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

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Running Head: Carbohydrate dependence during intense running

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Abstract

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 $CHO \cdot kg \cdot BM^{-1}$), and during (44 $g \cdot h^{-1}$) [CFED]; 2) CFED plus NA ingestion [CFED-NA]; 3) fasted with placebo ingestion during [FAST] 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. Plasma free fatty acids (FFA) and glycerol concentrations were suppressed following NA ingestion irrespective of pre-exercise nutritional intake but were higher throughout exercise in FAST compared to all other trials ($P<0.05$). Rates of whole body CHO oxidation were unaffected by NA ingestion for CFED and FAST, but were lower in FAST compared to CFED-NA ($P<0.05$). 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 alter patterns of substrate oxidation in competitive athletes. While there was a small, but obligatory use of fat-, the oxidation of CHO-based fuels predominates during half-marathon running.

Key words: Carbohydrate, high-intensity running, nicotinic acid, substrate utilization, performance.

Introduction

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, as well as implementing acute competition strategies that ensure optimal substrate availability to meet the energy cost of the event.

While 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 maximising fat oxidation (adipose and intra-muscular triglycerides [TGs], 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 since CHO- 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 six months were recruited for this study. Participant characteristics were: age 31 ± 5 (SD) y; body mass (BM) 70.8 ± 5.5 kg; $\dot{V}O_2$max 64.1 ± 3.4 ml·kg^{-1}·min^{-1}; personal best half-marathon time (PB) 80:50 ± 4:12 min: s. At the time of the investigation, participants were running ~82 ± 32 km·wk^{-1}. 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 prior to commencing the performance trials. The study was approved by the Human Research Ethics Committee of the Australian Catholic University.

Preliminary testing and familiarisation

Each participant completed an incremental test to volitional fatigue on a motorized treadmill (Pulsar 3p, HP Cosmos, Nussdorf-Traunstein, Germany) to determine $\dot{V}O_2$max. The test commenced at a speed of 12 km·h^{-1} with a 1% incline and increased by 2 km·h^{-1} every two min until a speed of 16 km·h^{-1} was reached. Thereafter, the treadmill gradient was increased by 2% every two 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, Utah, USA) and the instantaneous rates of O_2 consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$) and the 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 L syringe. An individual's $\dot{V}O_2$max was determined as the highest 30 s average which typically coincided with an inability to maintain the prescribed pace, an RER > 1.15 or a subjective rating of maximal effort (RPE). To familiarize participants to the trial protocol they completed a 10 km treadmill run within the 10 days prior to the first performance trial. The treadmill was set at a speed of 95% of individual best half-marathon (21.1 km) time attained in the last 6 months, with a gradient of 1%, to better simulate the metabolic cost of overground running (2). 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 4 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 months, with a gradient of 1% (2). The 4 performance trials were completed following a pre-exercise meal with different nutritional value: CHO ingestion before (2 g CHO·kg·BM^{-1}) and during (44 $g.h^{-1}$) (CFED); CFED plus NA ingestion (CFED-NA); overnight fasted, PLC meal before and PLC during (FAST); FAST with NA ingestion (FAST-NA).

Exercise and diet control

Participants were instructed to refrain from any vigorous physical activity in the 48 h prior to a performance trial and to abstain from exercise in the 24 h before a trial. During this time, dietary standardization was achieved by providing participants with individualized pre-packaged meals and snacks (daily intake of 8 g CHO·kg·BM^{-1}, 2 g protein·kg·BM^{-1} and 1 g fat·kg·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·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 lab at 0700 h after a 10-12 h overnight fast (**Figure 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 prior (0 min) to the performance trial. NA (10 mg·kg·BM^{-1} or 5 mg·kg·BM^{-1}) or PLC (200 mg maltodextrin) capsules were administered 30 min (10 mg·kg·BM^{-1}) and 15 min (5 mg·kg·BM^{-1}) prior to the performance trial. Intermittent administration of NA was chosen to minimise the risk of negative circulatory effects which typically occur with a single bolus dose (27). Participant's BM was recorded prior to completing a 5-10 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), rate of perceived exertion (RPE) (Borg 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 a final expired gas sample could be collected. Isotonic CHO (SiS GO Isotonic Gel, Blackburn, UK, 44 g CHO·h^{-1}) or PLC gels and NA or PLC capsules were administered every 25 min and 30 min, respectively. Water was consumed *ad libitum* and the total volume consumed throughout each trial 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^{TM}. Following initial analysis, samples were centrifuged at 1500 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 Non-esterified-fatty acid (NEFA) assay kit (Wako Pure Chemical Industries, Ltd, Osaka, Japan) and glycerol concentration using a glycerol assay kit (Sigma-Aldrich, Ltd, 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^{-1}) 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 non-protein RER equations below 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.

172 $$\text{CHO oxidation } (g \cdot min^{-1}) = 4.585\ \dot{V}CO_2\ (L \cdot min^{-1}) - 3.226\ \dot{V}O_2\ (L \cdot min^{-1})$$

173 $$\text{Fat oxidation } (g \cdot min^{-1}) = 1.695\ \dot{V}O_2\ (L \cdot min^{-1}) - 1.701\ \dot{V}CO_2\ (L \cdot min^{-1})$$

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

182 *Statistical analysis*

183 Statistical analysis was undertaken using SPSS (Version 20 for Windows, SPSS Inc,
184 Chicago, IL). Data from the 4 trials were analyzed using a linear mixed model (time x
185 treatment). When a significant main effect was reported, a one way ANOVA was used (time
186 or treatment) with Bonferroni post hoc analysis. Statistical significance was set at $P<0.05$. All
187 data are represented as mean ± SD. Data for distance run was also analyzed for magnitude-
188 based effect sizes between conditions using a custom spreadsheet (19). Data was log-
189 transformed to account for non-uniformity and effect size ± 90% confidence interval (ES ±
190 90% CI) calculated and classified as either trivial (-0.2-0.2, ES) small (0.2-0.6 ES), moderate
191 (0.6-1.2 ES) or large (1.2-2 ES). Where the 90% CI overlapped small positive (0.2) and
192 negative (-0.2) values, the effect was considered “unclear”.

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Results

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

Running distance covered

There were small but statistically non-significant differences in the distance run such that CFED > CFED-NA > FAST > FAST-NA (**Figure 2**; P=0.067). ES statistics revealed a moderate reduction in distance run in FAST-NA (ES -0.96 ± 0.61) compared to CFED and a small reduction in FAST compared to 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; -0.16 ± 0.53 respectively). No difference was measured for the time to completion between trials (**Table 2**; P=0.053).

Blood metabolites

A significant treatment x time interaction was observed for both plasma FFA (P<0.001) and plasma glycerol concentrations (P<0.01) from rest until post exercise (**Figure 3**). There was no difference in FFA or glycerol concentrations at rest between treatments. The 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 to the CFED, CFED-NA and FAST-NA trials until the completion of exercise (**Figure 3A**; 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 post exercise (0.29 ± 0.05 vs. 0.50 ± 0.21 mmol·L^{-1} respectively, P<0.001). Following 20 min of exercise, glycerol concentrations remained higher in the FAST trial than the CFED, CFED-NA and FAST-NA trials until exercise completion (**Figure 3B**; 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 post exercise (0.46 ± 0.16 to 0.54 ± 0.18 mmol·L^{-1}, P<0.05), such that they remained significantly higher than the CFED-NA trial during this period (P<0.01).

A significant treatment x time interaction was observed for blood glucose and lactate concentrations (**Figure 4**; P<0.001). Glucose concentrations increased above rest following the ingestion of a CHO meal in the CFED and CFED-NA trials (**Figure 4A**; CFED: 1.80 ± 0.39; CFED-NA: 1.67 ± 0.50 mmol·L^{-1}, P<0.001). Thereafter a decrease in glucose concentrations to below 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 to the FAST and FAST-NA trials (P<0.02). In all 4 trials, glucose concentrations increased until 40 min of exercise and remained relatively stable thereafter until post exercise.

For all performance trials, lactate concentrations increased in the first 20 min of exercise above baseline (**Figure 4B**), where FAST was lower than CFED, CFED-NA and FAST-NA trials (P<0.02) and CFED-NA was higher than 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·L^{-1}, P<0.001). No difference was observed in post-exercise 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 were lower in FAST compared to the CFED-NA trial (338.48 ± 34.71 vs. 297.15 ± 45.88 umol·kg·min^{-1}, respectively, P=0.010), such that there was a main treatment effect (P=0.007). Rates of fat oxidation were higher in the FAST trial compared to the CFED-NA trial (16.78 ± 8.74 vs. 8.92 ± 6.65 umol·kg·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 in comparison to the CFED and CFED-NA trials (310.22 ± 49.95 vs. 358.48 ± 46.36 vs. 371.89 ± 27.06 g, P=0.025, P=0.002 respectively). Estimated total energy expenditure was lower in the FAST trial than the CFED-NA trial (6539 ± 747 vs. 7164 ± 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 (**Figure 5**). RER was lower in the FAST trial compared to 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 4 trials (P=0.137) (Table 2). 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 2). VO_2 and VCO_2 increased in

the 4 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 $L.min^{-1}$ respectively, $P<0.05$) and HR, RR and RPE increased from 20 min to exercise completion (165 ± 8 to 173 ± 9 bpm, 44 ± 6 to 51 ± 9 bpm, 13 ± 1 to 17 ± 2, 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 4 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 the 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 pre-event nutrition (a CHO-rich pre-race meal or following an overnight fast), intense exercise was CHO-dependent, with fat oxidation providing only a small contribution towards total energy expenditure. This is the first study to administer NA to well-trained runners to suppress blood-borne FA 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 (**Figure 1**) 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 to when CHO fed, respectively. The small decrement in distance covered measured in the overnight fasted trials in comparison to the CHO fed trials (6.6%) supports the importance of ingesting CHO in the hours prior to and during high-intensity running to increase CHO availability, turnover and oxidation rates and ultimately optimise performance. Indeed, it has long been know that high CHO availability can delay the onset of fatigue during prolonged intense exercise (10). While 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 pre-exercise CHO was consumed was well below the 10-15%, 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 which have previously investigated the NA-induced suppression of fat availability on exercise performance have focussed on cycling protocols (5, 14, 21, Torrens S.L. et al., unpublished observations). Torrens S.L. 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 in comparison to a control trial. Equally, no differences in cycling performance were observed during a ~30 min cycling TT (320 W, ~80% $\dot{V}O_2$max) or a 3.5 mile cycling TT (~12 min) when NA was ingested in a CHO-fed state in comparison to a control trial (16, 25). The findings of these studies might be considered predictable, based on

the nature (short duration, high-intensity) or mode (cycling) of exercise, both of which favour high rates of CHO oxidation (1, 8, 24, 31). 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 it is recognised that rates of fat oxidation are 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%, **Figure 6**). The mean rates of CHO oxidation for all four conditions was ~4 $g \cdot min^{-1}$ which amounts to a total of ~350 g of CHO for the exercise task (**Table 1**). Such a value is well within the 400-500 g of muscle glycogen stored from the CHO loading diet (8 $g \cdot kg \cdot BM^{-1}$ CHO) 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 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 $km \cdot h^{-1}$ vs. 12.2 $km \cdot h^{-1}$) 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 pre-exercise CHO intake compared to 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 pre-exercise CHO intake. However, even under conditions that should favour 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 the 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 (**Figure 3**). However, there was an additive effect of pre-exercise 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 to pre-exercise CHO feeding alone. These findings support the results of Murray et al. (25) who reported higher circulating plasma FFAs during submaximal cycling (~70% $\dot{V}O_2max$) when ingesting CHO compared to the co-ingestion of CHO plus NA. Although the administration of NA in the current study suppressed adipose tissue lipolysis as evidenced by the reduction in plasma FFAs, total fat oxidation during the running protocol was estimated to be ~21 g with no difference observed between trials (**Table 1**). As there was only a small contribution from plasma FFAs to total fat utilized, it is likely that a large proportion of the fat oxidized was from intramuscular triglycerides (IMTG) (24, 31). Consequently, the small yet obligatory contribution of endogenous fat substrates when running at high intensity, irrespective of nutritional status pre-exercise should not go unrecognized.

Bergstrom 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 compared to a control trial. 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 pre-exercise CHO intake on lowering blood glucose concentrations (**Figure 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 pre-exercise 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) have 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) or 'slow' (completion time ≤3 h, 30 min, 65% $\dot{V}O_2$max) runners. These workers reported RER values and energy contribution from CHO substrates were significantly higher throughout the marathon in the faster runners (0.99, ~85-90% vs. 0.90, ~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). While a recent study has 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 x 4 min work bouts at ~90-94% of $\dot{V}O_2$max) were reduced in well trained compared to recreational runners (0.88 vs. 0.95, respectively). However, these workers failed to demonstrate steady-state conditions during exercise, or correct for bicarbonate kinetics, so it is not known if 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 only contribute a maximum of 38% of total energy expenditure in their well trained runners (18), demonstrating CHO rather than fat dependence. Our results support the original findings of O'Brien et al (24), 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-, 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 pre-event CHO status and CHO availability during exercise. While 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 maximise rates of CHO oxidation and optimise race performance.

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Disclosures

All authors report no conflict of interest.

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514 **Figure Legends**

515 **Figure 1**. Schematic figure of study design. CHO, carbohydrate; PLC, placebo, NA,
516 nicotinic acid.

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

520 **Figure 3.** Plasma FFA (A) and glycerol concentrations (B) during all experimental trials.
521 CFED, carbohydrate trial; CFED-NA, carbohydrate with nicotinic acid trial; FAST, fasted
522 trial; FAST-NA, fasted with nicotinic acid trial. Values are means ± SD. Significantly
523 different ($P<0.05$), *FAST to CFED, CFED-NA, FAST-NA; a CFED, CFED-NA, FAST-NA
524 to rest, b CFED to CFED-NA, c FAST to rest.

525 **Figure 4.** Blood glucose (A) and lactate (B) concentrations during all experimental trials.
526 CFED, carbohydrate trial; CFED-NA, carbohydrate with nicotinic acid trial; FAST, fasted
527 trial; FAST-NA, fasted with nicotinic acid trial. Values are means ± SD. Significantly
528 different ($P<0.05$), *CFED & CFED-NA to FAST & FAST-NA; a CFED, CFED-NA, FAST-
529 NA to rest, b CFED to CFED-NA, # FAST to FAST-NA, ^ FAST to CFED, CFED-NA,
530 FAST-NA.

531 **Figure 5**. Respiratory exchange ratio during all experimental trials. CFED, carbohydrate trial;
532 CFED-NA, carbohydrate with nicotinic acid trial; FAST, fasted trial; FAST-NA, fasted with
533 nicotinic acid trial. Values are means ± SD. Significantly different between treatments
534 ($P<0.05$), *CFED-NA to FAST.

535 **Figure 6.** Estimated energy expenditure during half-marathon running for all experimental
536 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. Significantly different between treatments ($P<0.05$), *CFED-NA to FAST.

Table 1. Metabolic responses for the four experimental trials

Treatment	CHO ($g \cdot min^{-1}$)	Fat ($g \cdot min^{-1}$)	CHO ($umol \cdot kg.min^{-1}$)	Fat ($umol \cdot kg.min^{-1}$)	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

CFED, carbohydrate trial; CFED-NA, carbohydrate with nicotinic acid trial; FAST, fasted trial; FAST-NA, fasted with nicotinic acid trial. Values are means ± SD *Significantly different to FAST trial ($P<0.05$).

551 **Table 2**. Respiratory parameters, RPE and average run time until completion for the four experimental trials

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Treatment	$\dot{V}O_2$ ($L \cdot min^{-1}$)	$\dot{V}CO_2$ ($L \cdot min^{-1}$)	HR (bpm)	RR (bpm)	RPE	Time (min)	% $\dot{V}O_2max$
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

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; % $\dot{V}O_2max$, percentage of maximal oxygen uptake. Values are means ± SD.