However, this interpretation may not translate to prolonged running. Practically, runners have a more limited capacity than cyclists to carry and ingest foods during locomotion and anecdotally suffer from greater gastrointestinal (GI) discomfort (possibly due to the movement of the trunk churning stomach contents; Peters et al., 2000; Rudzki et al., 1995). Moreover, mechanistically, if the ergogenic effect of carbohydrate ingestion during cycling simply requires the maintenance of systemic glucose availability, then a single bolus ingested late during exercise can achieve this. Conversely, if carbohydrate ingestion benefits running performance by sparing muscle glycogen, then that effect must be imparted from the early stages of exercise (Tsintzas et al., 1996b); otherwise, the previous overreliance on muscle glycogen when no carbohydrates were ingested will not be compensated for by a later carbohydrate bolus.

This study compared the effects of ingesting carbohydrates frequently (FREQ) with a single late bolus (BOL) during exercise on running capacity and muscle glycogen utilization in trained male runners. We hypothesized that time to exhaustion (TTE) will be significantly greater in the FREQ condition and that net muscle glycogen utilization (i.e., magnitude of depletion) will be higher in the BOL condition prior to the carbohydrate bolus ingestion.

Methods

Participants

Six trained male runners participated in this study (mean ± *SD*: age, 21 ± 3 years; body mass, 69.9 ± 5.1 kg; height, 1.83 ± 0.03 m; $\dot{V}O_2$max, 61 ± 4 ml·kg^{-1}·min^{-1}). Preceding participation, the participants were briefed and provided written informed consent. This study was conducted in accordance with the Declaration of Helsinki and approved by the National Health Service South West—Frenchay Research Ethics Committee (18/SW/0177) and registered at clinicaltrials.gov (NCT03749785).

Preliminary Measurements

Submaximal and maximal oxygen uptakes were assessed on a motorized treadmill (Ergo ELG70; Woodway, Weil am Rein, Germany) using a modified, two-stage, incremental protocol (Taylor et al., 1955). On a separate occasion, ≥10 days prior to the experimental trials, the participants completed a familiarization trial. The participants consumed water ad libitum throughout each trial, following an overnight fast, and refrained from caffeine and alcohol ingestion for 12 hr and strenuous exercise for 24 hr preceding the trial. The participants completed 24-hr diet and exercise diaries before the trial, to enable a replication of these for the experimental trials. The initial speed was set in consultation with each participant to find a speed that would elicit fatigue after ~90 min, and small adjustments were made if required during the first 45 min. If the TTE during familiarization was between 75 and 105 min, then the final agreed speed that day was recorded and used for experimental trials; in the cases where the TTE during familiarization fell <75 min or >105 min, then minor adjustments were made to the final treadmill speed to ensure that the TTE would be closer to 90 min. This ensured that the carbohydrates would be ingested at a similar time prior to the initiation of fatigue. During all trials, the TTE was defined as the third volitional endpoint, excluding two 2-min walks at 4.4 km/hr (Walk 1/Walk 2). This approach in our laboratory reduces the coefficient of variation to 5.4% (Alghannam et al., 2016).

Experimental Design

The participants twice ran to exhaustion in a randomized order, separated by 16 ± 5 days. Each participant replicated the familiarization pretrial instructions and arrived at the laboratory at the same time of day (±2 hr) for both trials. Runs were performed at a previously established fixed intensity (mean ± *SD*; 13.4 ± 0.9 km/hr; 77 ± 4% $\dot{V}O_{2max}$). During both trials, the participants ingested a total of 75 g sucrose (granulated sugar; Silver Spoon, London, United Kingdom) and consumed 1,090 ml of fluid. The sucrose was mixed either into every bottle in 5 g portions (FREQ) or all in the final 250 ml drink (BOL), with the drinks taste matched through the addition of artificial sweetener (Aspartame, Saccharin; Figure 1). Muscle biopsies of *vastus lateralis* were obtained at rest, 75 min into the run, and exhaustion to assess the muscle glycogen concentrations.

Experimental Protocol

Postvoid body mass was recorded using electronic weighing scales (BC543 Monitor; Tanita, Tokyo, Japan) before beginning the experimental protocol (Figure 1). Muscle biopsies were sampled using the Bergstrom needle technique (Bergstrom, 1975). In any given trial, all muscle biopsies were sampled from distal to proximal from separate 0.5 cm incisions separated by 3 cm on the same leg to minimize biochemical artifacts (Van Thienen et al., 2014). The opposite leg was used in the second trial, with a counterbalancing of dominant/nondominant limbs. All incisions were made at rest under a local anesthetic (~5 ml of 1% lidocaine, Hameln Pharmaceuticals Ltd., Brockworth, United Kingdom), using a surgical blade, and were dressed prior to the run. Consequently, the times taken to obtain samples (mean ± *SD*; 75 min: 346 ± 229 s; exhaustion: 396 ± 150 s) and the time spent off the treadmill at 75 min (577 ± 326 s) were minimized. An indwelling cannula was inserted at rest into an antecubital vein to enable 5 ml blood samples to be drawn while running. Ratings of perceived exertion (RPE) and GI discomfort were assessed using 6–20 Borg scales (Borg, 1982; Stocks et al., 2016). One-minute expired gas samples were collected using the Douglas bag technique, with corrections for ambient fluctuations (Betts & Thompson, 2012), to calculate whole-body carbohydrate and lipid oxidation (Jeukendrup & Wallis, 2005). All drinks were prepared by one researcher (M. Wood) to maintain blinding of a separate researcher (C. Menzies) who provided verbal encouragement throughout. Blinding effectiveness was assessed via an exit interview, which revealed that three participants were able to correctly identify each condition.

Expired Gas, Blood, and Muscle Analysis

Expired gas samples were collected and analyzed following the procedures previously described in our laboratory (Chrzanowski-Smith et al., 2018). The blood samples were immediately dispensed into a tube containing the anticoagulant ethylenediaminetetraacetic acid and centrifuged at 2,500 *g* for 10 min at 4 °C (Heraeus Megafuge 16R, Thermo Fisher Scientific, Loughborough, United Kingdom). Plasma was aliquoted and stored at −80 °C until later analysis of glucose, NEFA, and lactate concentrations using a spectrophotometric analyzer (Daytona; Randox Laboratories Ltd.,

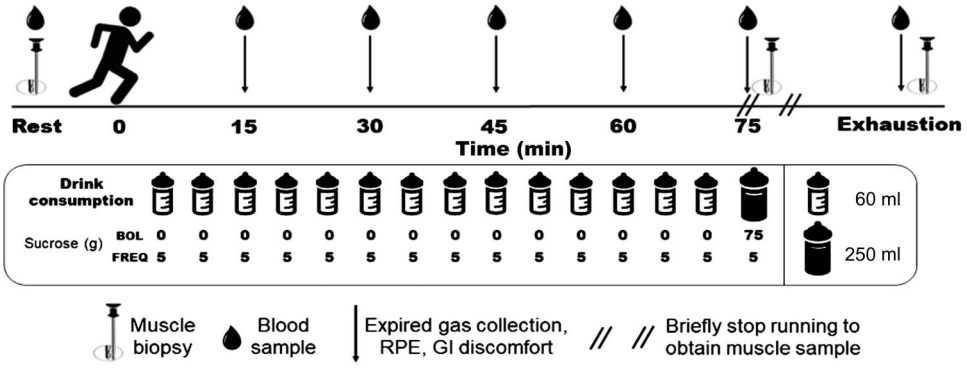

Figure 1 — Schematic of the protocol used during both experimental conditions. BOL = bolus; FREQ = frequently; RPE = ratings of perceived exertion; GI = gastrointestinal.

Crumlin, United Kingdom). The intra- and interassay coefficients of variation for these metabolites were <5% and <9%, respectively.

Each muscle sample was immediately removed from the biopsy needle and snap frozen in liquid nitrogen before being stored at −80 °C. After study completion, the samples were then placed overnight in a freeze dryer (Pirani 10; Edwards, West Sussex, United Kingdom) at approximately −50 °C. Visible blood and connective tissue were removed from the freeze-dried samples, which were then reduced to a fine powder using an agate pestle and mortar. The precise powder quantity obtained from each sample was determined using an electronic balance scale (AE240; Mettler, Greifensee, Switzerland) and stored at −80 °C alongside silica until later analysis for the determination of muscle glycogen. Briefly, the muscle powder was digested in 0.1 mM NaOH and neutralized with a HCl–citrate buffer, pH = 5.0. The glycogen present in the supernatant was hydrolyzed with α-amyloglucosidase and analyzed for glucosyl units by an enzymatic method, using a spectrophotometric plate reader (SpectraMax 190; Molecular Devices, San Jose, CA; Harris, Hultman, & Nordesjö, 1974). The intra-assay coefficient of variation was calculated at 5.8%, and all samples were analyzed in duplicate in a single assay. Total mixed muscle glycogen concentrations are reported as mmol glucosyl units per kilogram of dry muscle (dm) to avoid changes in concentration due to fluid shift during exercise. The contribution of muscle glycogen to whole-body carbohydrate metabolism was then calculated as the product of the measured utilization rate (g·kg dm^{-1}·min^{-1}) in *vastus lateralis* and 7.5% of the body mass, as described by Betts et al. (2008). The extramuscular (i.e., blood glucose) carbohydrate contribution was calculated as the difference between the rate of muscle glycogen degradation and the overall carbohydrate oxidation rate.

Statistical Analysis

Individual participants are represented by adjoining lines in the figures. Unless stated otherwise, the data presented are mean ± *SD*, the mean difference (Δ), and 95% confidence interval (CI). Within the figures, error bars depict normalized confidence intervals (nCIs) corrected for interindividual variation using the specific error term from the pairwise contrast at each timepoint (Masson & Loftus, 2003). Rather than describing the variability of individual values around the mean in each condition, the magnitude of these CIs provides a visual representation of the contrast between means such that, in general, plotted means whose CIs overlap by no more than half one side of an interval would typically generate a p value <.05 if using a paired t test at that timepoint. Correlations were explored using Pearson's r and interpreted according to Cohen (1988). No order effects were detected between trials for any variable. Analysis was conducted in IBM SPSS Statistics (version 24.0; IBM, Armonk, NY) and Microsoft Excel (version 16.23; Microsoft, Redmond, WA).

Results

Exercise Capacity

TTE was greater for all participants in FREQ compared with BOL (105.7 ± 3.0 vs. 96.4 ± 5.0 min; Δ 9.3 min; 95% CI [2.8, 15.8] min; Figure 2). This difference was also found at Walk 1 (94.7 ± 4.0 vs. 86.6 ± 4.9 min; Δ 8.1 min; 95% CI [1.9, 14.4] min) and Walk 2 (99.1 ± 3.2 vs. 91.0 ± 4.6 min; Δ 8.1 min; 95% CI [1.8, 14.5] min).

Substrate Utilization

The concentrations of glycogen measured in the *vastus lateralis* at each timepoint are illustrated in Figure 3 and demonstrate a decreased rate of muscle glycogen utilization in FREQ, compared with BOL, between rest and 75 min of exercise (104.2 ± 29.7 vs. 142.4 ± 55.5 mmol glucosyl units·kg dm^{-1}; Δ 38.2 mmol glucosyl units·kg dm^{-1}; 95% CI [−1.6, 78.1] mmol glucosyl units·kg dm^{-1}). There were no differences between the conditions in total whole-body carbohydrate oxidation, lipid oxidation, or total energy expenditure from rest to 75 min; accordingly, at a whole-body level, all participants used less muscle glycogen (1,719 ± 520 vs. 2,361 ± 979 kJ; Δ −641 kJ; 95% CI [−1,320, 37] kJ) and thus oxidized more extramuscular carbohydrate (2,733 ± 705 vs. 2,008 ± 1,258 kJ; Δ 726 kJ; 95% CI [17, 1,434] kJ) during FREQ versus BOL (Figure 4). There were no differences at exhaustion in muscle glycogen concentrations or carbohydrate and lipid oxidation between conditions.

Blood Metabolites

The plasma glucose concentrations were similar between conditions at all timepoints until 75 min (Figure 5). At exhaustion, the plasma glucose concentration was lower in FREQ than BOL (6.07 ± 0.97 vs. 7.12 ± 1.05 mmol/L; Δ −1.06 mmol/L; 95% CI [−1.74, −0.37] mmol/L), due to a decrease in concentration between 75 min and exhaustion that was not observed in the BOL condition. There were no differences at any timepoint between the conditions in plasma lactate concentrations (Figure 5). However,

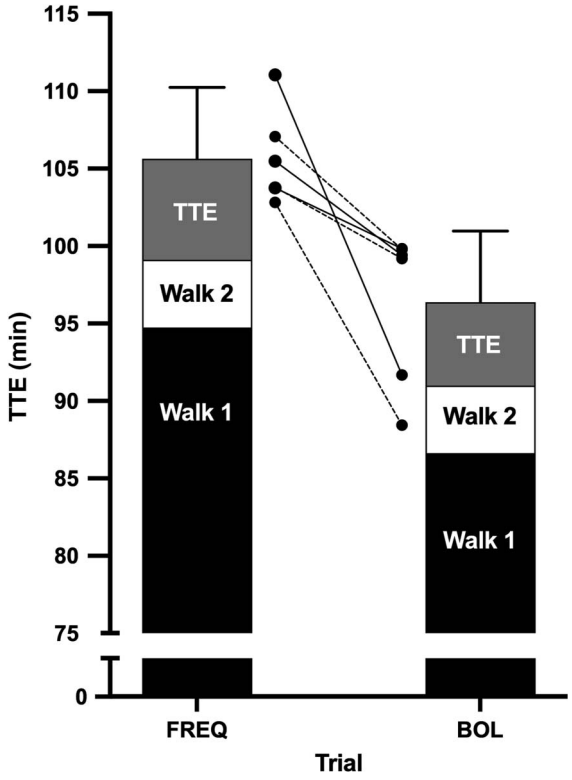

Figure 2 — Mean (bar) for Walk 1 (black), Walk 2 (white), and TTE (gray). Error bars represent 95% nCI around TTE. Solid lines represent individual differences in TTE. Nonblinded participants are represented by dashed lines. TTE = time to exhaustion; BOL = bolus; FREQ = frequently; 95% nCI = 95% normalized confidence interval.

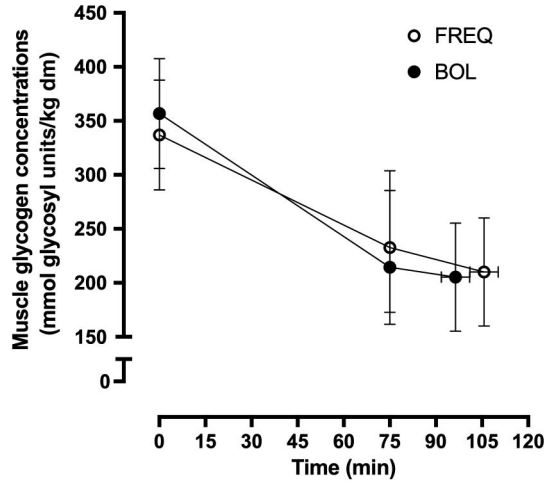

Figure 3 — Muscle glycogen concentrations at rest, 75 min, and exhaustion. Filled circles represent BOL; open circles represent FREQ. Error bars represent 95% nCI. BOL = bolus; FREQ = frequently; 95% nCI = 95% normalized confidence interval; dm = dry muscle.

at exhaustion, the plasma lactate concentration during BOL was lower compared with 75 min in the same condition (4.66 ± 1.72 vs. 2.95 ± 1.08 mmol/L; Δ −1.71 mmol/L; 95% CI [–0.70, 2.97] mmol/L). There were no differences in NEFA concentrations between conditions at any timepoint (Figure 5).

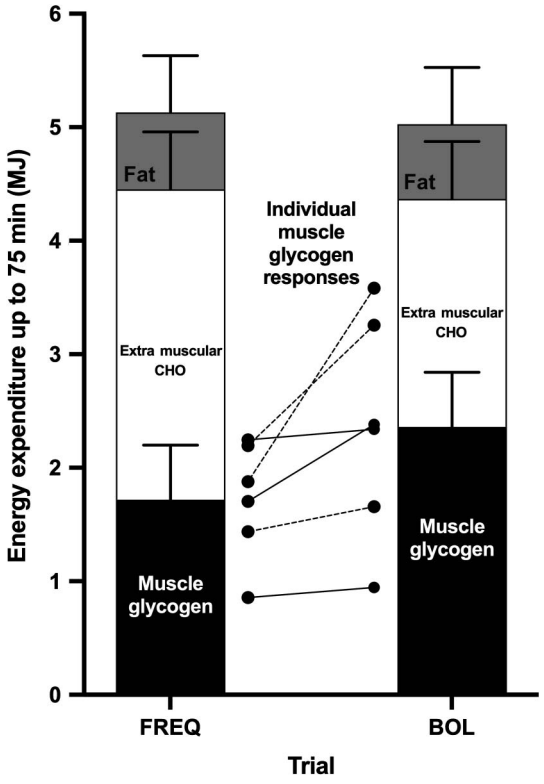

Figure 4 — Substrate utilization up to 75 min. Mean (bar) substrate utilization for muscle glycogen (black), extramuscular CHO (white), and fat (gray). Error bars represent 95% nCI. Lines represent individual differences in muscle glycogen utilization. Nonblinded participants are represented by dashed lines. BOL = bolus; CHO = carbohydrate; FREQ = frequently; 95% nCI = 95% normalized confidence interval.

Perceptual Measures

RPE and GI discomfort are summarized in Table 1. RPE increased throughout exercise and was greater during BOL after 45 and 60 min, but similar at all other timepoints. GI discomfort was greater during BOL at all measured timepoints.

Correlations

The differences between conditions in muscle glycogen utilization from rest to 75 min (glycogen spared) and TTE showed a moderate positive correlation ($r = .58$). TTE was not related to resting glycogen concentration ($r = -.03$), total glycogen utilization ($r = .09$), or GI discomfort ($r = -.10$).

Discussion

Running capacity was improved when ingesting carbohydrates in small, frequent doses compared with a single large bolus late in exercise. Muscle glycogen utilization was greater with no carbohydrate ingestion during the early running phase compared with frequent carbohydrate ingestion. These findings support the stated hypotheses and provide novel evidence that the timing of carbohydrate ingestion can influence exercise capacity and metabolism during running.

All participants had a greater TTE in FREQ than BOL, with a mean difference of 9.3 ± 6.2 min between conditions, representing a 10% mean increase in exercise capacity. As the participants

ingested equal amounts of carbohydrate in both conditions, it is unsurprising that this magnitude is less than previously reported when comparing either regular feeding (25–33%; Coyle et al., 1986; Tsintzas et al., 1996a) or a single bolus (21%; Coggan & Coyle, 1989) to a noncaloric placebo. However, the present magnitude is similar to the 14% increase in running capacity when comparing early carbohydrate feeding to water only (Tsintzas et al., 1996b). This similarity is likely due to the sparing of endogenous carbohydrate stores during the early running phase during the FREQ condition, with an inability to meet the energy requirements in the latter phase of the BOL and water conditions. In the present study, the mean energy requirement for running at the desired speed was 68 kJ/min, with only 9 kJ/min provided by fat oxidation. The remaining demand must be met by carbohydrate oxidation. In the BOL condition, muscle glycogen was depleted to a greater extent, and the maximal rate of exogenous carbohydrate appearance is *circa* 25 kJ/min. Therefore, the bolus feed late in exercise could not provide sufficient energy to meet the demands of running at the desired speed.

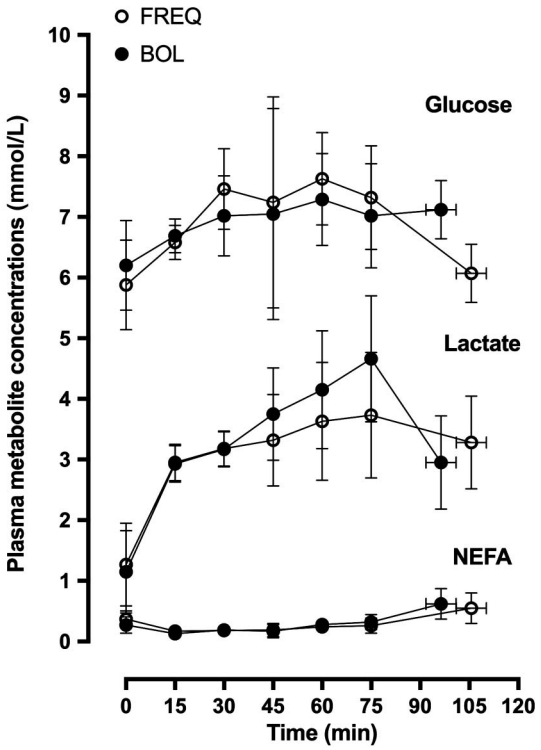

Figure 5 — Plasma concentrations of glucose (top), lactate (middle), and NEFA (bottom). Filled circles represent BOL; open circles represent FREQ. Error bars represent 95% nCI (n.b. blood samples were not obtained from one participant beyond 30 min in one condition and subsequently blood data are presented as $n = 5$ beyond that timepoint for both conditions). BOL = bolus; FREQ = frequently; NEFA = nonesterified fatty acids; 95% nCI = 95% normalized confidence interval.

At the point of exhaustion, muscle glycogen concentrations were ~200 mmol glucosyl units/kg dm in both conditions. This value has been suggested to represent a critical threshold at which there is a decrease in sarcoplasmic reticulum Ca^{2+} release (Ørtenblad et al., 2011), which causes decreased muscular force production and fatigue (Chin & Allen, 1997). Therefore, in the present study, muscle glycogen sparing delayed the arrival at this threshold and thus could represent the differential factor in running capacity between conditions. Based on the observed glycogen sparing, it would be tempting to calculate a theoretical difference in TTE and compare this with the observed response. However, this comparison is inappropriate, as the present study only measured total muscle glycogen concentration in the *vastus lateralis*. Muscle glycogen utilization is greater in Type 1 muscle fibers (Tsintzas et al., 1996a) and in the *triceps surae* muscles during running (Costill et al., 1974). Moreover, decreased sarcoplasmic reticulum Ca^{2+} release may be specific to the location of muscle glycogen, with the greatest association relating to the intramyofibrillar glycogen (Ørtenblad et al., 2011). Without these parameters, a theoretical difference in running capacity is not advised. Nevertheless, the moderate relationship observed between glycogen sparing and TTE ($r = .58$) supports the notion that glycogen sparing was a key factor in the observed increase in TTE during the FREQ condition.

The absence of a stronger relationship could suggest that other factors were involved in volitional exercise termination. Though severe GI discomfort can result in exercise termination (Hoffman & Fogard, 2011), no relationship was found between GI discomfort and exercise termination in the present study. Similarly, no differences in NEFA concentrations throughout exercise were observed, meaning that any NEFA-induced inhibition of glucose uptake to the exercising muscle was not systematically different between conditions. High glucose concentrations in the present study were likely a result of the high exercise intensity (77% $\dot{V}O_2$max). The similarity of blood glucose concentrations, despite the absence of feeding, is certainly surprising in the context of the cycling-based literature (Coyle et al., 1986), but is consistent with previous

Table 1 Median (Range) of RPE and GI Discomfort Throughout Exercise

	Time (min)					
	15	30	45	60	75	Fatigue
RPE						
FREQ	13 (12–14)	14.5 (13–16)	15 (14–16)	16.5 (15–18)	17.5 (16–20)	20 (17–20)
BOL	12 (10–13)	14.5 (13–16)	16.5 (15–18)	17 (16–20)	18 (17–20)	20 (18–20)
	4/0/2	**2/2/2**	**0/1/5**	**0/1/5**	**0/4/2**	**1/4/1**
GI discomfort						
FREQ	6.5 (6–8)	7.5 (6–11)	8.5 (6–12)	10 (6–13)	10.5 (7–18)	8 (6–14)
BOL	8 (6–10)	9.5 (6–12)	10 (6–13)	12 (6–14)	12 (6–16)	10 (9–14)
	0/3/3	**0/1/5**	**0/1/5**	**0/1/5**	**1/0/5**	**1/1/4**

Note. Rows in bold show the number of participants who reported RPE or GI discomfort to be FREQ > BOL/FREQ = BOL/FREQ < BOL, respectively. RPE = ratings of perceived exertion; GI = gastrointestinal; FREQ = frequently; BOL = bolus.

running studies (Tsintzas et al., 1996a, 1996b). Despite a decrease in plasma glucose concentrations at exhaustion in the FREQ condition, this was still well above the values considered for hypoglycemia-induced fatigue (6.1 vs. ~3.5 mmol/L; Coggan & Coyle, 1991), although it has been speculated that rapid decrements in blood glucose late in exercise may be relevant to fatigue, even when euglycemia is maintained (Claassen et al., 2005). This may have resulted in an earlier termination of exercise in the FREQ condition and, therefore, may explain some of the additional variance in running capacity between conditions. This decrease may have been caused by participants no longer ingesting carbohydrate after 75 min and thus reinforces the notion that, once feeding has begun, it should continue for the remainder of the exercising period (Coyle, 2004). This highlights that, although this study demonstrates that running capacity is greater with frequent feeding versus a single late bolus, recommendations on optimal timing for carbohydrate ingestion during prolonged running cannot be made until more research is conducted.

In conclusion, this study found that ingesting carbohydrate as 75 g sucrose in small doses of 5 g every 5 min enhances running capacity compared with ingestion of the same amount of carbohydrate in a single bolus after 75 min. Moreover, during the first 75 min, muscle glycogen was spared in the frequent feeding condition, and this was associated with an increased exercise capacity. This study indicates that failure to ingest carbohydrates from the outset of prolonged running increases reliance on limited endogenous muscle glycogen stores—the ergolytic effects of which cannot be rectified by subsequent carbohydrate ingestion late in exercise.

Acknowledgments

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