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Altering fatty acid availability does not impair prolonged, continuous running to fatigue: evidence for carbohydrate dependence

Jill J. Leckey,[1] Louise M. Burke,[2] James P. Morton,[3] and John A. Hawley[1,3]

[1]*Centre for Exercise and Nutrition, Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, Australia;* [2]*Sports Nutrition, Australian Institute of Sport, Canberra, Australia; and* [3]*Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, United Kingdom*

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Leckey JJ, Burke LM, Morton JP, Hawley JA. Altering fatty acid availability does not impair prolonged, continuous running to fatigue: evidence for carbohydrate dependence. *J Appl Physiol* 120: 107–113, 2016. First published November 19, 2015; doi:10.1152/japplphysiol.00855.2015.—We determined the effect of suppressing lipolysis via administration of nicotinic acid (NA) on fuel substrate selection and half-marathon running capacity. In a single-blinded, Latin square design, 12 competitive runners completed four trials involving treadmill running until volitional fatigue at a pace based on 95% of personal best half-marathon time. Trials were completed in a fed or overnight fasted state: *1*) carbohydrate (CHO) ingestion before (2 g CHO·kg^{-1}·body mass^{-1}) and during (44 g/h) [CFED]; *2*) CFED plus NA ingestion [CFED-NA]; *3*) fasted with placebo ingestion during [FAST]; and *4*) FAST plus NA ingestion [FAST-NA]. There was no difference in running distance (CFED, 21.53 ± 1.07; CFED-NA, 21.29 ± 1.69; FAST, 20.60 ± 2.09; FAST-NA, 20.11 ± 1.71 km) or time to fatigue between the four trials. Concentrations of plasma free fatty acids (FFA) and glycerol were suppressed following NA ingestion irrespective of preexercise nutritional intake but were higher throughout exercise in FAST compared with all other trials ($P < 0.05$). Rates of whole-body CHO oxidation were unaffected by NA ingestion in the CFED and FAST trials, but were lower in the FAST trial compared with the CFED-NA trial ($P < 0.05$). CHO was the primary substrate for exercise in all conditions, contributing 83-91% to total energy expenditure with only a small contribution from fat-based fuels. Blunting the exercise-induced increase in FFA via NA ingestion did not impair intense running capacity lasting ~85 min, nor did it alter patterns of substrate oxidation in competitive athletes. Although there was a small but obligatory use of fat-based fuels, the oxidation of CHO-based fuels predominates during half-marathon running.

carbohydrate; high-intensity running; nicotinic acid; substrate utilization; performance

THE MAJOR GOAL OF ENDURANCE training is to induce physiological adaptations that increase an athlete's ability to sustain the highest average power output or speed of movement for a given distance or time (14), reduce the oxygen cost ($\dot{V}O_2$) of locomotion, and maintain a higher fractional utilization of maximal oxygen uptake ($\dot{V}O_{2max}$) during training and competition (9). Such adaptations depend in part on the rate at which chemical energy [i.e., fat and carbohydrate (CHO)] can be converted into mechanical energy for skeletal muscle contraction (14). In most endurance events, a mix of substrates and energy-producing pathways contribute to work outputs, and athletes pursue training/dietary strategies that increase the overall capacity of these pathways, and they implement acute competition strategies that ensure optimal substrate availability to meet the energy cost of the event.

Although the absolute oxidation rate of all energy substrates increases at the high exercise intensities and power outputs sustained by athletes in training and competition, CHO-based fuels are the predominant energy source (4, 6, 17). However, recent attention has focused on diet-exercise strategies that reduce skeletal muscle dependence on CHO-based fuels (i.e., muscle and liver glycogen, blood glucose, lactate) before and during exercise while concomitantly maximizing fat oxidation [adipose and intramuscular triglycerides (TGs), and blood-borne free fatty acids (FFAs) and TGs] (33). It has been proposed that such strategies will enhance performance by promoting greater utilization of fat-based fuels whose availability is relatively unlimited (33). However, even when these strategies promote rates of fat oxidation that are substantially higher than those achieved by the effects of endurance training alone, there is no clear evidence of a performance benefit (7, 13, 30). Indeed, rates of muscle fat oxidation are inadequate to support the high relative (70–90% $\dot{V}O_{2max}$) and absolute work rates sustained by competitive athletes during running or cycling events lasting <2 h (17, 22, 32, 34).

An alternative strategy to test the role of fat availability to the performance of endurance sports is to investigate scenarios in which the muscle's access to fatty substrates is impaired. Accordingly, in the present study we administered the pharmacological agent nicotinic acid (NA) during simulated half-marathon running in both fed and overnight-fasted states. We hypothesized that suppressing lipolysis via NA ingestion would not alter substrate selection or have a detrimental effect on half-marathon running capacity because CHO-based and not fat-based fuels support optimal endurance exercise up to ~90 min.

METHODS

Participants. Twelve competitive male runners who had completed a half-marathon within the previous 6 mo were recruited for this study. Participant characteristics were as follows: age, 31 ± 5 (SD) y; body mass (BM), 70.8 ± 5.5 kg; $\dot{V}O_{2max}$, 64.1 ± 3.4 ml·kg^{-1}·min^{-1}; personal best half-marathon time, 80.50 ± 4.12 min. At the time of the investigation, participants were running ~82 ± 32 km/wk. Participants were fully informed of all experimental procedures and possible risks before providing their written, informed consent. All participants completed a medical history questionnaire to ensure they were free from illness and injury before commencing the performance trials. The study was approved by the Human Research Ethics Committee of the Australian Catholic University.

Preliminary testing and familiarization. Each participant completed an incremental test to volitional fatigue on a motorized treadmill (Pulsar 3p; HP Cosmos, Nussdorf-Traunstein, Germany) to

Address for reprint requests and other correspondence: J.A Hawley, Centre for Exercise and Nutrition, Mary MacKillop Institute for Health Research, Australian Catholic Univ., Melbourne, VIC 3065, Australia (e-mail: john.hawley@acu.edu.au).

determine $\dot{V}O_{2max}$. The test commenced at a speed of 12 km/h with a 1% incline and increased by 2 km/h every 2 min until a speed of 16 km/h was reached. Thereafter, the treadmill gradient was increased by 2% every 2 min until the participant reached volitional fatigue, determined as the inability to maintain the prescribed speed. During the maximal test and the subsequent described performance trials, expired gas was collected via open-circuit spirometry (TrueOne 2400; Parvo Medics, Sandy, UT) and the instantaneous rates of O_2 consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$), and respiratory exchange ratio (RER) were calculated every 30 s from conventional equations (28). Before each test, gas analyzers were calibrated with commercially available gas mixtures (16% O_2, 4% CO_2), and volume flow was calibrated using a 3-liter syringe. An individual's $\dot{V}O_{2max}$ was determined as the highest 30-s average that typically coincided with an inability to maintain the prescribed pace, an RER >1.15 or a subjective rating of maximal effort (rate of perceived exertion, RPE). To familiarize participants to the trial protocol, they completed a 10-km treadmill run within the 10 days before the first performance trial. To better simulate the metabolic cost of overground running (2) the treadmill was set at a speed of 95% of individual best half-marathon (21.1 km) time attained in the last 6 mo with a gradient of 1%. Expired gas was collected at 15 and 30 min, and a CHO gel and placebo (PLC) capsules were administered at 25 min.

Overview of study design. In a single-blinded, Latin square design, each participant completed four performance trials in a randomized order separated by 10–14 d. Participants were blinded to the order of the trials. Each trial required running to volitional fatigue (i.e., the inability to maintain the prescribed speed) at a speed of 95% of their best half-marathon time attained in the last 6 mo with a gradient of 1% (2). The four performance trials were completed following a preexercise meal with different nutritional values: CHO ingestion before (2 g CHO·kg^{-1}·BM^{-1}) and during (44 g/h) (CFED); CFED plus NA ingestion (CFED-NA); overnight fasted, PLC meal before, and PLC during (FAST); and FAST with NA ingestion (FAST-NA).

Exercise and diet control. Participants were instructed to refrain from any vigorous physical activity in the 48 h before a performance trial and to abstain from exercise in the 24 h before a trial. During this time, dietary standardization was achieved by giving participants individualized prepackaged meals and snacks (daily intake of 8 g CHO·kg^{-1}·BM^{-1}, 2 g protein·kg^{-1}·BM^{-1}, and 1 g fat·kg^{-1}·BM^{-1}) (21) and by instructing them to abstain from caffeine (i.e., coffee, tea, energy drinks) and alcohol. On the day of a trial, participants were provided a standardized meal consisting of jelly and 600 ml of fluid (2 g CHO·kg^{-1}·BM^{-1}) or a visually identical, taste-matched PLC of negligible energy value.

Protocol. On the morning of a performance trial, participants reported to the laboratory at 7:00 A.M. after a 10- to 12-h overnight fast (Fig. 1). A cannula (22G; Terumo, Tokyo, Japan) was inserted into the antecubital vein of the left arm and a baseline blood sample (6 ml) was taken. Following each blood draw, the cannula was flushed with saline (5 ml NaCl) to keep the vein patent. Participants then ingested either the CHO or PLC meal and rested for 120 min. Further blood samples were taken at −100 min, −12 min, and immediately before (0 min) the performance trial. NA (10 mg·kg^{-1}·BM^{-1} or 5 mg·kg^{-1}·BM^{-1}) or PLC (200 mg maltodextrin) capsules were administered 30 min (10 mg·kg^{-1}·BM^{-1}) and 15 min (5 mg·kg^{-1}·BM^{-1}) prior to the performance trial. Intermittent administration of NA was chosen to minimize the risk of negative circulatory effects that typically occur with a single bolus dose (27). Each participant's BM was recorded before they completed a 5- to 10-min warm up on the motorized treadmill at a self-selected pace, which remained the same for each individual for each trial. Participants commenced the performance trial 120 min following breakfast. During the performance trial, participants were unable to see elapsed time or distance, but were informed to run until they could no longer maintain the prescribed pace.

Blood samples (6 ml), RPE using the scale proposed by Borg in 1973, heart rate (HR) (Polar Electro OY, Kempele, Finland), and expired gas were collected at 20-min intervals. Participants were instructed to inform the principal investigator when they were close to "fatigue" so that a final expired gas sample could be collected. Isotonic CHO (44 g CHO/h, SiS GO Isotonic Gel; Blackburn, UK) or

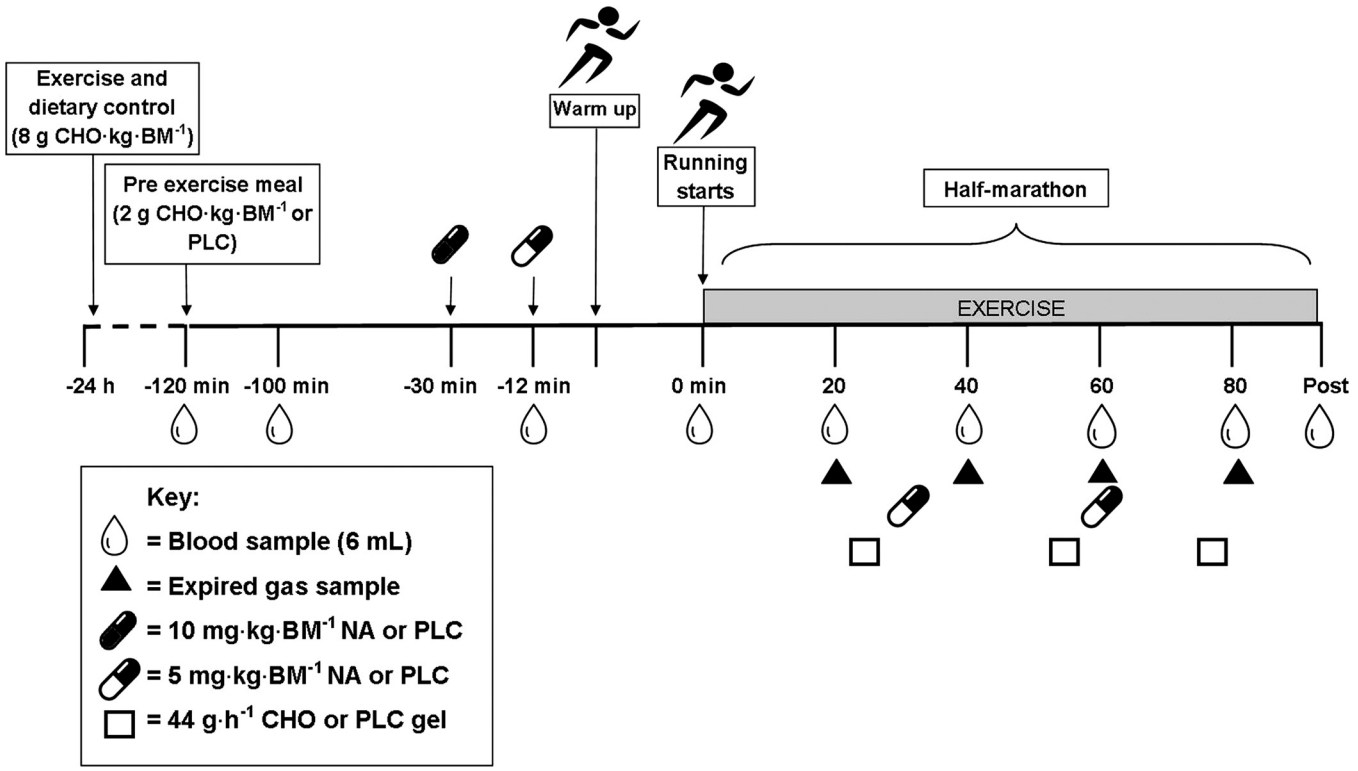

Fig. 1. Schematic figure of study design. BM, body mass; CHO, carbohydrate; NA, nicotinic acid; PLC, placebo.

PLC gels and NA or PLC capsules were administered every 25 min and 30 min, respectively. Participants consumed water ad libitum, and the total volume consumed throughout each trial was measured. On completion of a trial, participants filled out a questionnaire comprising a descriptive 9-point gastrointestinal discomfort scale ("no problem at all" to "worst it's ever been") to rate any distress experienced during the run (29).

Blood analysis. Blood samples (6 ml) were collected into vacutainers containing EDTA and immediately analyzed for blood lactate and glucose concentrations using YSI 2300 STAT Plus (Yellow Springs, OH). Following initial analysis, samples were centrifuged at 1,500 *g* for 10 min at 4°C, and aliquots of plasma were stored at −80°C for later FFA and glycerol analysis. Samples were analyzed for FFA concentration using a nonesterified fatty acids (NEFA) assay kit (Wako Pure Chemical Industries, Osaka, Japan) and glycerol concentration using a glycerol assay kit (Sigma-Aldrich, Australia) as per the manufacturer's instructions.

Rates of whole body substrate oxidation and total energy expenditure. Rates of whole body CHO and fat oxidation (g/min) were calculated from each steady-state gas sample collected during the performance trial using conventional equations (28). The calculations were made from $\dot{V}O_2$ and $\dot{V}CO_2$ measurements using the nonprotein RER equations, which are based on the assumption that $\dot{V}O_2$ and $\dot{V}CO_2$ accurately reflect tissue O_2 consumption and CO_2 production: CHO oxidation (g/min) = 4.585 $\dot{V}CO_2$ (liter/min) − 3.226 $\dot{V}O_2$ (liter/min); and fat oxidation (g/min) = 1.695 $\dot{V}O_2$ (liter/min) − 1.701 $\dot{V}CO_2$ (liter/min).

Rates of fatty acid oxidation ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) were determined by converting the rate of triacylglycerol oxidation ($g \cdot kg^{-1} \cdot min^{-1}$) to its molar equivalent, assuming the average molecular mass of human triacylglycerol to be 855.3 g/mol, and multiplying the molar rate of triacylglycerol oxidation by 3 because each molecule contains 3 μmol fatty acid. Rates of CHO oxidation ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) were determined by converting the rate of CHO oxidation (g/min) to its molar equivalent assuming 6 mol of O_2 is consumed and 6 mol of CO_2 is produced for each mol (180 g) oxidized. Total energy expenditure was estimated for each trial assuming an energy yield of 17.57 kJ and 39.33 kJ for 1 g of CHO and fat, respectively.

Statistical analysis. Statistical analysis was undertaken using SPSS (version 20 for Windows; SPSS, Chicago, IL). Data from the four trials were analyzed using a linear mixed model (time × treatment). When a significant main effect was reported, a one-way ANOVA was used (time or treatment) with Bonferroni post hoc analysis. Statistical significance was set at $P < 0.05$. All data are represented as means ± SD. Data for distance run was also analyzed for magnitude-based effect sizes between conditions using a custom spreadsheet (19). Data were log-transformed to account for nonuniformity and effect size ± 90% confidence interval [effect size (ES) ± 90% CI] calculated and classified as either trivial (−0.2 to 0.2, ES) small (0.2–0.6 ES), moderate (0.6–1.2 ES), or large (1.2–2.0 ES). Where the 90% CI overlapped small positive (0.2) and negative (−0.2) values, the effect was considered to be unclear.

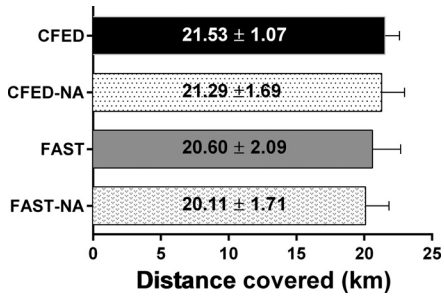

Fig. 2. Running distance covered during experimental trials. CFED, carbohydrate trial; CFED-NA, carbohydrate with nicotinic acid trial; FAST, fasted trial; FAST-NA, fasted with nicotinic acid trial. Values are means ± SD.

RESULTS

Twelve participants commenced this study but one participant was unable to complete the FAST trial due to illness, and another participant did not complete two of the prescribed performance trials with NA ingestion (CFED-NA and FAST-NA) because of side effects (i.e., dizziness, abdominal cramps). The preexercise data for the latter two trials have been included in analyses.

Running distance covered. There were small but statistically nonsignificant differences in the distance run such that CFED > CFED-NA > FAST > FAST-NA (Fig. 2, $P = 0.067$). ES statistics revealed a moderate reduction in distance run in FAST-NA (ES −0.96 ± 0.61) compared with CFED, and a small reduction in FAST compared with CFED (ES −0.54 ± 0.65). The difference in distance run in CFED vs. CFED-NA and FAST vs. FAST-NA was unclear (ES −0.24 ± 0.64 and −0.16 ± 0.53, respectively). No difference was measured for the time to completion between trials (Table 1; $P = 0.053$).

Blood metabolites. A significant treatment × time interaction was observed for both plasma FFA ($P < 0.001$) and plasma glycerol concentrations ($P < 0.01$) from rest until after exercise (Fig. 3). There was no difference in FFA or glycerol concentrations at rest between treatments. Ingestion of NA suppressed lipolysis and blunted the typical exercise-induced increase in FFA concentrations in the CFED-NA and FAST-NA trials. Following the onset of exercise, FFA concentrations remained higher in the FAST trial compared with the CFED, CFED-NA, and FAST-NA trials until the completion of exercise (Fig. 3*A*; $P < 0.05$). FFA concentrations increased in the CFED trial between 60 and 80 min of exercise ($P < 0.05$), but such an increase was not observed in the CFED-NA trial. FFA concentrations were lower in the CFED than the FAST trial after exercise (0.29 ± 0.05 vs. 0.50 ± 0.21 mmol/liter, respectively, $P < 0.001$). Following 20 min of

Table 1. *Respiratory parameters, RPE and average run time until completion for the four experimental trials*

Treatment	$\dot{V}O_2$, liter/min	$\dot{V}CO_2$, liter/min	HR, bpm	RR, bpm	RPE	Time, min	% $\dot{V}O_2$max
CFED	3.57 ± 0.42	3.41 ± 0.38	167 ± 9	46 ± 9	14 ± 1	1:26:32 ± 0:06:01	78.5 ± 3.7
CFED-NA	3.61 ± 0.39	3.49 ± 0.34	170 ± 9	47 ± 7	15 ± 1	1:25:32 ± 0:03:49	79.7 ± 3.4
FAST	3.51 ± 0.39	3.33 ± 0.46	169 ± 10	47 ± 7	15 ± 2	1:23:10 ± 0:05:41	77.8 ± 5.1
FAST-NA	3.56 ± 0.37	3.42 ± 0.36	169 ± 11	47 ± 8	15 ± 2	1:20:57 ± 0:08:08	79.1 ± 3.7

bpm, beats per minute; CFED, carbohydrate trial; CFED-NA, carbohydrate with nicotinic acid trial; FAST, fasted trial; FAST-NA, fasted with nicotinic acid trial. $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, carbon dioxide production; HR, heart rate; RR, respiratory rate; RPE, rate of perceived exertion; % $\dot{V}O_2$max, percentage of maximal oxygen uptake. Values are means ± SD.

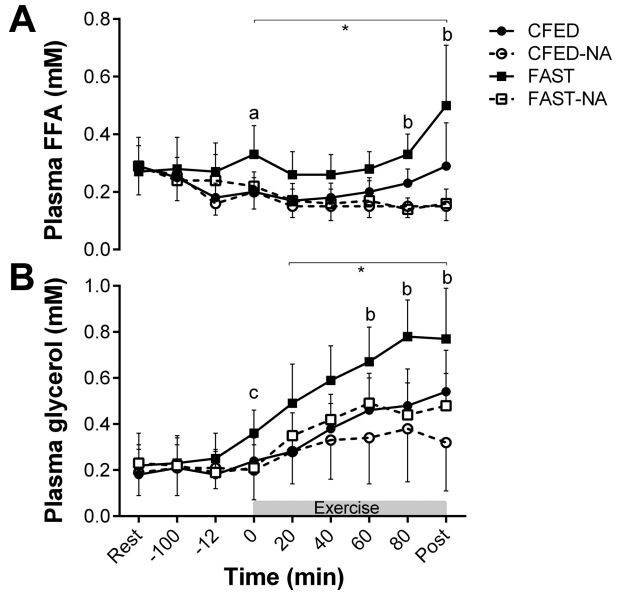

Fig. 3. Plasma free fatty acids (FFA) (*A*) and glycerol (*B*) concentrations during all experimental trials. Values are means ± SD. Significantly different ($P < 0.05$): *FAST to CFED, CFED-NA, FAST-NA; [a]CFED, CFED-NA, FAST-NA to rest; [b]CFED to CFED-NA; [c]FAST to rest.

exercise, glycerol concentrations remained higher in the FAST trial than the CFED, CFED-NA, and FAST-NA trials until exercise completion (Fig. 3*B*, $P < 0.05$). Increases in glycerol concentrations during the first 40 min of exercise were similar in the CFED, CFED-NA, and FAST-NA trials. From 60 min of exercise, glycerol concentrations continued to elevate in the CFED trial until after exercise (0.46 ± 0.16 to 0.54 ± 0.18 mmol/liter, $P < 0.05$) such that they remained significantly higher than the CFED-NA trial during this period ($P < 0.01$).

A significant treatment × time interaction was observed for blood glucose and lactate concentrations (Fig. 4; $P < 0.001$). Glucose concentrations increased above rest following ingestion of a CHO meal in the CFED and CFED-NA trials (CFED, 1.80 ± 0.39; CFED-NA, 1.67 ± 0.50 mmol/liter; $P < 0.001$; Fig. 4*A*). Thereafter, a decrease in glucose concentrations to levels below those at rest was observed in the CFED and CFED-NA trials until exercise commenced ($P < 0.001$). At 20 min of exercise, glucose concentrations were lower in the CFED and CFED-NA trials compared with the FAST and FAST-NA trials ($P < 0.02$). In all four trials, glucose concentrations increased until 40 min of exercise and remained relatively stable thereafter until postexercise.

For all performance trials, lactate concentrations increased in the first 20 min of exercise above baseline (Fig. 4*B*): levels in the FAST trial were lower than those in the CFED, CFED-NA, and FAST-NA trials ($P < 0.02$), and levels in the CFED-NA trial were higher than in the CFED trial ($P < 0.02$). From 20 to 80 min of exercise no change was observed in lactate concentrations in the CFED, FAST, and FAST-NA trials, although there was a decrease in the CFED-NA trial (3.24 ± 0.68 to 2.54 ± 1.24 mmol/liter, $P < 0.001$). No difference was observed in postexercise lactate concentrations between treatments.

CHO and fat oxidation during exercise. Rates of whole body CHO oxidation were similar in the CFED, CFED-NA, and FAST-NA trials, but they were lower in the FAST trial compared with the CFED-NA trial (338.48 ± 34.71 vs. 297.15 ± 45.88 umol·kg^{-1}·min^{-1}, respectively, $P = 0.010$), such that there was a main treatment effect ($P = 0.007$) (Table 2). Rates of fat oxidation were higher in the FAST trial compared with the CFED-NA trial (16.78 ± 8.74 vs. 8.92 ± 6.65 umol·kg^{-1}·min^{-1}, $P = 0.023$) and there was a main effect of treatment ($P = 0.008$). No difference in fat oxidation was observed in the CFED, CFED-NA, and FAST-NA trials.

There was a significant effect of treatment for total CHO oxidized during each trial ($P < 0.001$) but no difference for total fat oxidized. Total CHO oxidation was lower in the FAST trial compared with the CFED and CFED-NA trials (310.22 ± 49.95 vs. 358.48 ± 46.36 g and 310.22 ± 49.95 vs. 371.89 ± 27.06 g, $P = 0.025$ and $P = 0.002$, respectively). Estimated total energy expenditure was lower in the FAST trial than the CFED-NA trial (6,539 ± 747 vs. 7,164 ± 609 kJ, $P = 0.011$); as such, there was a significant effect of treatment ($P = 0.010$) on estimated total energy expenditure.

Respiratory parameters and RPE. There was a main effect of time ($P = 0.042$) and treatment ($P = 0.004$) for RER (Fig. 5). RER was lower in the FAST trial compared with the CFED-NA trial (0.94 ± 0.03 vs. 0.97 ± 0.02, $P = 0.016$), although no difference in RER was observed within the CFED (0.96 ± 0.03) and CFED-NA trials or the FAST and FAST-NA (0.96 ± 0.02) trials.

There was no difference in relative exercise intensity between the four trials ($P = 0.137$) (Table 1). There was a main effect of time for $\dot{V}O_2$ and $\dot{V}CO_2$, HR, RR, and RPE for all trials ($P < 0.05$), but no treatment effect for these variables (Table 1). $\dot{V}O_2$ and $\dot{V}CO_2$ increased in the four trials from 60 min to exercise completion (3.55 ± 0.38 to 3.62 ± 0.38, 3.38 ± 0.36 to 3.46 ± 0.40 liter/min, respectively, $P < 0.05$) and HR, RR, and RPE increased from 20 min to exercise completion [165 ±

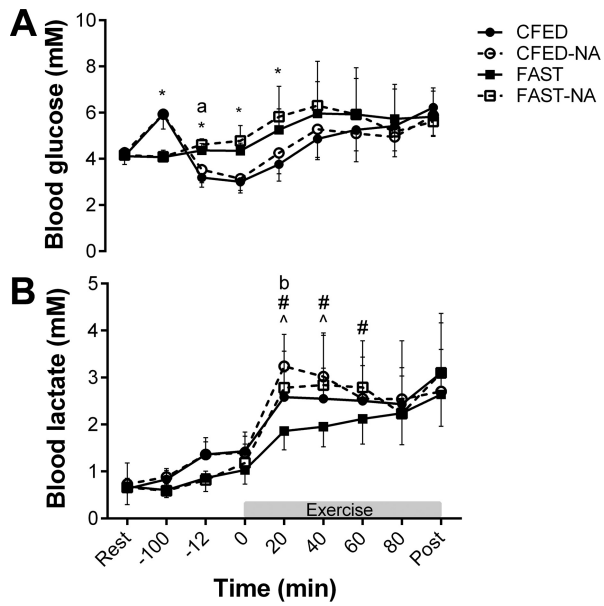

Fig. 4. Blood glucose (*A*) and lactate (*B*) concentrations during all experimental trials. Values are means ± SD. Significantly different ($P < 0.05$): *CFED and CFED-NA to FAST and FAST-NA; [a]CFED, CFED-NA, and FAST-NA to rest; [b]CFED to CFED-NA; #FAST to FAST-NA; ^FAST to CFED, CFED-NA, and FAST-NA.

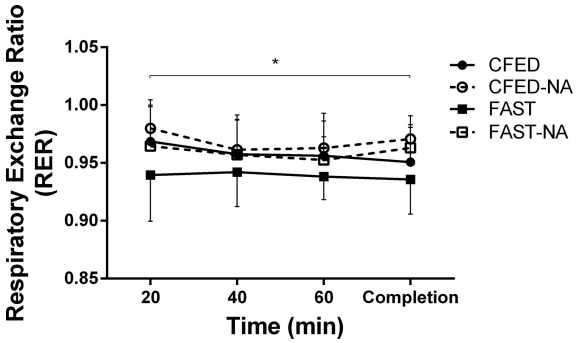

Fig. 5. Respiratory exchange ratio during all experimental trials. Values are means ± SD. *Significantly different between treatments ($P < 0.05$), CFED-NA to FAST.

8 to 173 ± 9, 44 ± 6 to 51 ± 9, and 13 ± 1 to 17 ± 2 beats per min (bpm), respectively, $P < 0.05$].

Fluid intake, body mass loss, and gastrointestinal distress. There were no differences in the average fluid consumed (330 ± 171 ml, $P = 0.680$) or loss in BM (1.73 ± 0.32 kg, $P = 0.081$) during the four experimental trials. No significant difference was reported between trials ($P = 0.241$), with gastrointestinal stress rated as "no problem at all" in the CFED and FAST trials to "very very minor" in the CFED-NA and FAST-NA trials.

DISCUSSION

The novel finding of the present study was that the suppression of lipolysis and exercise-induced increase in plasma FFA concentrations via NA ingestion did not impair half-marathon running capacity in competitive male athletes. Indeed, regardless of substrate priming by preevent nutrition (a CHO-rich prerace meal or following an overnight fast), intense exercise was CHO-dependent, with fat oxidation providing only a small contribution toward total energy expenditure. This is the first study to administer NA to well-trained runners to suppress blood-borne fatty acid availability during high-intensity running.

A primary goal of the current investigation was to determine whether blunting the normal exercise-induced rise in plasma FFA would have a detrimental effect on the performance of an endurance running event (viz. half-marathon) in competitive athletes. Although time-to-fatigue protocols measure exercise capacity rather than performance per se, the protocol implemented in this study was necessary to allow steady-state measures of whole body rates of substrate oxidation. Our primary finding of no difference in the running distance covered between the four trials when running at ~80% $\dot{V}O_{2\,max}$ (Fig. 2) supports our original hypothesis that fat oxidation plays only a minor role in endurance events lasting ~90 min when CHO availability is high. We observed a step-wise reduction in the mean distance covered whereby CFED > CFED-NA > FAST > FAST-NA, although such differences failed to reach statistical significance. Indeed, ES statistics revealed small to moderate reductions in performance when exercising fasted or fasted with NA compared with when CHO fed, respectively. The small decrement in distance covered measured in the overnight fasted trials compared with the CHO fed trials (6.6%) supports the importance of ingesting CHO in the hours before and during high-intensity running to increase CHO availability, turnover, and oxidation rates and ultimately optimize performance. Indeed, it has long been known that high-CHO availability can delay the onset of fatigue during prolonged, intense exercise (10). Although a ~7% difference in the distance covered appears a worthwhile improvement for an athlete, it is important to note that the magnitude of the increase in distance covered in the trials in which preexercise CHO was consumed was well below the 10–15% range, which has been estimated as a meaningful variation when using a time-to-volitional fatigue trial of similar exercise duration (20).

The majority of studies that have previously investigated the NA-induced suppression of fat availability on exercise performance have focused on cycling protocols (5, 14, 21, Torrens et al., unpublished observations). Torrens et al. (unpublished observations) reported no difference in cycling performance when participants completed a 90-min cycling time trial (TT) (~300 W, 82% $\dot{V}O_{2\,max}$) following the ingestion of NA in a CHO-fed state compared with a control trial. Equally, no differences in cycling performance were observed during a cycling TT of ~30 min (320 W, ~80% $\dot{V}O_{2max}$) or a 3.5-mile cycling TT (~12 min) when NA was ingested in a CHO-fed state compared with a control trial (16, 25). The findings of these studies might be considered predictable on the basis of the nature (short-duration, high-intensity) or mode (cycling) of exercise, both of which favor high rates of CHO oxidation (1, 8, 31, 32a). The current study adds to the body of knowledge by confirming the importance of CHO as a substrate for sporting activities at higher exercise intensities and during running, where rates of fat oxidation are recognized to be higher at the same relative intensities than observed during cycling (1, 8). The half-marathon event was chosen for investigation because endogenous fat and CHO stores would be highly available as energy substrates under control conditions (15), and thus a change in performance and fuel use associated with a change in substrate availability would indicate the importance of this fuel source.

The second major finding of the current study was that participants were reliant on CHO substrates to fuel muscular work under all experimental conditions, as indicated by the predominant contribution of CHO to total energy expenditure (83–91%, Fig. 6). The mean rate of CHO oxidation for all four conditions was ~4 g/min, which amounts to a total of ~350 g of CHO for the exercise task (Table 2). Such a value is well within the 400–500 g of muscle glycogen stored from the CHO loading diet (8 g $CHO \cdot kg^{-1} \cdot BM^{-1}$) consumed by the trained runners in the 24 h prior to our half-marathon protocol (15). We note that the absolute rates of CHO oxidation in the present

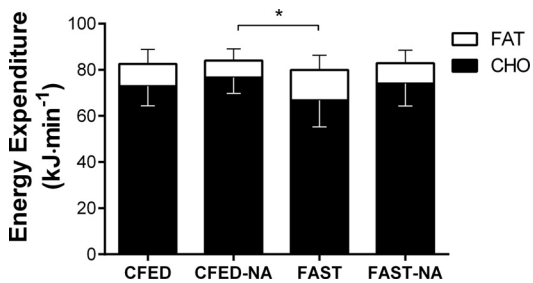

Fig. 6. Estimated energy expenditure during half-marathon running for all experimental trials. Values are means ± SD. *Significantly different between treatments ($P < 0.05$), CFED-NA to FAST.

Table 2. *Metabolic responses for the four experimental trials*

Treatment	CHO, g/min	Fat, g/min	CHO, umol·kg^{-1}·min^{-1}	Fat, umol·kg^{-1}·min^{-1}	Total CHO, g	Total Fat, g	Total Energy Expenditure, kJ
CFED	4.15 ± 0.57	0.25 ± 0.18	322.02 ± 43.77	11.82 ± 8.51	358.48 ± 46.36*	20.98 ± 13.64	7123 ± 804
CFED-NA	4.36 ± 0.46*	0.19 ± 0.15*	338.48 ± 34.71*	8.92 ± 6.65*	371.89 ± 27.06*	16.02 ± 11.26	7164 ± 609*
FAST	3.80 ± 0.70	0.34 ± 0.17	297.15 ± 45.88	16.78 ± 8.74	310.22 ± 49.95	27.68 ± 14.14	6539 ± 747
FAST-NA	4.17 ± 0.57	0.23 ± 0.16	324.28 ± 38.04	11.34 ± 7.47	337.43 ± 35.71	18.62 ± 12.35	6661 ± 769

Values are means ± SD. *Significantly different to FAST trial, $P < 0.05$.

study are substantially higher than those reported by Lee et al. (23) during a half-marathon in which CHO was consumed. However, the well-trained status and faster running speeds (~15 km/h vs. 12.2 km/h) of our participants along with the higher energy demand of exercise explains such differences. Greater amounts of CHO (~55 g) were oxidized in the trials involving preexercise CHO intake compared with overnight-fasted conditions; this is explained by greater CHO availability and the priming of the hormonal environment to increase rates of CHO utilization (11). The blunting of FFA availability with NA led to an equal increase in total CHO oxidation, regardless of preexercise CHO intake. However, even under conditions that should favor fat oxidation (overnight fasting, absence of exogenous CHO intake during exercise), CHO remained the predominant fuel source (83% total energy expenditure).

It has long been known that ingestion of NA alters fuel availability and hence muscle substrate selection during exercise (5). A blunting of the typical exercise-induced rise in FFAs has been demonstrated in previous studies that have administered NA in cycling protocols (16, 25) and was clearly demonstrated in the present study, independent of CHO status (Fig. 3). However, there was an additive effect of preexercise CHO and NA on fat metabolism during exercise, as evidenced by the reduction in plasma FFA and glycerol concentrations after 60 min and 80 min of running, respectively, compared with preexercise CHO feeding alone. These findings support the results of Murray et al. (25) who reported higher circulating plasma FFA during submaximal cycling (~70% $\dot{V}O_{2max}$) when ingesting CHO compared with coingestion of CHO plus NA. Although administration of NA in the current study suppressed adipose tissue lipolysis as evidenced by the reduction in plasma FFA, total fat oxidation during the running protocol was estimated to be ~21 g, with no difference observed between trials (Table 2). Because plasma FFA made only a small contribution to total fat utilized, it is likely that a large proportion of the fat oxidized was from intramuscular triglycerides (31, 32a). Consequently, the small yet obligatory contribution of endogenous fat substrates when running at high intensity irrespective of nutritional status preexercise should not go unrecognized.

Bergström et al. (5) reported higher respiratory quotient (RQ) values measured via arteriovenous oxygen difference across the working leg and thus greater CHO utilization during submaximal cycling exercise following administration of NA. The higher RQ was associated with a 33% increase in muscle glycogen utilization, greater arterial blood lactate concentrations, and a reduction in arterial FFA and glycerol concentrations. The measurement of whole body RER in the present study makes it difficult to isolate the energy contribution from individual CHO sources. However, NA ingestion was associated with a greater increase in blood lactate concentrations at the onset of exercise regardless of the effect of preexercise CHO intake on lowering blood glucose concentrations (Fig. 4). This provides indirect evidence for a greater reliance on endogenous CHO sources (i.e., muscle and liver glycogen) as previously reported (5).

When investigating the interaction between training status, exercise intensity, and preexercise nutritional state on substrate oxidation, Bergman and Brooks (3) reported that substrate oxidation during graded cycling was largely determined by the relative intensity of exercise. O'Brien et al. (26) also previously demonstrated the importance of exercise intensity during simulated marathon running in "fast" (completion time ≤2 h 43 min, 73% $\dot{V}O_{2\,max}$) and "slow" (completion time ≤3 h 30 min, 65% $\dot{V}O_{2\,max}$) runners. Those researchers reported that RER values and energy contribution from CHO substrates were both significantly higher throughout the marathon in the fast runners (0.99, approximately 85–90% vs. 0.90, approximately 60–70%), even under conditions in which rates of fat oxidation would be expected to be maximized (i.e., overnight fasted, no CHO feeding during exercise). Although a recent study highlighted the importance of fat oxidation during high-intensity exercise (18), the results of that investigation should be interpreted with caution. Hetlelid et al. (18) reported that RER values during interval-run training (6 × 4-min work bouts at approximately 90–94% of $\dot{V}O_{2max}$) were reduced in well-trained runners compared with recreational runners (0.88 vs. 0.95, respectively). However, Hetlelid et al. (18) failed to demonstrate steady-state conditions during exercise, or to correct for bicarbonate kinetics, so it is not known whether breath $\dot{V}O_2$ and $\dot{V}CO_2$ values accurately reflect tissue oxygen consumption and CO_2 production (12). Furthermore, even if the rates of fat oxidation were valid in that study (18), they would still contribute only a maximum of 38% of total energy expenditure in well-trained runners (18), demonstrating CHO rather than fat dependence. Our results support the original findings of O'Brien et al. (26), who reported CHO dependency in both CHO fed and overnight fasted conditions when running a half-marathon, and further reinforce the fact that when highly trained athletes compete in endurance events lasting up to 3 h, CHO-based, not fat-based fuels, are the predominant fuel for the working muscles, and CHO, not fat availability, becomes rate limiting for performance (17).

In conclusion, the results of the current study show that well-trained runners are CHO-dependent when running a half-marathon at race pace. Furthermore, when CHO availability is high, blunting the exercise-induced increase in FFA via NA ingestion did not impair intense exercise capacity in competitive athletes. During exercise of this intensity and duration, fat oxidation constitutes only a small percentage of overall energy expenditure independent of preevent CHO status and CHO availability during exercise. Although there is a small but

obligatory use of fat-based fuels during intense endurance exercise lasting ~90 min, the oxidation of CHO-based fuels predominate. Therefore, endurance athletes should undertake dietary strategies that ensure high-CHO availability before and during competition to maximize rates of CHO oxidation and optimize race performance.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.J.L., L.M.B., J.P.M., and J.A.H. conception and design of research; J.J.L. performed experiments; J.J.L. analyzed data; J.J.L., L.M.B., J.P.M., and J.A.H. interpreted results of experiments; J.J.L. prepared figures; J.J.L. and J.A.H. drafted manuscript; J.J.L., L.M.B., J.P.M., and J.A.H. edited and revised manuscript; J.J.L., L.M.B., J.P.M., and J.A.H. approved final version of manuscript.

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