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

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11 Running Head: Carbohydrate dependence during intense running

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27 **Abstract**

28 We determined the effect of suppressing lipolysis via administration of nicotinic acid (NA)
29 on fuel substrate selection and half-marathon running capacity. In a single-blinded Latin
30 square design, 12 competitive runners completed four trials involving treadmill running until
31 volitional fatigue at a pace based on 95% of personal best half-marathon time. Trials were
32 completed in a fed or overnight fasted state: 1) Carbohydrate (CHO) ingestion before (2 g
33 CHO·kg·BM⁻¹), and during (44 g·h⁻¹) [CFED]; 2) CFED plus NA ingestion [CFED-NA]; 3)
34 fasted with placebo ingestion during [FAST] 4) FAST plus NA ingestion [FAST-NA]. There
35 was no difference in running distance (CFED 21.53 ± 1.07, CFED-NA 21.29 ± 1.69, FAST
36 20.60 ± 2.09, FAST-NA 20.11 ± 1.71 km) or time to fatigue between the four trials. Plasma
37 free fatty acids (FFA) and glycerol concentrations were suppressed following NA ingestion
38 irrespective of pre-exercise nutritional intake but were higher throughout exercise in FAST
39 compared to all other trials (P<0.05). Rates of whole body CHO oxidation were unaffected
40 by NA ingestion for CFED and FAST, but were lower in FAST compared to CFED-NA
41 (P<0.05). CHO was the primary substrate for exercise in all conditions, contributing 83-91%
42 to total energy expenditure with only a small contribution from fat-based fuels. Blunting the
43 exercise-induced increase in FFA via NA ingestion did not impair intense running capacity
44 lasting ~85 min nor alter patterns of substrate oxidation in competitive athletes. While there
45 was a small, but obligatory use of fat-, the oxidation of CHO-based fuels predominates during
46 half-marathon running.

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

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52 **Introduction**

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

63 While the absolute oxidation rate of all energy substrates increases at the high
64 exercise intensities and power outputs sustained by athletes in training and competition,
65 CHO-based fuels are the predominant energy source (4, 6, 17). However, recent attention has
66 focused on diet-exercise strategies that reduce skeletal muscle dependence on CHO-based
67 fuels (i.e. muscle and liver glycogen, blood glucose, lactate) before and during exercise,
68 while concomitantly maximising fat oxidation (adipose and intra-muscular triglycerides
69 [TGs], blood-borne free fatty acids [FFAs] and TGs) (33). It has been proposed that such
70 strategies will enhance performance by promoting greater utilization of fat-based fuels,
71 whose availability is relatively unlimited (33). However, even when these strategies promote
72 rates of fat oxidation that are substantially higher than those achieved by the effects of
73 endurance training alone, there is no clear evidence of a performance benefit (7, 13, 30).
74 Indeed, rates of muscle fat oxidation are inadequate to support the high relative (70-90%
75 $\dot{V}O_{2max}$) and absolute work rates sustained by competitive athletes during running or cycling
76 events lasting < 2 h (17, 22, 32, 34).

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

84 **Methods**

85 *Participants*

86 Twelve competitive male runners who had completed a half-marathon within the
87 previous six months were recruited for this study. Participant characteristics were: age 31 ± 5
88 (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-
89 marathon time (PB) $80:50 \pm 4:12$ min: s. At the time of the investigation, participants were
90 running $\sim 82 \pm 32$ km·wk⁻¹. Participants were fully informed of all experimental procedures
91 and possible risks before providing their written, informed consent. All participants
92 completed a medical history questionnaire to ensure they were free from illness and injury
93 prior to commencing the performance trials. The study was approved by the Human Research
94 Ethics Committee of the Australian Catholic University.

95 *Preliminary testing and familiarisation*

96 Each participant completed an incremental test to volitional fatigue on a motorized
97 treadmill (Pulsar 3p, HP Cosmos, Nussdorf-Traunstein, Germany) to determine $\dot{V}O_{2\max}$.
98 The test commenced at a speed of 12 km·h⁻¹ with a 1% incline and increased by 2 km·h⁻¹
99 every two min until a speed of 16 km·h⁻¹ was reached. Thereafter, the treadmill gradient was
100 increased by 2% every two min until the participant reached volitional fatigue, determined as

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101 the inability to maintain the prescribed speed. During the maximal test and the subsequent
102 described performance trials, expired gas was collected via open-circuit spirometry (TrueOne
103 2400, Parvo Medics, Utah, USA) and the instantaneous rates of O₂ consumption ($\dot{V}O_2$), CO₂
104 production ($\dot{V}CO_2$) and the respiratory exchange ratio (RER) were calculated every 30 s from
105 conventional equations (28). Before each test, gas analyzers were calibrated with
106 commercially available gas mixtures (16% O₂, 4% CO₂) and volume flow was calibrated
107 using a 3 L syringe. An individual's $\dot{V}O_{2max}$ was determined as the highest 30 s average
108 which typically coincided with an inability to maintain the prescribed pace, an RER > 1.15 or
109 a subjective rating of maximal effort (RPE). To familiarize participants to the trial protocol
110 they completed a 10 km treadmill run within the 10 days prior to the first performance trial.
111 The treadmill was set at a speed of 95% of individual best half-marathon (21.1 km) time
112 attained in the last 6 months, with a gradient of 1%, to better simulate the metabolic cost of
113 overground running (2). Expired gas was collected at 15 and 30 min and a CHO gel and
114 placebo (PLC) capsules were administered at 25 min.

115 *Overview of study design*

116 In a single blinded Latin square design, each participant completed 4 performance
117 trials in a randomized order separated by 10-14 d. Participants were blinded to the order of
118 the trials. Each trial required running to volitional fatigue (i.e. the inability to maintain the
119 prescribed speed) at a speed of 95% of their best half-marathon time attained in the last 6
120 months, with a gradient of 1% (2). The 4 performance trials were completed following a pre-
121 exercise meal with different nutritional value: CHO ingestion before (2 g CHO·kg·BM⁻¹) and
122 during (44 g·h⁻¹) (CFED); CFED plus NA ingestion (CFED-NA); overnight fasted, PLC meal
123 before and PLC during (FAST); FAST with NA ingestion (FAST-NA).

124 *Exercise and diet control*

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125 Participants were instructed to refrain from any vigorous physical activity in the 48 h
126 prior to a performance trial and to abstain from exercise in the 24 h before a trial. During this
127 time, dietary standardization was achieved by providing participants with individualized pre-
128 packaged meals and snacks (daily intake of 8 g CHO·kg·BM⁻¹, 2 g protein·kg·BM⁻¹ and 1 g
129 fat·kg·BM⁻¹) (21) and by instructing them to abstain from caffeine (i.e. coffee, tea, energy
130 drinks) and alcohol. On the day of a trial, participants were provided a standardized meal
131 consisting of jelly and 600 mL of fluid (2 g CHO·kg·BM⁻¹) or a visually identical, taste
132 matched PLC of negligible energy value.

133 *Protocol*

134 On the morning of a performance trial, participants reported to the lab at 0700 h after
135 a 10-12 h overnight fast (**Figure 1**). A cannula (22G, Terumo, Tokyo, Japan) was inserted
136 into the antecubital vein of the left arm and a baseline blood sample (6 mL) was taken.
137 Following each blood-draw, the cannula was flushed with saline (5 mL NaCl) to keep the
138 vein patent. Participants then ingested either the CHO or PLC meal and rested for 120 min.
139 Further blood samples were taken at -100 min, -12 min and immediately prior (0 min) to the
140 performance trial. NA (10 mg·kg·BM⁻¹ or 5 mg·kg·BM⁻¹) or PLC (200 mg maltodextrin)
141 capsules were administered 30 min (10 mg·kg·BM⁻¹) and 15 min (5 mg·kg·BM⁻¹) prior to the
142 performance trial. Intermittent administration of NA was chosen to minimise the risk of
143 negative circulatory effects which typically occur with a single bolus dose (27). Participant's
144 BM was recorded prior to completing a 5-10 min warm up on the motorized treadmill at a
145 self-selected pace, which remained the same for each individual for each trial. Participants
146 commenced the performance trial 120 min following breakfast. During the performance trial,
147 participants were unable to see elapsed time or distance, but were informed to run until they
148 could no longer maintain the prescribed pace.

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149 Blood samples (6 mL), rate of perceived exertion (RPE) (Borg 1973), heart rate (HR) (Polar
150 Electro OY, Kempele, Finland) and expired gas were collected at 20 min intervals.
151 Participants were instructed to inform the principal investigator when they were close to
152 “fatigue”, so a final expired gas sample could be collected. Isotonic CHO (SiS GO Isotonic
153 Gel, Blackburn, UK, 44 g CHO·h⁻¹) or PLC gels and NA or PLC capsules were administered
154 every 25 min and 30 min, respectively. Water was consumed *ad libitum* and the total volume
155 consumed throughout each trial measured. On completion of a trial, participants filled out a
156 questionnaire comprising a descriptive 9-point gastrointestinal discomfort scale (“no problem
157 at all” to “worst it’s ever been”) to rate any distress experienced during the run (29).

158 *Blood analysis*

159 Blood samples (6 mL) were collected into vacutainers containing EDTA and
160 immediately analyzed for blood lactate and glucose concentrations using YSI 2300 STAT
161 PlusTM. Following initial analysis, samples were centrifuged at 1500 g for 10 min at 4 °C and
162 aliquots of plasma were stored at -80°C for later FFA and glycerol analysis. Samples were
163 analyzed for FFA concentration using a Non-esterified-fatty acid (NEFA) assay kit (Wako
164 Pure Chemical Industries, Ltd, Osaka, Japan) and glycerol concentration using a glycerol
165 assay kit (Sigma-Aldrich, Ltd, Australia) as per the manufacturer’s instructions.

166 *Rates of whole body substrate oxidation and total energy expenditure*

167 Rates of whole body CHO and fat oxidation (g.min⁻¹) were calculated from each
168 steady-state gas sample collected during the performance trial using conventional equations
169 (28). The calculations were made from $\dot{V}O_2$ and $\dot{V}CO_2$ measurements using the non-protein
170 RER equations below which are based on the assumption that $\dot{V}O_2$ and $\dot{V}CO_2$ accurately
171 reflect tissue O₂ consumption and CO₂ production.

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172 CHO oxidation ($\text{g}\cdot\text{min}^{-1}$) = $4.585 \dot{V}\text{CO}_2 (\text{L}\cdot\text{min}^{-1}) - 3.226 \dot{V}\text{O}_2 (\text{L}\cdot\text{min}^{-1})$

173 Fat oxidation ($\text{g}\cdot\text{min}^{-1}$) = $1.695 \dot{V}\text{O}_2 (\text{L}\cdot\text{min}^{-1}) - 1.701 \dot{V}\text{CO}_2 (\text{L}\cdot\text{min}^{-1})$

174 Rates of FA oxidation ($\mu\text{mol}\cdot\text{kg}\cdot\text{min}^{-1}$) were determined by converting the rate of
175 triacylglycerol oxidation ($\text{g}\cdot\text{kg}\cdot\text{min}^{-1}$) to its molar equivalent, assuming the average
176 molecular mass of human triacylglycerol to be $855.3 \text{ g}\cdot\text{mol}^{-1}$, and multiplying the molar rate
177 of triacylglycerol oxidation by three, because each molecule contains 3 μmol FA. Rates of
178 CHO oxidation ($\mu\text{mol}\cdot\text{kg}\cdot\text{min}^{-1}$) were determined by converting the rate of CHO oxidation
179 ($\text{g}\cdot\text{min}^{-1}$) 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 \pm 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 \pm 90% confidence interval (ES \pm
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”.

193

194

195 **Results**

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

201 *Running distance covered*

202 There were small but statistically non-significant differences in the distance run such that
203 CFED > CFED-NA > FAST > FAST-NA (**Figure 2**; $P=0.067$). ES statistics revealed a
204 moderate reduction in distance run in FAST-NA ($ES -0.96 \pm 0.61$) compared to CFED and a
205 small reduction in FAST compared to CFED ($ES -0.54 \pm 0.65$). The difference in distance
206 run in CFED vs CFED-NA and FAST vs FAST-NA was “unclear” ($ES -0.24 \pm 0.64$; $-0.16 \pm$
207 0.53 respectively). No difference was measured for the time to completion between trials
208 (**Table 2**; $P=0.053$).

209 *Blood metabolites*

210 A significant treatment x time interaction was observed for both plasma FFA
211 ($P<0.001$) and plasma glycerol concentrations ($P<0.01$) from rest until post exercise (**Figure**
212 **3**). There was no difference in FFA or glycerol concentrations at rest between treatments. The
213 ingestion of NA suppressed lipolysis and blunted the typical exercise-induced increase in
214 FFA concentrations in the CFED-NA and FAST-NA trials. Following the onset of exercise,
215 FFA concentrations remained higher in the FAST trial compared to the CFED, CFED-NA
216 and FAST-NA trials until the completion of exercise (**Figure 3A**; $P<0.05$). FFA
217 concentrations increased in the CFED trial between 60 and 80 min of exercise ($P<0.05$) but
218 such an increase was not observed in the CFED-NA trial. FFA concentrations were lower in

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219 the CFED than the FAST trial post exercise (0.29 ± 0.05 vs. 0.50 ± 0.21 mmol·L⁻¹
220 respectively, $P < 0.001$). Following 20 min of exercise, glycerol concentrations remained
221 higher in the FAST trial than the CFED, CFED-NA and FAST-NA trials until exercise
222 completion (**Figure 3B**; $P < 0.05$). Increases in glycerol concentrations during the first 40 min
223 of exercise were similar in the CFED, CFED-NA and FAST-NA trials. From 60 min of
224 exercise, glycerol concentrations continued to elevate in the CFED trial until post exercise
225 (0.46 ± 0.16 to 0.54 ± 0.18 mmol·L⁻¹, $P < 0.05$), such that they remained significantly higher
226 than the CFED-NA trial during this period ($P < 0.01$).

227 A significant treatment x time interaction was observed for blood glucose and lactate
228 concentrations (**Figure 4**; $P < 0.001$). Glucose concentrations increased above rest following
229 the ingestion of a CHO meal in the CFED and CFED-NA trials (**Figure 4A**; CFED: $1.80 \pm$
230 0.39 ; CFED-NA: 1.67 ± 0.50 mmol·L⁻¹, $P < 0.001$). Thereafter a decrease in glucose
231 concentrations to below rest was observed in the CFED and CFED-NA trials until exercise
232 commenced ($P < 0.001$). At 20 min of exercise, glucose concentrations were lower in the
233 CFED and CFED-NA trials compared to the FAST and FAST-NA trials ($P < 0.02$). In all 4
234 trials, glucose concentrations increased until 40 min of exercise and remained relatively
235 stable thereafter until post exercise.

236 For all performance trials, lactate concentrations increased in the first 20 min of
237 exercise above baseline (**Figure 4B**), where FAST was lower than CFED, CFED-NA and
238 FAST-NA trials ($P < 0.02$) and CFED-NA was higher than the CFED trial ($P < 0.02$). From 20
239 to 80 min of exercise no change was observed in lactate concentrations in the CFED, FAST
240 and FAST-NA trials, although there was a decrease in the CFED-NA trial (3.24 ± 0.68 to
241 2.54 ± 1.24 mmol·L⁻¹, $P < 0.001$). No difference was observed in post-exercise lactate
242 concentrations between treatments.

243 *CHO and fat oxidation during exercise*

244 Rates of whole body CHO oxidation were similar in the CFED, CFED-NA and
245 FAST-NA trials, but were lower in FAST compared to the CFED-NA trial (338.48 ± 34.71
246 vs. 297.15 ± 45.88 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}^{-1}$, respectively, $P=0.010$), such that there was a main
247 treatment effect ($P=0.007$). Rates of fat oxidation were higher in the FAST trial compared to
248 the CFED-NA trial (16.78 ± 8.74 vs. 8.92 ± 6.65 $\mu\text{mol}\cdot\text{kg}\cdot\text{min}^{-1}$, $P=0.023$) and there was a
249 main effect of treatment ($P=0.008$). No difference in fat oxidation was observed in the CFED,
250 CFED-NA and FAST-NA trials.

251 There was a significant effect of treatment for total CHO oxidized during each trial
252 ($P<0.001$) but no difference for total fat oxidized. Total CHO oxidation was lower in the
253 FAST trial in comparison to the CFED and CFED-NA trials (310.22 ± 49.95 vs. $358.48 \pm$
254 46.36 vs. 371.89 ± 27.06 g, $P=0.025$, $P=0.002$ respectively). Estimated total energy
255 expenditure was lower in the FAST trial than the CFED-NA trial (6539 ± 747 vs. 7164 ± 609
256 kJ, $P=0.011$), as such there was a significant effect of treatment ($P=0.010$) on estimated total
257 energy expenditure.

258 *Respiratory parameters and RPE*

259 There was a main effect of time ($P=0.042$) and treatment ($P=0.004$) for RER (**Figure**
260 **5**). RER was lower in the FAST trial compared to the CFED-NA trial (0.94 ± 0.03 vs. $0.97 \pm$
261 0.02 , $P=0.016$) although no difference in RER was observed within the CFED (0.96 ± 0.03)
262 and CFED-NA trials or the FAST and FAST-NA (0.96 ± 0.02) trials.

263 There was no difference in relative exercise intensity between the 4 trials ($P=0.137$)
264 (Table 2). There was a main effect of time for $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$, HR, RR and RPE for all trials
265 ($P<0.05$), but no treatment effect for these variables (Table 2). VO_2 and VCO_2 increased in

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266 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
267 ± 0.40 L.min⁻¹ respectively, $P < 0.05$) and HR, RR and RPE increased from 20 min to exercise
268 completion (165 ± 8 to 173 ± 9 bpm, 44 ± 6 to 51 ± 9 bpm, 13 ± 1 to 17 ± 2 , respectively,
269 $P < 0.05$).

270 *Fluid intake, body mass loss and gastrointestinal distress*

271 There were no differences in the average fluid consumed (330 ± 171 mL, $P = 0.680$) or
272 loss in BM (1.73 ± 0.32 kg, $P = 0.081$) during the 4 experimental trials. No significant
273 difference was reported between trials ($P = 0.241$), with gastrointestinal stress rated as ‘no
274 problem at all’ in the CFED and FAST trials to ‘very very minor’ in the CFED-NA and
275 FAST-NA trials.

276 **Discussion**

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

284 A primary goal of the current investigation was to determine whether blunting the
285 normal exercise-induced rise in plasma FFA would have a detrimental effect on the
286 performance of an endurance running event (viz. half marathon) in competitive athletes.
287 Although time to fatigue protocols measure exercise capacity rather than performance *per se*,
288 the protocol implemented in this study was necessary to allow steady-state measures of
289 whole-body rates of substrate oxidation. Our primary finding of no difference in the running

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290 distance covered between the four trials when running at $\sim 80\%$ $\dot{V}O_{2\max}$ (**Figure 1**) supports
291 our original hypothesis that fat oxidation plays only a minor role in endurance events lasting
292 ~ 90 min when CHO availability is high. We observed a step-wise reduction in the mean
293 distance covered whereby CFED > CFED-NA > FAST > FAST-NA, although such
294 differences failed to reach statistical significance. Indeed, ES statistics revealed small to
295 moderate reductions in performance when exercising fasted or fasted with NA compared to
296 when CHO fed, respectively. The small decrement in distance covered measured in the
297 overnight fasted trials in comparison to the CHO fed trials (6.6%) supports the importance of
298 ingesting CHO in the hours prior to and during high-intensity running to increase CHO
299 availability, turnover and oxidation rates and ultimately optimise performance. Indeed, it has
300 long been known that high CHO availability can delay the onset of fatigue during prolonged
301 intense exercise (10). While a $\sim 7\%$ difference in the distance covered appears a worthwhile
302 improvement for an athlete, it is important to note that the magnitude of the increase in
303 distance covered in the trials in which pre-exercise CHO was consumed was well below the
304 10-15%, which has been estimated as a meaningful variation when using a time to volitional
305 fatigue trial of similar exercise duration (20).

306 The majority of studies which have previously investigated the NA-induced
307 suppression of fat availability on exercise performance have focussed on cycling protocols (5,
308 14, 21, Torrens S.L. et al., unpublished observations). Torrens S.L. et al. (unpublished
309 observations) reported no difference in cycling performance when participants completed a
310 90 min cycling time trial (TT) (~ 300 W, 82% $\dot{V}O_{2\max}$) following the ingestion of NA in a
311 CHO-fed state in comparison to a control trial. Equally, no differences in cycling
312 performance were observed during a ~ 30 min cycling TT (320 W, $\sim 80\%$ $\dot{V}O_{2\max}$) or a 3.5
313 mile cycling TT (~ 12 min) when NA was ingested in a CHO-fed state in comparison to a
314 control trial (16, 25). The findings of these studies might be considered predictable, based on

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315 the nature (short duration, high-intensity) or mode (cycling) of exercise, both of which favour
316 high rates of CHO oxidation (1, 8, 24, 31). The current study adds to the body of knowledge
317 by confirming the importance of CHO as a substrate for sporting activities at higher exercise
318 intensities and during running, where it is recognised that rates of fat oxidation are higher at
319 the same relative intensities than observed during cycling (1, 8). The half-marathon event
320 was chosen for investigation because endogenous fat and CHO stores would be highly
321 available as energy substrates under control conditions (15) and thus a change in performance
322 and fuel use associated with a change in substrate availability would indicate the importance
323 of this fuel source.

324 The second major finding of the current study was that participants were reliant on
325 CHO substrates to fuel muscular work under all experimental conditions as indicated by the
326 predominant contribution of CHO to total energy expenditure (83-91%, **Figure 6**). The mean
327 rates of CHO oxidation for all four conditions was $\sim 4 \text{ g}\cdot\text{min}^{-1}$ which amounts to a total of
328 $\sim 350 \text{ g}$ of CHO for the exercise task (**Table 1**). Such a value is well within the 400-500 g of
329 muscle glycogen stored from the CHO loading diet ($8 \text{ g}\cdot\text{kg}\cdot\text{BM}^{-1}$ CHO) consumed by the
330 trained runners in the 24 h prior to our half-marathon protocol (15). We note that the absolute
331 rates of CHO oxidation in the present study are substantially higher than those reported by
332 Lee et al. (23) during a half-marathon in which CHO was consumed. However, the well-
333 trained status and faster running speeds ($\sim 15 \text{ km}\cdot\text{h}^{-1}$ vs. $12.2 \text{ km}\cdot\text{h}^{-1}$) of our participants along
334 with the higher energy demand of exercise explains such differences. Greater amounts of
335 CHO ($\sim 55 \text{ g}$) were oxidized in the trials involving pre-exercise CHO intake compared to
336 overnight-fasted conditions; this is explained by greater CHO availability and the priming of
337 the hormonal environment to increase rates of CHO utilization (11). The blunting of FFA
338 availability with NA led to an equal increase in total CHO oxidation, regardless of pre-
339 exercise CHO intake. However, even under conditions that should favour fat oxidation

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340 (overnight fasting, absence of exogenous CHO intake during exercise), CHO remained the
341 predominant fuel source (83 % total energy expenditure).

342 It has long been known that the ingestion of NA alters fuel availability and hence
343 muscle substrate selection during exercise (5). A blunting of the typical exercise-induced rise
344 in FFAs has been demonstrated in previous studies that have administered NA in cycling
345 protocols (16, 25) and was clearly demonstrated in the present study, independent of CHO
346 status (**Figure 3**). However, there was an additive effect of pre-exercise CHO and NA on fat
347 metabolism during exercise, as evidenced by the reduction in plasma FFA and glycerol
348 concentrations after 60 min and 80 min of running, respectively, compared to pre-exercise
349 CHO feeding alone. These findings support the results of Murray et al. (25) who reported
350 higher circulating plasma FFAs during submaximal cycling ($\sim 70\% \dot{V}O_{2\max}$) when ingesting
351 CHO compared to the co-ingestion of CHO plus NA. Although the administration of NA in
352 the current study suppressed adipose tissue lipolysis as evidenced by the reduction in plasma
353 FFAs, total fat oxidation during the running protocol was estimated to be ~ 21 g with no
354 difference observed between trials (**Table 1**). As there was only a small contribution from
355 plasma FFAs to total fat utilized, it is likely that a large proportion of the fat oxidized was
356 from intramuscular triglycerides (IMTG) (24, 31). Consequently, the small yet obligatory
357 contribution of endogenous fat substrates when running at high intensity, irrespective of
358 nutritional status pre-exercise should not go unrecognized.

359 Bergstrom et al. (5) reported higher respiratory quotient (RQ) values measured via
360 arteriovenous oxygen difference across the working leg and thus greater CHO utilization
361 during submaximal cycling exercise following administration of NA compared to a control
362 trial. The higher RQ was associated with a 33% increase in muscle glycogen utilization,
363 greater arterial blood lactate concentrations and a reduction in arterial FFA and glycerol
364 concentrations. The measurement of whole body RER in the present study makes it difficult

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365 to isolate the energy contribution from individual CHO sources. However, NA ingestion was
366 associated with a greater increase in blood lactate concentrations at the onset of exercise,
367 regardless of the effect of pre-exercise CHO intake on lowering blood glucose concentrations
368 (**Figure 4**). This provides indirect evidence for a greater reliance on endogenous CHO
369 sources (i.e. muscle and liver glycogen) as previously reported (5).

370 When investigating the interaction between training status, exercise intensity and pre-
371 exercise nutritional state on substrate oxidation, Bergman and Brooks (3) reported that
372 substrate oxidation during graded cycling was largely determined by the relative intensity of
373 exercise. O'Brien et al. (26) have also previously demonstrated the importance of exercise
374 intensity during simulated marathon running in 'fast' (completion time ≤ 2 h, 43 min, 73%
375 $\dot{V}O_2\text{max}$) or 'slow' (completion time ≤ 3 h, 30 min, 65% $\dot{V}O_2\text{max}$) runners. These workers
376 reported RER values and energy contribution from CHO substrates were significantly higher
377 throughout the marathon in the faster runners (0.99, ~85-90% vs. 0.90, ~60-70%), even under
378 conditions in which rates of fat oxidation would be expected to be maximized (i.e., overnight
379 fasted, no CHO feeding during exercise). While a recent study has highlighted the
380 importance of fat oxidation during high-intensity exercise (18), the results of that
381 investigation should be interpreted with caution. Hetlelid et al. (18) reported that RER values
382 during interval run training (6 x 4 min work bouts at ~90-94% of $\dot{V}O_2\text{max}$) were reduced in
383 well trained compared to recreational runners (0.88 vs. 0.95, respectively). However, these
384 workers failed to demonstrate steady-state conditions during exercise, or correct for
385 bicarbonate kinetics, so it is not known if breath $\dot{V}O_2$, and $\dot{V}CO_2$ values accurately reflect
386 tissue oxygen consumption and CO_2 production (12). Furthermore, even if the rates of fat
387 oxidation were valid in that study (18), they would still only contribute a maximum of 38%
388 of total energy expenditure in their well trained runners (18), demonstrating CHO rather than
389 fat dependence. Our results support the original findings of O'Brien et al (24), who reported

390 CHO dependency in both CHO fed and overnight fasted conditions when running a half-
391 marathon, and further reinforce the fact that when highly trained athletes compete in
392 endurance events lasting up to 3 h, CHO-, not fat-based fuels are the predominant fuel for the
393 working muscles and CHO, not fat availability becomes rate limiting for performance (17).

394 In conclusion, the results of the current study show that well-trained runners are CHO
395 dependent when running a half-marathon at race pace. Furthermore, when CHO availability
396 is high, blunting the exercise-induced increase in FFA via NA ingestion did not impair
397 intense exercise capacity in competitive athletes. During exercise of this intensity and
398 duration, fat oxidation constitutes only a small percentage of overall energy expenditure
399 independent of pre-event CHO status and CHO availability during exercise. While there is a
400 small but obligatory use of fat-based fuels during intense endurance exercise lasting ~90 min,
401 the oxidation of CHO-based fuels predominate. Therefore, endurance athletes should
402 undertake dietary strategies that ensure high-CHO availability before and during competition
403 to maximise rates of CHO oxidation and optimise race performance.

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410 **Disclosures**

411 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 \pm 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 \pm 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 \pm 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 \pm 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,

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537 fasted trial; FAST-NA, fasted with nicotinic acid trial. Values are means \pm SD. Significantly
538 different between treatments ($P < 0.05$), *CFED-NA to FAST.

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Table 1. Metabolic responses for the four experimental trials

Treatment	CHO (g·min⁻¹)	Fat (g·min⁻¹)	CHO (umol·kg·min⁻¹)	Fat (umol·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

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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

Treatment	$\dot{V}O_2$ (L·min ⁻¹)	$\dot{V}CO_2$ (L·min ⁻¹)	HR (bpm)	RR (bpm)	RPE	Time (min)	% $\dot{V}O_{2max}$ ⁵⁵²
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.