Carbohydrate dependence during prolonged simulated cycling time-trials

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Running Head: Carbohydrate dependence during intense cycling.

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Abstract

Purpose: We determined the effect of supressing lipolysis via administration of Nicotinic acid (NA) and pre-exercise feeding on rates of whole-body substrate utilisation and cycling time-trial performance (TT). **Methods:** In a randomised, single-blind, crossover design, 8 trained male cyclists/triathletes completed two series of TT's in which they performed a predetermined amount of work calculated to last ~60, 90 and 120 min. TT's were undertaken after a standardised breakfast (2 $g \cdot kg^{-1}$ BM of carbohydrate [CHO]) and ingestion of capsules containing either NA or Placebo (PL). **Results:** Plasma [free fatty acids] were supressed with NA but increased in the later stages ofTT90 and TT120 with PL ($p<0.05$). There was no treatment effect on time to complete TT60 (60.4 ±4.1 vs. 59.3 ±3.4 min) or TT90 (90.4 ±9.1 vs. 89.5 ±6.6 min) for NA and PL, respectively. However, TT120 was slower with NA (123.1 ±5.7 vs. 120.1 ±8.7 min, $p<0.001$), which coincided with a decline in plasma [glucose] during the later stages of this ride ($p<0.05$). For TT's of the same duration, rates of whole-body CHO oxidation were unaffected by NA, but decreased with increasing TT time ($p<0.05$). CHO was the predominant substrate for all TT's contributing between 83-96% to total energy expenditure, although there was a small use of lipid-based fuels for all rides. **Conclusion:** 1) NA impaired cycling TT performance lasting 120 min, 2) cycling TT's lasting from 60 to 120 min are CHO dependent, and 3) there is an obligatory use of lipid-based fuels in TT's lasting 1-2 h.

Key words: Fat, high-intensity cycling, nicotinic acid, substrate utilisation, performance.

Abbreviations:

BM – body mass

CHO – carbohydrate

FA – fatty acid

FFA – free fatty acids

HR – heart rate

IMTG – intramuscular triglyceride

LMM – linear mixed model

NA – nicotinic acid

PL – placebo

PPO – peak power output

RER – respiratory exchange ratio

RPE – rating of perceived exertion

TG – triglycerides

TT – time trial

$\dot{V}O_2max$ – maximal oxygen consumption

W – Watts

Introduction

The question of what fuels support muscle contraction during exercise has been a topic of long-standing interest to physiologists since the early 1900's (for a review see Hawley et al. 2015), and today it is well accepted that during continuous activities lasting more than a few minutes duration, both intra- and extra-muscular carbohydrate and lipid substrates are the predominant substrates oxidised by the working muscles. Furthermore, there is consensus that at the intensities at which competitive endurance athletes train and race (i.e. >65% of $\dot{V}O_2max$), the primary determinant of the balance of carbohydrate (muscle and liver glycogen, blood glucose and lactate) and lipid (adipose and intramuscular triglycerides, blood-borne free fatty acids [FFAs] and triglycerides) fuels for the working muscles is the relative exercise intensity (Bergman et al. 1999; Romijn et al. 1993; van Loon et al. 2001). However, the energy available from endogenous carbohydrate stores are limited, while fat stores, even in highly trained individuals, are abundant (Martin and Klein 1998). Consequently, increasing the contribution from fat-based fuels to the total energy requirements of prolonged, intense endurance exercise could 'spare' endogenous carbohydrate reserves and improve endurance capacity. Several studies have used a combination of dietary and other interventions to increase fat availability before and during exercise (Burke et al. 2000; Burke and Hawley 2002; Carey et al. 2001) and while such strategies result in robust increases in fat oxidation and reduce muscle and liver glycogen utilisation, they fail to improve performance in endurance tasks lasting several hours (Burke et al. 2000; Carey et al. 2001; Havemann et al. 2006).

In contrast to increasing fat availability before or during endurance exercise, several studies have determined the effect of reducing fatty acid (FA) availability on substrate metabolism and performance. This has typically been achieved by administration of drugs that block lipolysis such as Nicotinic acid (NA) (Bergström et al. 1969; Murray et al. 1995) or Acipimox (Zderic et al. 2004). Murray, et al. (1995) reported that inhibiting the exercise-induced rise in FFA concentration (via NA administration) had no effect on a cycling time-trial (TT) lasting ~15 min, while Hawley, et al. (2000) showed little effect of either increasing (via a high-fat meal and intravenous heparin administration) or decreasing (via NA ingestion) fat availability on ~30 min cycling TT performance. These studies (Hawley et al. 2000; Murray et al. 1995) both used intense cycling protocols lasting <30 min during which CHO-based fuels would be expected to be the major energy source for oxidation by the working muscles (Romijn et al. 1993), independent of fat availability. However, as exercise duration increases, reliance on CHO-based fuels declines while that from fat-based fuels increase (Brooks and Mercier 1994; Romijn et al. 1993). Consequently, if FA availability and oxidation is obligatory during maximal exercise lasting $\geq$60 min, blocking lipolysis may impact negatively on performance. Hence the aim of the present study

was to determine the effect of inhibiting the endurance exercise-induced rise in plasma FFA levels before and during simulated cycling TTs lasting ~60, 90 and 120 min. We hypothesised that reducing FA availability would not impair performance of TTs of these durations and that CHO-based fuels would be the major source of fuel for oxidative metabolism.

Methods

Subjects

Eight trained male cyclists/triathletes aged 18-40 y, with a $\dot{V}O_{2max}$ >60 mL·kg·min^{-1} and ≥2 y TT racing experience, were recruited to participate in this study. At the time of investigation, all subjects were cycling a minimum of 200 km·wk^{-1}. Subjects were informed of the nature of the study and possible risks involved before giving their written consent. The study was approved by the Human Research Ethics Committee of the Australian Catholic University (Reference number 2014 51V). The study was prospectively registered with the Australian New Zealand Clinical Trials Registry (ACTRN12614000629639).

Preliminary testing

Two weeks prior to commencing their first experimental trial, subjects performed a maximal, incremental cycle test to voluntary exhaustion on an electromagnetically-braked ergometer (Lode Excalibur Sport, Groningen, The Netherlands) to determine $\dot{V}O_2$max and peak sustained power output (PPO, the peak work-load attained at the completion of the test [W]), as described previously (Hawley and Noakes 1992). Briefly, the test protocol commenced at 3.33 W·kg^{-1} for 150 s and thereafter was increased by 50 W for a further 150 s. Subsequently, increases of 25 W every 150 seconds occurred until volitional fatigue or a drop in cadence below 70 rev·min^{-1}. During the maximal test and portions of the experimental trials expired breath was measured for oxygen and carbon dioxide expired using a calibrated online gas analyser (True One 2400 Parvo Medics Metabolic Measurement System). $\dot{V}O_2$max was determined as the highest 30s average $\dot{V}O_2$ consumption. All testing sessions were conducted under standard laboratory conditions (18-22 °C, 40-50% relative humidity), and subjects were fan cooled during all exercise sessions.

Exercise and dietary control

In the 48 h prior to each experimental trial subjects abstained from any vigorous physical activity. Any exercise completed during this time was of low intensity (<60% of $\dot{V}O_2$max) and short duration (≤1 h) and recorded by the subject each week in a training log. The same training was then repeated before subsequent trials. Subjects were provided with a pre-packaged standardised diet containing 8 g CHO·kg^{-1} BM for the 24 h

before each trial and requested to abstain from caffeine-containing beverages (i.e., tea, coffee, cola, energy drinks) and alcohol consumption during this period. On each trial day, subjects consumed a standardised breakfast of 2 g CHO·kg^{-1} BM 2 h before the start of a TT. Dietary records and training logs were kept to ensure compliance.

Familiarisation session

All subjects completed a familiarisation session one week prior to the start of their first experimental trial to habituate to laboratory conditions and the experimental protocol. Using the same protocol as experimental trials (described subsequently) subjects completed a ~30 min TT. All familiarisation sessions and experimental trials were undertaken on the same ergometer. Blood samples were not taken during the familiarisation session and NA was not administered.

Overview of the experimental design

An overview of the experimental protocol is shown in *Figure 1*. In brief, in a randomised, single-blind, crossover design, eight trained male cyclists/triathletes undertook two series of three simulated TTs during which they completed specific amounts of mechanical work calculated to take ~60, 90 and 120 min. In one series of rides subjects were administered NA, while the second series acted as a placebo control. In total, participants completed six TT's over an 8 wk period. On the day of a trial, subjects arrived at the laboratory at ~0700 h after a 10 h overnight fast. After voiding, a Teflon catheter (20-22G; Terumo, Japan) was inserted into the antecubital vein of one arm to allow for serial blood sampling. After a resting blood sample (6 mL) was taken, subjects ingested the standardised breakfast and rested for 105 min. At this time subjects were weighed and commenced a warm up (15 min at 60% of individual PPO (~70% $\dot{V}O_2$max)) followed by 1 min rest. Subjects were then informed of the length of the TT to be completed that day and were given a brief count down to the start of each ride. Subjects were encouraged to complete the designated work as fast as possible and provided with financial incentives for the fastest total time to complete all six TTs (for first, second and third place, respectively). Water was ingested *ad libitum* during the TT and total fluid intake was recorded. BM was measured upon completion of each TT. Expired air was measured at 20 min intervals during the TTs at the same time as ratings of perceived exertion (RPE) using the Borg Scale (Borg 1982) and heart rate (HR) using telemetry (Polar, Kempele, Finland).

Blood sampling

Blood samples (6 mL) were taken upon arrival at the lab, 20 min after the commencement of the ingestion of breakfast, at the start of the warm up, at the start of each TT and at the completion of each third of

155 the total workload to be completed in that TT (*Figure 1*). Following each blood sample, and at regular 20-30
156 min intervals, catheters were flushed with 3-5 mL saline (0.9% NaCl $g \cdot L^{-1}$). Blood samples were collected in 6
157 mL EDTA tubes and spun at 3500 RPM for 10 min at 4 ºC. The resultant plasma was aliquoted and stored at -80
158 ºC for later analyses.

159 *Respiratory gases measurement*

160 During the familiarisation session and experimental trials, expired air was measured for three
161 consecutive min at the 5 min time-point of the warm up, at the 5 min time-point of the TT and at regular 20 min
162 intervals during each TT to estimate rates of substrate oxidation from the non-protein respiratory exchange ratio
163 (RER = $\dot{V}CO_2/\dot{V}O_2$). The final expired gas collection for each TT was taken during the last 100 kJ of work. For
164 each time-point, the first 30 s of data was removed and therefore the final 150 s of data was averaged. Mean
165 RER values were calculated across all measurement time-points within a TT.

166 *Nicotinic acid/placebo administration*

167 NA or placebo treatments were administered in capsule form. NA was given 30 min (10 $mg \cdot kg^{-1}$BM)
168 and 15 min (5 $mg \cdot kg^{-1}$BM) prior to the start of a designated trial, with a further 5 $mg \cdot kg^{-1}$BM ingested every 30
169 min during TTs. Placebo capsules were indistinguishable from NA capsules in taste and colour, and were
170 provided to subjects at the same time-points. Dosages were prescribed based on previous NA studies and taking
171 into consideration exercise intensity and duration (Gollnick et al. 1981; Grundy et al. 1981; Murray et al. 1995;
172 Hawley et al. 2000).

173 *Time trial protocol*

174 Simulated cycling TTs were undertaken with the ergometer set in a cadence-dependent power-output
175 (linear) mode for subjects to complete set amounts of mechanical work for different TT durations. Power output
176 was therefore function of cadence and a fixed factor (alpha value) as described in the following equation: Power
177 (W) = $[\text{Cadence (rpm)}]^2$ x [alpha (W/rpm^2)]. A custom-determined, alpha value was assigned to each individual
178 for each trial duration based on the calculations of total workload and expected cadence as described below. The
179 amount of work to be completed during each TT duration was calculated according to each subject's PPO and
180 based on previous data (Jeukendrup et al. 1996) and pilot testing. We estimated that subjects would be able to
181 maintain average power-outputs equivalent to ~75, 74, and 73% of individual PPO for the 60, 90 and 120 min
182 TTs, respectively. Accordingly, the mechanical power-output to be completed was determined as follows:

183 ~TT60 min TT Work (joules) = 0.75 x PPO x 3600 s

184 ~TT90 min TT Work (joules) = 0.74 x PPO x 5400 s

185 ~TT120 min TT Work (joules) = 0.73 x PPO x 7200 s

186

187 Alpha values for the linear mode were calculated for subjects to cycle at a cadence of 100, 97 and 95
188 rev·min^{-1} for the 60, 90 and 120 min TTs respectively to complete the trial in the estimated time. Cadence for
189 each duration was based on previous validation of the 60 min TT protocol (Jeukendrup et al. 1996), accounting
190 the effect of exercise duration on cadence selection (Hansen and Smith 2009), and corroborated during pilot
191 testing. To simulate race conditions and allow optimal pacing strategy, subjects were provided with real-time
192 feedback pertaining to power output, HR, cadence, elapsed time and total mechanical work.

193 *Analytical techniques*

194 Plasma FFA concentrations were measured using a non-esterified-fatty acid (NEFA) assay kit (Wako
195 Pure Chemical Industries, Ltd, Osaka, Japan). Plasma glucose and lactate concentrations were analysed using a
196 YSI 2300 STAT Plus™ analyser (Yellow Springs, USA). Whole-body rates of CHO and fat oxidation (g·min^{-1})
197 were determined for each steady-state gas measurement point from the rates of CO_2 production ($\dot{V}CO_2$) and O_2
198 consumption ($\dot{V}O_2$) using the non-protein RER values (Péronnet and Massicotte 1991). These equations are
199 based on the assumption that $\dot{V}O_2$ and $\dot{V}CO_2$ accurately reflect tissue O_2 consumption and CO_2 production. In
200 well-trained subjects, similar to those employed in the current investigation, indirect calorimetry has been
201 shown as a valid method for quantifying rates of substrate oxidation during strenuous exercise at 85% $\dot{V}O_2$max
202 (Romijn et al. 1992).

203

204 *Statistical analysis*

205 Statistical analysis was undertaken using two way (duration × treatment) repeated measures ANOVAs
206 (IBM SPSS Statistics, version 22). Where ANOVA revealed significant differences of time, post-hoc analyses
207 (Student–Newman–Keuls) were conducted. Linear mixed models (LMM) were used to analyse data which had
208 more than one time point (i.e. blood glucose, lactate, FFA, and power output). Subsequent post-hoc comparisons
209 (Bonferroni) between the two treatment groups were conducted within the LMM. Statistical significance was
210 set at $p<0.05$. All data are presented as mean ±SD, with 95% confidence intervals (CI) where appropriate.

211

Results

Subjects

One subject was unable to complete the workload for NA TT120 and therefore his data was excluded from all trials. Consequently, seven subjects were included in the final analysis (age 26 ±6 y, body mass (BM) 76.1 ±13.1 kg, peak power output (PPO) 409 ±57 W, maximal oxygen consumption ($\dot{V}O_2max$) 4.9 ±0.7 $L \cdot min^{-1}$).

Blood parameters

Plasma free fatty acid concentrations

Plasma FFA concentrations were similar before all TTs (*Figure 2*). There were main effects for both treatment ($p<0.003$), trial duration ($p=0.044$) and time ($p<0.001$), as well as an interaction effect of treatment × duration × time ($p<0.001$), on plasma FFA concentrations. There was a significant increase in plasma FFA concentrations at the end of the PL TT90 and PL TT120 compared to the respective NA trials (TT90: $p<0.001$, 95%CI: 113 – 257 mEq/L; TT120: $p<0.001$, 95%CI: 149 – 303 mEq/L). During TT90 and TT120, FFA concentrations were higher for PL compared to NA after two-thirds of the total work to be completed (TT90: $p<0.01$, 95%CI: 23 – 167 mEq/L; TT120: $p<0.002$, 95%CI: 43 – 197 mEq/L) and at completion (TT90: $p<0.001$, 95%CI: 113 – 257 mEq/L; TT120: $p<0.001$, 95%CI: 149 – 303 mEq/L).

Plasma glucose concentrations

There was a main effect of time ($p<0.001$) and trial duration ($p<0.001$) for plasma glucose concentration (*Figure 3*), with no treatment or interaction effects. Plasma glucose concentration increased above rest following the ingestion of breakfast (-100 min) in all trials ($p<0.04$) after which there was a decrease below resting values until exercise was commenced ($p<0.001$). After one-third of the total work was completed for the respective TTs plasma glucose levels had returned to baseline values. In contrast, at the end of the NA TT120, there was a drop in plasma glucose concentration compared to baseline ($p=0.048$, 95%CI: -2.02 – -0.004 mM) and the one-third of the total work time point ($p=0.007$; 95%CI: -2.20 – -0.18 mM). Compared to PL, NA also reduced plasma glucose concentration at the end of TT90 ($p=0.03$; 95%CI: -1.27 – -0.06 mM) and TT120 ($p=0.03$; 95%CI: -1.32 – -0.07 mM).

Plasma lactate concentrations

There were main effects of duration ($p<0.001$) and time ($p<0.001$), but no treatment or interaction effect, on plasma lactate concentrations (*Figure 4*). At the onset of exercise, plasma lactate concentration increased above rest at the onset of exercise and remained elevated throughout exercise ($p<0.05$) for all trials.

With the exception of NA TT120, plasma lactate concentrations were elevated at the end of all TTs ($p<0.001$) compared to values measured after completion of one-third of the total work. Plasma lactate concentration in NA TT120 was reduced ($p<0.001$; 95%CI: -4.0 – -1.5 mM) at the end of the experimental ride compared to PL.

Respiratory parameters and rates of substrate oxidation

Table 1 and *2* display the respiratory and metabolic data, respectively, for all experimental trials. There was a main effect of TT duration ($p<0.03$), with no effect of treatment observed, for all variables. An interaction effect of duration × treatment was observed for absolute $\dot{V}O_2$ ($p=0.045$) and the fractional utilisation of $\dot{V}O_2max$ ($p=0.037$). Absolute $\dot{V}O_2$ and the fractional utilisation of $\dot{V}O_2max$ were lower in TT120 compared to TT60 ($p<0.015$) and TT90 ($p=0.004$) but no difference was observed between TT60 and TT90. Compared to PL, the absolute $\dot{V}O_2$ and the percentage of $\dot{V}O_2max$ utilised was lower for NA during TT120 ($p=0.01$, *Table 1*).

There was an incremental decrease in RER values with increasing trial duration such that TT60 was higher than TT90 ($p=0.002$) and TT120 ($p=0.001$), and TT90 was higher than TT120 ($p=0.001$). Accordingly, rates of CHO oxidation were higher in TT60 than both TT90 ($p=0.006$) and TT120 ($p=0.001$) and were higher in TT90 than TT120 ($p<0.001$). Consequently, rates of fat oxidation were lower in TT60 than TT90 ($p=0.002$) and TT120 ($p<0.001$). Average rates of fat oxidation were lower in TT90 than TT120 ($p<0.001$). The estimated amount of CHO oxidised during TT60, TT90 and TT 120 min was 301 ± 37 vs. 289 ± 38 g; 425 ± 64 vs. 413 ± 54 g; and 486 ± 30 vs. 502 ± 82 g for NA and PL, respectively. No effect of treatment on the relative energy contribution from CHO and fat for TT60, TT90 or TT120 (*Table 2*) was observed. However, there was an incremental decline in the amount of CHO oxidised and total energy expended (*Table 2*), where TT120 > TT90 > TT60 ($p<0.001$).

Time, power output and rate of perceived exertion

For absolute and relative power output, there were main effects of trial duration ($p<0.001$) and treatment ($p<0.001$) and time ($p<0.02$), with a significant interaction of treatment × duration × time for absolute power output ($p=0.034$) and a trend for an interaction ($p=0.058$) for relative power output (*Table 3*). No differences in time to complete the prescribe work were measured for TT60 or TT90 trials between treatments. However, there was a significant effect of NA on time to complete TT120 (-2.4%, $p<0.005$, 95%CI: -2.3 – -8.2 min; *Figure 5*). NA decreased both the absolute ($p<0.001$; 95%CI: -35 – -12 W) and relative power output ($p<0.001$; 95%CI: -8 – -3%) compared to PL in the final third of work completed (i.e. 3/3 time point).

Mean absolute power output during the TTs was not different across time for NA TT60, PL TT60, PL TT90, and PL TT120. Absolute power output in the final one-third of work was greater than for both the first or

second third of work completed for NA TT90 (p=0.016, 95%CI: 2 – 29 W; and p=0.009, 95%CI: 3 – 30 W; respectively). Similarly, relative power output (%PPO) was greater in the final third of work than the first or second third of work completed for NA TT90 (p=0.021, 95%CI: 0.5 – 7.0% and p=0.012, 95%CI: 0.7 – 7%; respectively). For NA TT120, absolute and relative power output in the final third of work (i.e. 3/3) were greater than the first third (i.e. 1/3) of work completed (p=0.025, 95%CI: 1 – 30 W; and p=0.042, 95%CI: 0.1 – 7.0%; respectively). The final third of work in PL TT120 was significantly greater than in NA TT120 for both absolute and relative power output (p<0.001, 95%CI: 12.0 – 35.0 W, and p=0.001, 95%CI: 2.6 – 8.2%, respectively). There was no treatment or time effect on average RPE (*Table 1*).

Discussion

The first novel finding from the current study was that inhibiting lipolysis and the normal endurance exercise-induced rise in FFA availability (via NA ingestion) significantly impaired performance of a simulated cycling TT lasting 120 min. In contrast, administration of NA had no effect on TT's of 60 or 90 min duration. A second finding was that when following sport nutrition guidelines (i.e., a high CHO diet 24 h before and pre-event CHO meal), cycling TT's lasting 60, 90 and 120 min were dependent on CHO for skeletal muscle energy metabolism, contributing between 83 and 93% of total energy (*Table 2*). However, for all rides there was a small, but obligatory use of fat-based fuels.

The impaired TT120 performance (-2.4%, p<0.001, *Figure 5*) after NA administration was in contrast to one of our original hypotheses. Subjects were blinded to all treatment conditions and reported no differences in their RPE during any of the rides (*Table 1*). However, we cannot exclude the possibility that NA may have had a direct effect on skeletal muscle substrate turnover and oxidation. Bergstrom et al. (1969) previously reported that the arterio-venous respiratory quotient during leg exercise is higher after ingestion of NA and demonstrated that the reduced delivery of FFA to the muscles was compensated by an increased metabolism of muscle glycogen. We cannot distinguish between the different CHO-based fuels oxidised among treatments from whole-body RER measures (*Table 1*). However, a greater use of endogenous muscle (and liver) glycogen with NA seems plausible. Indirect support for this notion comes from the plasma glucose and lactate concentrations observed at the end of the different TTs. Plasma glucose concentrations at the final sampling point in TT120 with NA administration were lower compared to all other TTs and had declined to below resting (pre-exercise) values. Plasma lactate responses were also different with NA ingestion: at the end of TT120, plasma lactate levels failed to rise in response to an increase in power output (a sustained all-out sprint during

the final minutes of the ride) compared to values observed during other TT's. These differential lactate levels show the importance CHO availability for supporting supramaximal sprint during the final stages of an event.

Based on the training status of our subjects and the CHO-based nutritional intervention, estimated muscle glycogen stores would have been in the range of~450-550 g (Hawley and Burke 1997). As expected, the longer the TT duration, the greater the total amount of CHO oxidised. In the present study, ~300 g of CHO was utilised in TT60 suggesting that muscle glycogen availability was not rate-limiting for performance. For TTs lasting 90 min, total CHO oxidation amounted to ~400 g and endogenous muscle glycogen stores alone would have been adequate to fuel the metabolic demands of exercise. Only during TTs lasting 120 min did the total rates of CHO oxidation (~500 g) approach the upper limits of endogenous muscle glycogen stores. It could be argued that the increased demand on glycogen and the subsequent inability to maintain blood glucose was a result of NA inhibiting the supply of glycerol from adipose tissue lipolysis for gluconeogenesis. However, the maximal rate of gluconeogenesis from glycerol during prolonged exercise in trained individuals is minimal (only 1-2 µmol glucose·min·kg^{-1}) and even complete inhibition of gluconeogenesis from glycerol would account for only a 2-3 g of glucose during 120 min of cycling for a 75 kg individual (Coggan et al. 1995).

A second finding of the current study was that performance of self-paced endurance cycling TT's lasting between 60 and 120 min was highly dependent on the oxidation of CHO-based fuels, which contributed between 83 and 93% of total energy expenditure (*Table 2*). In a recent investigation, we reported that altering FA availability did not impair prolonged (~90 min), continuous running to fatigue (Leckey et al. 2015). In that study, blunting the exercise-induced increase in FFA via nicotinic acid ingestion did not alter patterns of substrate utilisation during a half marathon run, and carbohydrate was the primary fuel for the exercising muscles, contributing ~87% to total energy (Leckey et al. 2015). Our data showing that high rates of CHO oxidation are required to sustain high-intensity cycling (≥80% $\dot{V}O_2$max), regardless of plasma FFA availability, are also in agreement with the results from previous studies (Cole et al. 2014; Hawley et al. 2000; Romijn et al. 1993; van Loon et al. 2001). Indeed the rates of whole-body CHO and fat oxidation measured during the current investigation are also similar to those reported by others for highly-trained endurance cyclists working at similar absolute (~300 W) and relative (>80% of $\dot{V}O_2$max) power outputs (Romijn et al. 1993; van Loon et al. 2001).

Clearly at the intensities at which competitive endurance athletes' train and race (>70-75% of $\dot{V}O_2$max), the exercising muscles are dependent on CHO for oxidative metabolism (Bergström et al. 1967) independent of training and nutritional status (Bergman and Brooks 1999) or fat availability (Romijn et al. 1995). Indeed, the ability to oxidise both endogenous (Cole et al. 2014) and exogenous (Cox et al. 2010) CHO-

based fuels at high rates during intense exercise appears to be a requirement for optimum endurance performance. O'Brien et al., (1993) reported that a during a simulated treadmill marathon, 'fast runners' (finishing time ≤2 h, 45 min) had significantly higher RER values (0.99 vs. 0.90, $p \leq 0.05$) throughout the marathon compared to a group of 'slow runners' (finishing time ≤3 h, 45 min). Indeed, even under conditions in which the oxidation of fat-based fuels would be expected to approach the upper limits (i.e., no pre-race CHO-loading, overnight fast, and no exogenous CHO supplementation during exercise), the contribution from CHO-based fuels to total energy was ~85-90% compared to ~60-70% for the fast and slow runners, respectively (O'Brien et al. 1993).

Finally, despite a dependence on CHO for muscle metabolism during all TT's, there was a small but obligatory contribution from fat-based fuels to total energy expenditure (6-17%) that increased with the duration of a TT (*Table 2*). Despite the administration of NA, we cannot rule out the possibility that blood-borne FFAs contributed, in part, to the rates of whole-body fat oxidation. However, based on the high absolute (~300 W) and relative (>80% of $\dot{V}O_2max$) exercise intensities sustained by our subjects for all TT's, IMTG's were likely to be the major source of lipid for oxidation by the muscle, at least during TT's lasting 60 and 90 min (Romijn et al. 1993; van Loon et al. 2001). Nevertheless, our data shows that even when lipolysis is supressed and CHO availability is high (i.e., high pre-exercise muscle and liver glycogen, high CHO availability before exercise) there is still a small requirement by the working muscle for fat-based fuels for oxidative metabolism.

In conclusion, inhibiting lipolysis and the normal endurance exercise-induced rise in FFA availability had little effect on simulated TT's lasting 60 or 90 min but impaired performance of TT's lasting 120 min. There was a small, but obligatory contribution from fat-based fuels to total energy expenditure, which increased with the duration of a TT. However, cycling TTs lasting 60 to 120 min were highly dependent on CHO for skeletal muscle energy metabolism.

Conflict of interest:

The authors declare that they have no conflict of interest.

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427

Figure Legends

Figure 1. Schematic figure of experimental design. After 48 h of exercise and 24 h of dietary control, each subject undertook two series of simulated time-trials (TTs) during which they completed specific amounts of mechanical work calculated to take ~ 60, 90 and 120 min. In one series of TTs subjects were administered 15 mg·kg body mass $(BM)^{-1}$ of nicotinic acid (NA) before the trial and 5 mg·kg BM^{-1} every 30 min during the ride, while the second series acted as a placebo (PL) control. Blood samples were obtained before the start of TTs and every 1/3 of total work and respiratory expired gases sampled during warm-up, 5 min after starting, every 20 min and during the last 100 kJ of work. Warm up, 15 min at 60% of Peak Power Output, 1 min rest.

Figure 2. Plasma free fatty acid concentrations measured during experimental periods across each of the six time trials (*n*=7) for A: 60 min time trials, B: 90 min time trials, and C: 120 min time trials. Values are means ±SD. NA, nicotinic acid; PL, placebo; 60, 60 min time-trial; 90, 90 min time-trial; 120,120 min time-trial. Different ($p<0.05$) vs † rest; ‡ one-third of exercise; ^{b} NA 60; ^{c} PL 90; ^{d} NA 90; ^{e} PL 120 and ^{f} NA 120 within time point. ⌐, referring to both treatments; where symbols are both above and below the SD bars, the symbols are for the corresponding point only.

Figure 3. Plasma glucose concentrations measured during experimental periods across each of the six time trials (*n*=7) for A: 60 min time trials, B: 90 min time trials, and C: 120 min time trials. Values are means (±SD). NA, nicotinic acid; PL, placebo; 60, 60 min time-trial; 90, 90 min time-trial; 120,120 min time-trial. Different ($p<0.05$) vs: †rest; ‡one-third of exercise; ^{c} PL 90; ^{d} NA 90; ^{e} PL 120 and ^{f} NA 120 within time point. ⌐, referring to both treatments; where symbols are both above and below the SD bars, the symbols are for the corresponding point only.

Figure 4. Plasma lactate concentrations measured during experimental periods across each of the six time trials (*n*=7) for A: 60 min time trials, B: 90 min time trials, and C: 120 min time trials. Values are means (±SD). NA, nicotinic acid; PL, placebo; 60, 60 min time-trial; 90, 90 min time-trial; 120,120 min time-trial. Different ($p<0.05$) vs: †rest; ‡one-third of exercise; ^{c} PL 90; ^{d} NA 90; ^{e} PL 120 and ^{f} NA 120 within time point. ⌐, referring to both treatments; where symbols are both above and below the SD bars, the symbols are for the corresponding point only.

458 *Figure 5.* Time trials time to completion. Bars represent the means. Individual points connected by lines
459 represent individual subjects (*n*=7).

Tables

Table 1. Respiratory data and rates of perceived exertion (RPE) averaged across measures taken during experimental periods across each of the six time trials by well-trained, male cyclists (*n*=7).

Condition	$\dot{V}O_2$ (L·min^{-1})		$\dot{V}CO_2$ (L·min^{-1})		RER		% $\dot{V}O_2$max		Average RPE
PL 60	4.13 (0.42)	§	3.94 (0.46)	§	0.97 (0.02)	#§	83 (5)	§	16 (1)
NA 60	4.10 (0.52)		3.95 (0.52)		0.98 (0.02)		82 (4)		16 (1)
PL 90	4.08 (0.49)	§	3.83 (0.52)	§	0.96 (0.02)	§	82 (5)	§	16 (1)
NA 90	4.11 (0.49)		3.88 (0.56)		0.97 (0.02)		82 (6)		16 (1)
PL 120	4.04 (0.48)*		3.81 (0.52)		0.94 (0.02)		82 (3)*		16 (1)
NA 120	3.79 (0.46)		3.57 (0.40)		0.94 (0.02)		77 (5)		16 (1)

Values are means (±SD). Different ($p<0.05$) vs. *, NA for same trial duration; #, 90 min; §, 120 min. CHO, carbohydrate oxidation; FAT, fat oxidation; NA, nicotinic acid; PL, placebo; RER, respiratory exchange ratio; $\dot{V}CO_2$, carbon-dioxide production; $\dot{V}O_2$, oxygen uptake; 60, 60 min time-trial; 90, 90 min time-trial; 120,120 min time-trial.

Table 2. Fuel oxidation data based on respiratory data for rates and contributions of carbohydrate (CHO) and fat oxidised averaged across measures taken during experimental periods across each of the six time trials (*n*=7).

Condition	CHO ($g \cdot min^{-1}$)		CHO ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)		Fat ($g \cdot min^{-1}$)		Fat ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)		Energy expenditure (kJ)		Fuel contribution (%) CHO		Fat	
PL 60	4.83 (0.63)	#	360 (42)	#	0.18 (0.10)	#	8.6 (4.8)	#	5461 ± 591	#	92 ± 4	#	8 ± 4	#
NA 60	4.99 (0.68)	§	360 (53)	§	0.14 (0.08)	§	6.8 (3.6)	§	5633 ± 602	§	94 ± 3	§	6 ± 3	§
PL 90	4.57 (0.73)	§	317 (54)	§	0.24 (0.13)	§	10.8 (5.4)	§	8027 ± 897	§	89 ± 5	§	11 ± 5	§
NA 90	4.72 (0.78)		343 (46)		0.22 (0.10)		10.0 (4.2)		8250 ± 1003		91 ± 4		9 ± 4	
PL 120	4.27 (0.75)		313 (38)		0.37 (0.16)		16.4 (6.4)		10543 ± 1170		83 ± 7		17 ± 7	
NA 120	3.96 (0.35)		293 (41)		0.36 (0.14)		15.1 (1.9)		10287 ± 896		83 ± 4		17 ± 4	

Values are means (±SD). Different ($p<0.05$) vs. *, NA for same trial duration; #, 90 min; §, 120 min. CHO, carbohydrate oxidation; FAT, fat oxidation; NA.

Table 3. Absolute and relative power output across the duration of each time trial during experimental periods across as performed (*n*=7).

Condition	Power (W)			Power (%PPO)		
	1/3	2/3	3/3	1/3	2/3	3/3
PL 60	309 ± 46	303 ± 44	311 ± 33	76 ± 5	75 ± 4	77 ± 4
NA 60	305 ± 48	297 ± 52	307 ± 46	75 ± 4	73 ± 5	76 ± 4
PL 90	302 ± 38	296 ± 41	304 ± 49	75 ± 5	73 ± 5	75 ± 6
NA 90	294 ± 46	293 ± 46	309 ± 49$^{‡£}$	72 ± 7	72 ± 6	76 ± 4$^{‡£}$
PL 120	304 ± 39	300 ± 41	304 ± 50*	75 ± 3	73 ± 3	74 ± 5*
NA 120	296 ± 43	292 ± 44	281 ± 37‡	72 ± 2	71 ± 3	69 ± 7‡

Values are means (± SD). Different ($p<0.05$) vs. *, NA for same trial duration and time point; ‡, one-third of exercise within trial duration; $^{£}$, two-thirds of exercise within trial duration. 60, 60 min time-trial; 90, 90 min time-trial; 120,120 min time-trial; W, watts; %PPO, % peak power output.