

Effect of altering substrate availability on metabolism and performance during intense exercise

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The purpose of this study was to determine the effect of altering substrate availability on metabolism and performance during intense cycling. Seven highly trained men ingested a random order of three isoenergetic meals 90 min before cycling at 80 % maximal oxygen uptake ($\text{VO}_{2\text{max}}$) for 20 min (about 310 W), followed by a 600 kJ time trial lasting about 30 min. Meals consisted of either 1.2 g saturated fat/kg body mass (BM) with 3500 U heparin intravenously (HIFAT) to elevate circulating plasma free fatty acid (FA) concentration, 2.5 g carbohydrate/kg BM (CHO) to elevate plasma glucose and insulin concentrations or 2.5 g carbohydrate + 20 mg nicotinic acid/kg BM (NA) to suppress lipolysis and reduce free FA concentration. HIFAT elevated free FA concentration (HIFAT 1.3 (SEM 0.2), CHO 0.2 (SEM 0.1), NA 0.1 (SEM 0.1) mM; $P < 0.001$), lowered the RER (HIFAT 0.94 (SEM 0.01), CHO 0.97 (SEM 0.01), NA 0.98 (SEM 0.01); $P < 0.01$) and increased the rate of fat oxidation (HIFAT 24 (SEM 3), CHO 12 (SEM 2), NA 8 (SEM 3) $\mu\text{mol/kg per min}$; $P < 0.01$) during the 20 min ride. Marked differences in fat availability and fuel utilisation, however, had little effect on performance in the subsequent time trial (HIFAT 320 (SEM 16), CHO 324 (SEM 15), NA 315 (SEM 13) W). We conclude: (1) increased fat availability during intense cycling increases the rate of fat oxidation; but (2) the reduction in the rate of carbohydrate oxidation in the presence of high circulating plasma free FA is unlikely to enhance intense exercise performance lasting about 1 h; (3) substrate selection during intense (about 80 % $\text{VO}_{2\text{max}}$) exercise is dominated by carbohydrate oxidation.

Carbohydrate: Cross-over concept: Fat: Nicotinic acid

During continuous, high-intensity exercise performed at about 85 % maximal O_2 uptake ($\text{VO}_{2\text{max}}$) and lasting about 1 h, muscle glycogen is the major energy source for muscle (Romijn *et al.* 1993). At such intensities, the rate of muscle glycogenolysis is rapid (Dyck *et al.* 1993, 1996) and the concentrations of plasma lactate (Romijn *et al.* 1995; Hawley *et al.* 1997) and catecholamines (Kjaer *et al.* 1991) are 2–3-fold higher compared with moderate intensity (60–70 % $\text{VO}_{2\text{max}}$) exercise. During intense exercise, the rate of appearance of fatty acids (FA) into the plasma are suppressed (Romijn *et al.* 1993; Sidossis *et al.* 1997) and

net contracting leg FA uptake is reduced (Kjaer *et al.* 1991), partly because of the failure of FA mobilisation to increase above resting basal levels (Romijn *et al.* 1993) and also due to insufficient blood flow and albumin delivery to carry FA from the peripheral adipocytes into the systemic circulation (Hodgetts *et al.* 1991).

In the search for strategies to enhance athletic performance, many studies have investigated a variety of nutritional procedures that increase fat availability and promote FA oxidation (for review see Hawley *et al.* 1998). In theory, any intervention that elevates the rate of

Abbreviations: BM, body mass; CHO, high-carbohydrate meal; FA, fatty acids; HIFAT, high-fat meal plus intravenous heparin; NA, high-carbohydrate meal plus nicotinic acid; $\text{VO}_{2\text{max}}$, maximal O_2 uptake.

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appearance of FA into the plasma above those levels normally observed during moderate-intensity exercise could have a positive effect on exercise capacity by slowing the rate of muscle glycogen depletion (Hawley, 2000). Indeed, FA can be oxidised to a significant extent during intense exercise if they are adequately provided to the muscle (Dyck *et al.* 1996). Under such conditions, the elevation of plasma free FA concentration by the intravenous infusion of a triacylglycerol emulsion (Dyck *et al.* 1993, 1996; Odland *et al.* 1998; Vukovich *et al.* 1993) or the consumption of a high-fat meal followed by infusion of heparin (Costill *et al.* 1977; Vukovich *et al.* 1993) reduces the rate of muscle glycogenolysis compared with either a placebo control trial or when subjects ingested carbohydrate before exercise.

Alternatively, increasing the concentration of free FA in the plasma during moderate to high-intensity exercise may increase FA availability without substantially affecting either substrate utilisation or exercise capacity. This is because during high-intensity exercise carbohydrate flux and oxidation regulate FA oxidation (Coyle *et al.* 1997; Sidossis *et al.* 1997; Bergman & Brooks, 1999) not *vice versa* (Randle *et al.* 1963). Accordingly, it has been hypothesised that elevating plasma free FA concentration during high-intensity exercise is unlikely to induce significant shifts in substrate oxidation (from carbohydrate to fat) or effect exercise capacity (Brooks & Mercier, 1994). However, as recently noted the absence of such an effect has not been determined experimentally (Coyle, 1997).

The aim of the present investigation, therefore, was to determine whether altering the availability of plasma free FA before strenuous exercise would modify the rates of substrate oxidation during a bout of intense cycling and then to examine if such perturbations might affect subsequent time trial performance. In order to increase plasma free FA availability, a high-fat meal plus heparin was administered. Heparin releases the enzyme lipoprotein lipase from endothelial binding sites into the circulation where it (presumably) has better contact with circulating triacylglycerol-rich particles and can exert its action on the hydrolysis of triacylglycerol (Camps *et al.* 1990). To elevate plasma glucose and insulin concentrations and decrease lipolysis and FA oxidation (Horowitz *et al.* 1997) carbohydrate was ingested, while we attempted to further suppress lipolysis by feeding nicotinic acid with carbohydrate (Murray *et al.* 1995). Nicotinic acid decreases lipolysis by causing an inhibition of adenylate cyclase activity and intracellular cAMP, and a resultant decrease in hormone-sensitive lipase activity (Dipalma & Thayer, 1991).

Methods

Subjects

Seven highly trained cyclists all who had a minimum of 5 years endurance training were recruited to participate in this study, which was approved by the Human Research Ethics Committee's of the Australian Institute of Sport and RMIT University. Their mean age, body mass (BM)

and $\text{VO}_{2\text{max}}$ were 26.4 (SEM 1.7) years, 72.6 (SEM 1.9) kg and 71.2 (SEM 3.1) ml/kg per min respectively. At the time of the investigation subjects were cycling 400 (SEM 53) km/week in Canberra at an altitude of 588 m. Subjects, who had all participated in previous studies and were familiar with all testing protocols, were fully informed of the nature and possible risks of the investigation before giving their written consent. Each subject completed a medical history including any previous bleeding abnormalities before commencing the experimental trials, and a resting blood sample (5 ml) was taken and analysed for platelet count. All subjects were free of any abnormal clotting characteristics.

Preliminary testing

On the first visit to the laboratory, each subject performed a maximal, incremental cycle test to exhaustion on an electromagnetically-braked ergometer (Lode Instruments, Groningen, The Netherlands). The test protocol has been described in detail previously (Hawley & Noakes, 1992). A first principles calibration rig was used to evaluate the accuracy and reliability of this ergometer. Expected power output (W) was within $\pm 2\%$ actual power output from 200–800 W when the pedal frequency was 90–140 rpm. During the maximal test and the 20 min experimental ride, subjects inspired air through a two-way Hans Rudolf valve attached to a custom built automated Douglas bag gas analysis system (Australian Institute of Sport, ACT, Australia) which incorporated O_2 and CO_2 analysers (Ametek N-22 electrochemical O_2 sensor, model S3A, and Ametek P-61B infrared CO_2 sensor; Applied Electrochemistry, Ametek Instruments, Pittsburgh, PA, USA) and two Tissot gasometers (Warren E. Collins Inc., Braintree, MA, USA) interfaced to an IBM personal computer by optical rotary encoders (RS 341-597; Berne, Switzerland) that calculated the rate of O_2 consumption, CO_2 production, minute ventilation and the RER every 30 s from conventional equations (Robergs & Roberts, 2000). Before each maximal test and all the subsequently described trials, the analysers were calibrated with commercially available gases of known O_2 and CO_2 content. Before and after the study, an automated high-capacity calibrator for open-circuit indirect calorimetry was used to simultaneously check the gas analysers, volume device and software of the custom built system (Gore *et al.* 1997). This device can calibrate to the high ventilation volumes (about 100 l/min) measured when well-trained athletes work for sustained periods at high (80–85% $\text{VO}_{2\text{max}}$) exercise intensities.

$\text{VO}_{2\text{max}}$ was defined as the highest O_2 uptake a subject attained during two consecutive 30 s sampling periods. The results of this maximal test were used to determine the power output that corresponded to 80% of each subject's $\text{VO}_{2\text{max}}$ to be used during the 20 min experimental rides.

Training and nutritional status of the subjects were controlled for the day before each experimental trial in order to regulate muscle and liver glycogen stores. All subjects reported to the laboratory 24 h before a trial, immediately upon completion of an easy 60 min cycle. Subjects rode the same route and at the same intensity before each trial. They were then provided with a standard

diet of 209 kJ/kg BM, composed of 63 % energy as carbohydrate (8 g/kg BM), 20 % as fat, and 17 % as protein, to be consumed that day. Subjects refrained from any further activity for this period. Such standardisation of the subject's training and diet has previously been shown to result in similar pre-exercise muscle glycogen stores (Flynn *et al.* 1987).

Experimental trials

Each subject completed a random order of three experimental trials: a fat-feeding (HIFAT) trial, a carbohydrate-feeding trial (CHO) and a trial where carbohydrate plus nicotinic acid (NA) was ingested. All subjects plus one investigator who supervised the performance rides (described subsequently) were blinded to the order of the trials. On the morning of an experiment subjects reported to the laboratory between 06.00 and 07.00 hours after an overnight fast. As blood was to be repeatedly sampled after a test meal and during exercise, a cannula (20G; Terumo, Tokyo, Japan) was inserted into an antecubital vein of one arm, and a resting sample (10 ml) was taken. Following each blood sample, the cannula was flushed with 5 ml NaCl (9 g/l) to keep the vein patent. Subjects then ingested one of the three test meals. For the HIFAT trial, subjects were required to consume a meal of 1.2 g saturated fat/kg BM. For the CHO and NA trials subjects consumed an isoenergetic carbohydrate meal (2.5 g/kg BM). The nutritional analyses of the drinks are given in Table 1. Immediately after finishing their test meal for that experiment, subjects rested for 90 min before exercise. Blood samples (10 ml) were taken 30 and 60 min after ingestion of a test meal, and 1 min before subjects began the experimental ride. In the HIFAT trial heparin was administered intravenously 1 h (2000 U) and 75 min (500 U) after the test meal, and 1 min (500 U) prior to the commencement of the experimental ride. In an attempt to further suppress adipocyte lipolysis during the NA trial, subjects ingested 10 mg nicotinic acid/kg BM (Rhone-Poulenc Rorer, Victoria, Australia) 1 h after finishing the test meal, and 5 mg/kg BM 15 min prior to the experimental ride. Intermittent administration of NA was chosen as this has been shown to reduce any negative circulatory effects of the drug experienced when a single bolus dose is given (Pernow *et al.* 1967; Pernow & Saltin, 1971). At the

same time points that subjects ingested nicotinic acid, they ingested placebo tablets containing about 500 mg sucrose during the HIFAT and CHO experiments. At the same time point that subjects received an intravenous infusion of heparin, they were given a sham infusion of saline during the NA and CHO experiments.

Exactly 90 min after ingestion of a test meal, the subject mounted the ergometer and began a standardised 5 min incremental warm-up. Upon completion of the warm-up the workload was immediately adjusted to 80 % $\text{VO}_{2\text{max}}$ and they began 20 min continuous cycling. During this ride, blood samples (10 ml) were taken after 10 and 20 min. Expired gas was collected throughout the last 10 min of the ride. A 20 min time period was chosen for measurements of metabolism because previous studies have shown that the majority of carbohydrate 'sparing' after elevated free FA concentration occurs during the first 15–20 min intense exercise (Dyck *et al.* 1993, 1996; Vukovich *et al.* 1993). Upon completion of this ride, subjects were allowed a standardised (3 min) rest while they remained seated on the ergometer. During this time, they consumed either placebo tablets during the HIFAT and CHO trials, or 5 mg nicotinic acid/kg during the NA trial. During this rest period, a further 500 U heparin was administered to subjects during the HIFAT trial. After exactly 3 min, subjects commenced a performance ride of 600 kJ which they were instructed to complete 'as fast as possible'. In a pilot study, we determined that a 600 kJ time-trial took our trained subjects about 30 min to complete at an intensity of about 80–82 % $\text{VO}_{2\text{max}}$ (JA Hawley, unpublished results). When combined with the 20 min experimental ride, cyclists would be exercising for a total time of 50–60 min at about 80 % $\text{VO}_{2\text{max}}$. After subjects had completed 50 % of the work, a blood sample (10 ml) was taken with a final sample drawn during the last 60 s of the ride. Throughout the performance ride, subjects were informed of their progress at 50 kJ intervals, and when they had completed 550 kJ, they were given feedback every 10 kJ until they finished the ride.

During the 20 min experimental rides, subjective ratings of perceived exertion were obtained after 10, 15 and 20 min using the modified Borg scale (Borg, 1975). During the performance ride, ratings of perceived exertion were obtained upon completion of every 100 kJ. Heart rates during the experimental trials were measured by telemetry (Polar Sports Tester; Polar Electro OY, Kempele, Finland).

Table 1. Composition of the pre-exercise test meals*

	High-fat meal	High-carbohydrate meal
Ingredient	Full cream (350 g fat/kg) 215 g Low-energy chocolate topping 50 g Full-cream milk 100 ml	Polyjoule™ † 85 g Regular chocolate topping 70 g Skimmed milk 400 ml Sustagen Sport powder™ ‡ 55 g
Analyses§		
Energy (kJ)	3458	3473
Fat (g)	83 (89 % energy)	2 (3 % energy)
Carbohydrate (g)	16 (7 % energy)	173 (82 % energy)
Protein (g)	9 (4 % energy)	32 (15 % energy)

* Based on a 70 kg subject.

† Baxter Health Care Proprietary Limited, Old Toongabbie, New South Wales, Australia.

‡ Bristol Myers, Ryalamere, New South Wales, Australia.

§ Source: NUTTAB Database, Australian Department of Community Services and Health, 1995.

Analytical techniques

Upon sampling, about 5 ml whole blood was placed into a tube containing fluoride heparin, mixed and spun at 800 *g* for 2 min. The resultant plasma was stored at -80°C for later analyses of glucose and lactate using automated analyses (ABL 615 Radiometer, Copenhagen, Denmark) and insulin by radioimmunoassay (Incstar, Stillwater, MN, USA). Blood for adrenaline, noradrenaline and free FA determination was placed in tubes containing ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid and GSH and spun in a centrifuge at 4°C for 15 min at 900 *g*. The plasma was stored at -80°C until analysed. Plasma adrenaline and noradrenaline were analysed using a single isotope [^3H]-radioenzymatic assay system (TRK995; Amersham, Bucks., UK) and plasma free FA was analysed using an enzymatic colorimetric method (Wako, NEFAC code 279-75409, Tokyo, Japan). A further 2 ml whole blood were placed in a tube containing lithium heparin, mixed, and spun down for 2 min. A 500 μl portion of plasma was transferred into a tube containing 500 μl ice-cold 3 M-HClO₄, mixed and spun in a centrifuge at 900 *g* at 0°C for 2 min. An 800 μl portion of the resultant supernatant was then added to a tube containing 200 μl 6 M-KOH mixed and spun in a centrifuge at 0°C for 2 min. The supernatant was stored at -80°C until analysed for glycerol using enzymatic analysis with fluorometric detection (Pinter *et al.* 1967).

Whole-body rates of carbohydrate and fat oxidation (*g*/min) were calculated throughout the last 10 min of each experimental ride from the rates of CO₂ production and O₂ consumption using the non-protein RER values, according to the following equations (Peronnet & Masicotte, 1991):

$$\begin{aligned} \text{CHO oxidation} &= 4.585 \text{ VCO}_2(\text{l}/\text{min}) \\ &\quad - 3.226 \text{ VO}_2(\text{l}/\text{min}), \end{aligned}$$

and

$$\begin{aligned} \text{fat oxidation} &= 1.695 \text{ VO}_2(\text{l}/\text{min}) \\ &\quad - 1.701 \text{ VCO}_2(\text{l}/\text{min}), \end{aligned}$$

where VCO₂ is the rate of CO₂ production and VO₂ is the rate of O₂ consumption. These equations are based on the assumption that VO₂ and VCO₂ accurately reflect tissue O₂ consumption and CO₂ production. In well-trained subjects

similar to those employed in the current investigation, indirect calorimetry has been shown to be a valid method for quantifying rates of substrate oxidation during strenuous exercise at about 85 % VO_{2max} (Romijn *et al.* 1992). Rates of FA oxidation ($\mu\text{mol}/\text{kg}$ per min) were determined by converting the rate of triacylglycerol oxidation (*g*/kg per min) to its molar equivalent assuming the average molecular mass of human triacylglycerol to be 855.3 *g*/mol and multiplying the molar rate of triacylglycerol oxidation by three, because each molecule contains 3 mmol FA. Rates of carbohydrate oxidation ($\mu\text{mol}/\text{kg}$ per min) were determined by converting the rate of carbohydrate oxidation (*g*/min) to its molar equivalent assuming 6 mol O₂ are consumed and 6 mol CO₂ produced for each mol (180 *g*) oxidised.

Statistical analyses

Data from the three trials were compared using a two-factor (treatment and time) ANOVA with repeated measures. Simple main effect analyses and Neumann-Keul post-hoc tests were undertaken when ANOVA revealed a significant interaction. Total carbohydrate and fat oxidation between trials, and time-trial performances were compared using a one-way ANOVA with treatment as the main factor and, where appropriate, with Neumann-Keul post-hoc test. Significant differences were accepted when $P < 0.05$ and all data are reported as mean values with standard errors of the means.

Results

Dietary control

Standardisation of each subject's diet was achieved: subjects consumed 577 (SEM 12) (CHO), 579 (SEM 12) (NA) and 575 (SEM 12) (HIFAT) *g* carbohydrate (8.0 (SEM 0.2) (CHO), 8.0 (SEM 0.1) (NA) and 7.9 (SEM 0.2) (HIFAT) *g*/kg BM) for the 24 h prior to each trial. As intended, the total energy intake was the same (CHO 15.2 (SEM 0.3), NA 15.2 (SEM 0.3) and HIFAT 14.8 (SEM 0.4) MJ).

Metabolic data

Table 2 displays the metabolic data collected during the three 20 min experimental rides. As intended, the rate of O₂

Table 2. Metabolic data measured during 20 min of intense cycling for 20 min at 80 (SEM 1) % maximal oxygen consumption§ (Mean values with standard errors of the means for seven subjects)

Treatment	VO ₂ (l/min)		VCO ₂ (l/min)		V _E (l/min)		RER		Energy (J/kg per min)		CHO _{ox} ($\mu\text{mol}/\text{kg}$ per min)		Fat _{ox} ($\mu\text{mol}/\text{kg}$ per min)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
CHO	4.15	0.12	4.01	0.11	91.0	3.4	0.97	0.01	1203	30	354	10	11.8	1.6
NA	4.15	0.14	4.05	0.13	99.3*	4.8	0.98	0.01	1210	30	368	14	8.1	2.7
HIFAT	4.19	0.12	3.93	0.11	90.9	2.3	0.94†	0.01	1205	25	312‡	8	24.0‡	3.2

VO₂, O₂ uptake; VCO₂, CO₂ production; V_E, ventilation; CHO_{ox}, rate of carbohydrate oxidation; FAT_{ox}, rate of fat oxidation; CHO, high-carbohydrate meal; NA, high-carbohydrate meal plus nicotinic acid; HIFAT, high-fat meal plus intravenous heparin.

Mean value was significantly different from CHO and HIFAT: * $P < 0.05$; mean value was significantly different from CHO and NA: † $P = 0.002$; mean values were significantly different from CHO and NA: ‡ $P = 0.002$.

§ For details of treatments and procedures see Table 1 and p. 831.

consumption averaged 80 (SEM 1) % of the subjects' $\text{VO}_{2\text{max}}$ and was not different between trials. RER values were lower after fat + heparin administration compared with the other dietary treatments (HIFAT 0.94 (SEM 0.01), CHO 0.97 (SEM 0.01) and NA 0.98 (SEM 0.01); $P = 0.002$). Accordingly, the mean rate of fat oxidation was greater in HIFAT (24 (SEM 3) $\mu\text{mol/kg}$ per min) compared with either CHO (12 (SEM 2) $\mu\text{mol/kg}$ per min, $P < 0.01$) or NA (8 (SEM 3) $\mu\text{mol/kg}$ per min, $P = 0.01$), while the rate of carbohydrate oxidation was concomitantly lower (HIFAT 312 (SEM 8), CHO 354 (SEM 10) and NA 368 (SEM 14) $\mu\text{mol/kg}$ per min, $P = 0.01$). As a result there was a 'sparing' of carbohydrate in the HIFAT trial compared with the other dietary treatments (HIFAT 81 (SEM 3), CHO 92 (SEM 3) and NA 96 (SEM 4) g/20 min, $P = 0.01$). This 'sparing' meant that the contribution to total energy from carbohydrate was reduced from 90 (SEM 1) % (CHO) and 93 (SEM 2) % (NA) to 79 (SEM 3) % in HIFAT ($P < 0.001$). NA elevated the ventilation rate (at STPD) compared with both CHO and HIFAT (NA 99.3 (SEM 4.8), CHO 91.0 (SEM 3.4) and HIFAT 90.9 (SEM 2.3) l/min; $P < 0.01$), yet despite this higher rate of ventilation there were no differences in either the subjects' heat rates (CHO 165 (SEM 3), NA 165 (SEM 3), HIFAT 164 (SEM 3) beats/min) or ratings of perceived exertion (CHO 13.0 (SEM 0.5), NA 13.8 (SEM 0.6), HIFAT 12.7 (SEM 0.3)) between treatments.

Plasma glucose, insulin and lactate concentrations

Fig. 1 displays the plasma glucose, insulin and lactate concentrations for the three dietary treatments. Resting values for these three variables were similar. After the CHO meal, glucose concentration increased slightly to 5.7 (SEM 0.5) mM, but was not different to either HIFAT or NA at any time point before exercise. In contrast, plasma insulin concentrations were rapidly elevated 30 min after ingestion of both CHO and NA compared with HIFAT (CHO 390 (SEM 51) and NA 289 (SEM 58) v. HIFAT 82 (SEM 1) pmol/l; $P < 0.0001$) and remained significantly higher throughout the 90 min pre-exercise period. Although lactate concentrations were similar after ingestion of the three test meals, they tended to be lower after HIFAT compared with either CHO or NA although such differences were not statistically significant.

Plasma glucose concentration rose steadily throughout the experimental ride so that after 20 min it had increased from about 4.5 mM to 5.3 (SEM 0.3), 5.6 (SEM 0.4) and 6.0 (SEM 0.2) mM for CHO, NA and HIFAT respectively (Fig. 1, $P < 0.0001$). In contrast, plasma insulin concentrations decreased rapidly after the first 10 min of the experimental ride during both CHO and NA (from 279 (SEM 36) and 179 (SEM 17) to about 60 pmol/l; $P < 0.0001$) where they remained for the rest of the ride (Fig. 1). As would be expected with the onset of the time trial, plasma lactate concentration increased such that compared with the start of the experimental ride, there was a significant interaction of time \times treatment for all three conditions for both 10 and 20 min time points (Fig. 1; $P < 0.05$). Plasma lactate concentrations remained steady at between 4.5 and 5.5 mmol/l for the duration of the experimental ride for both CHO and HIFAT conditions. However, plasma lactate concentrations in NA were higher at both the 10 min (NA 6.5 (SEM 1.1) v. CHO 4.7 (SEM 0.8) and

HIFAT 4.4 (SEM 0.8) mM; $P < 0.001$) and 20 min (NA 8.1 (SEM 1.6) v. CHO 5.5 (SEM 1.0) and HIFAT 5.3 (SEM 1.0) mmol/l; $P < 0.001$) time points (Fig. 1). Although there was a modest rise in plasma lactate concentration during the experimental ride (from 6.5 (SEM 1.1) to 8.1 (SEM 1.6) mmol/l) during NA, this increase was not significant.

The performance ride resulted in a modest hyperglycaemia such that at the end of the time trial, plasma glucose concentration had risen to between 6.1 and 6.8 mM for all three treatments (Fig. 1). However, such rises in glucose concentration were not different to those values observed at the end of the experimental ride. Plasma insulin concentrations remained relatively constant throughout the time trial and were similar for all three treatments at the end of the time trial (about 7 $\mu\text{U/ml}$). Plasma lactate concentrations rose from about 9.0 mM halfway through the performance ride to about 11–12 mM at the end of 600 kJ. This rise was likely due to the riding strategy of most subjects who employed a sustained 'sprint finish' during the last 120–150 s of the time trial.

Plasma free fatty acids and glycerol concentrations

Resting concentrations of plasma free FA and glycerol were similar for all three treatments (Fig. 2). As intended, HIFAT resulted in a significant increase in fat availability so that 75 min after ingestion, plasma free FA concentration had risen from 0.40 (SEM 0.03) to 1.31 (SEM 0.15) mM ($P < 0.0001$) compared with a slight decline for the corresponding time period for CHO and NA. Rises in plasma glycerol concentrations were more modest and were not statistically significant during the 90 min prior to exercise (Fig. 2).

Plasma free FA concentration remained elevated throughout the experimental ride and for the duration of the subsequent time trial after HIFAT compared with either CHO or NA ($P < 0.0001$, Fig. 2). Indeed, even at the end of the time trial, plasma free FA concentrations were higher for HIFAT than the other treatments (HIFAT 0.82 (SEM 0.12) v. CHO 0.16 (SEM 0.03) and NA 0.14 (SEM 0.04) mM; $P < 0.0001$). There were no differences in plasma free FA concentration at any time between CHO and NA. Plasma glycerol concentrations rose progressively with the onset of exercise and were significantly higher compared with the start of exercise after 10 min in HIFAT and after 20 min in CHO and NA (Fig. 2). Thereafter, they continued to increase so that by the end of the time trial they were 0.40 (SEM 0.02) (HIFAT), 0.19 (SEM 0.01) (CHO) and 0.15 (SEM 0.01) (NA) mM. At all exercise sample points, plasma glycerol concentration was significantly higher after HIFAT than either CHO or NA ($P < 0.0001$). There were no differences in plasma glycerol concentration at any time point between CHO and NA.

Plasma catecholamines

Plasma adrenaline and noradrenaline concentrations are shown in Table 3. There were no differences in resting values between all three treatments for adrenaline and noradrenaline. Although both plasma adrenaline and noradrenaline increased from rest during the 20 min

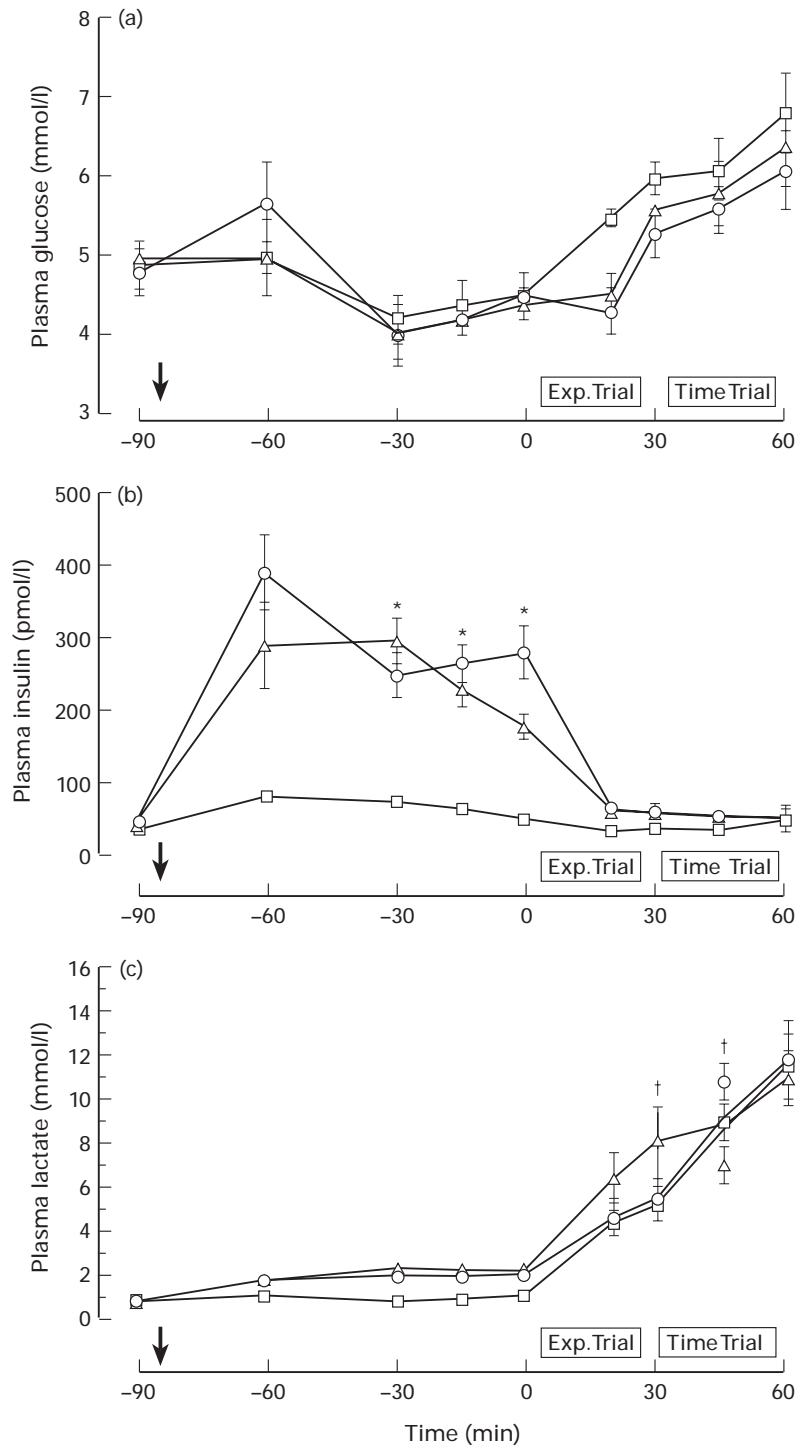


Fig. 1. (a) Plasma glucose, (b) insulin and (c) lactate concentrations during the experimental (Exp) and time trials. Values are means for seven subjects with standard errors of the means shown by vertical bars. For details of treatments and procedures see Table 1 and p. 831. Test meals were ingested 90 min before commencing a 20 min ride at about 80 % maximal O_2 uptake (308 (SEM 12) W), immediately followed by a 600 kJ time trial. \circ , CHO (2.6 g carbohydrate/kg); ∇ CHO + NA (2.6 g CHO + 20 mg nicotinic acid/kg); \square , HIFAT (1.2 g saturated fat/kg with 3500 U heparin intravenously). \downarrow , Time of feeding. Mean values were significantly different from HIFAT: * $P < 0.001$; mean values were significantly different from CHO and HIFAT: † $P < 0.05$.

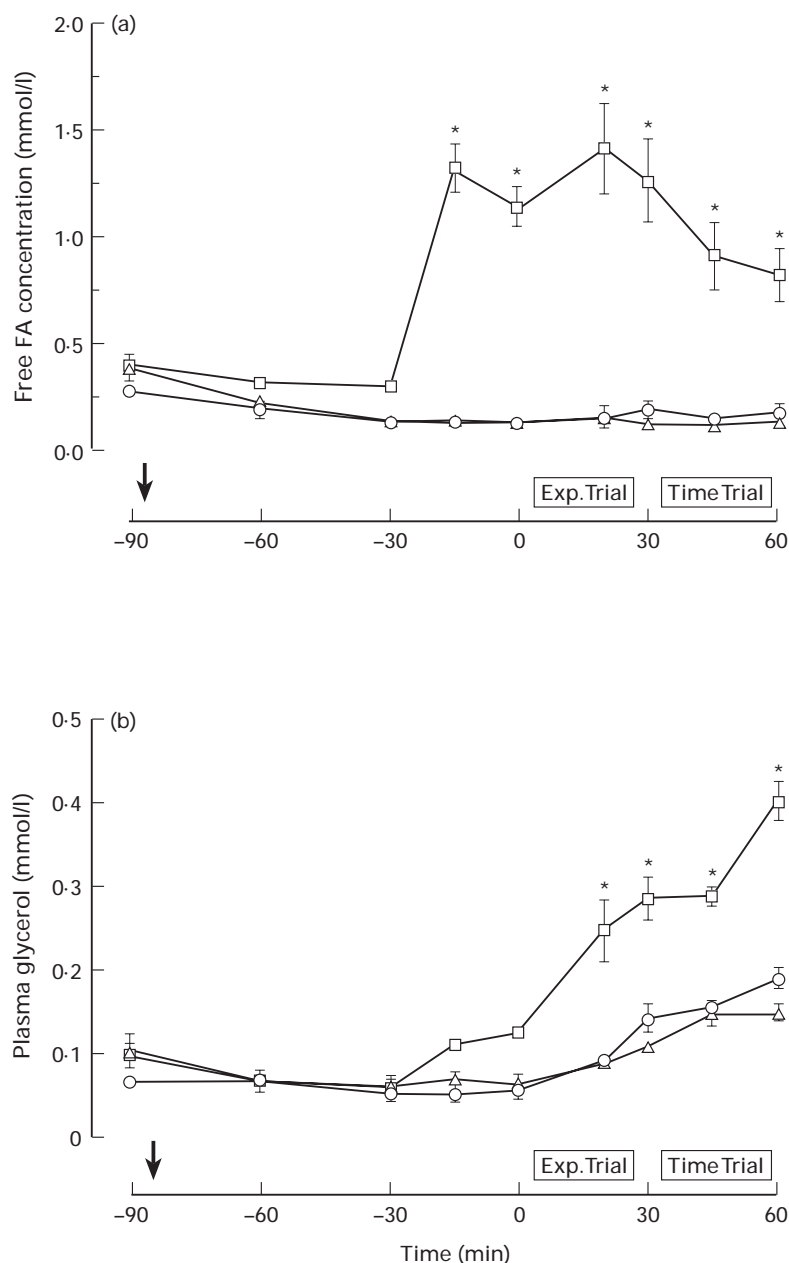


Fig. 2. (a) Plasma free fatty acids (FA) and (b) glycerol concentrations during the experimental (Exp) and time trials. Values are means for seven subjects with standard errors of the means shown by vertical bars. For details of treatments and procedures see Table 1 and p. 831. Test meals were ingested 90 min before commencing a 20 min ride at about 80 % maximal O_2 uptake (308 (SEM 12) W), immediately followed by a 600 kJ time trial. \circ , CHO (2.6 g carbohydrate/kg); ∇ , CHO + NA (2.6 g CHO + 20 mg nicotinic acid/kg); \square , HIFAT (1.2 g saturated fat/kg with 3500 U heparin intravenously). \downarrow , denotes time of feeding. Mean values were significantly different from CHO and NA; * $P < 0.001$.

experimental ride for all three dietary conditions ($P < 0.05$), there was no main treatment effect.

Time, power output, ratings of perceived exertion and heart rate during the time trial

The time taken to complete the time trial following the experimental ride was not different between experimental conditions (CHO 31.3, NA 32.1, and HIFAT 31.7 min).

Neither was there any difference for the time taken for any 100 kJ segments of the ride between trials. Accordingly, the average power output (CHO 324 (SEM 15), NA 315 (SEM 13), HIFAT 320 (SEM 16) W) and heart rate (CHO 174 (SEM 3), NA 172 (SEM 2), HIFAT 175 (SEM 3) beats/min) sustained for the duration of the time trial were similar. Ratings of perceived exertion rose steadily during all three time trials from 14 (SEM 1) units after the first 100 kJ to 19 (SEM 1) units at the end of the rides. There

Table 3. Effects of pre-exercise feedings on plasma catecholamine concentration[‡]

(Mean values with standard errors of the means for seven subjects)

Treatment	Adrenaline (nmol/l)				Noradrenaline (nmol/l)			
	Rest		Exercise [§]		Rest		Exercise [§]	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
CHO	0.53	0.10	1.30*	0.14	1.52	0.56	11.30†	1.43
NA	0.57	0.15	1.97*	0.52	1.47	0.37	13.31†	2.32
HIFAT	0.56	0.16	1.13*	0.18	1.22	0.22	9.61†	1.08

CHO, high-carbohydrate meal; NA, high-carbohydrate meal plus nicotinic acid; HIFAT, high-fat meal plus intravenous heparin.

Mean values were significantly different from rest: * $P < 0.05$, † $P < 0.0001$.

[‡] For details of treatments and procedures see Table 1 and p. 831.

[§] Exercise sample was taken 20 min from the start of the experimental ride at 80 % maximal O_2 uptake (308 (SEM 12) W).

were no differences in the ratings of perceived exertion between trials at any time point. Likewise, the loss in BM was similar for all conditions (CHO 1.2 (SEM 0.1), NA 1.1 (SEM 0.1) and HIFAT 1.1 (SEM 0.1) kg).

Discussion

The first finding of the present study was that the ingestion of isoenergetic fat and CHO meals 1 h before exercise resulted in dramatic changes in plasma substrate concentrations (Figs. 1 and 2) and marked differences in the rates of substrate oxidation during intense cycling. We deliberately chose ingestion of test meals rather than intravenous infusion of Intralipid or glucose as we wished to elevate circulating metabolite concentrations and induce changes in the regulatory plasma hormones that result from oral administration. Furthermore, a fat meal composed predominantly of saturated FA rather than unsaturated FA was chosen as the saturation of FA has been reported to have little effect on the subsequent decrease in muscle glycogen utilisation during exercise (Vukovich *et al.* 1993). As intended, ingestion of the fat meal resulted in higher plasma free FA concentrations immediately before and throughout intense exercise, with CHO ingestion effectively eliminating any rise in plasma free FA concentration both before and during the subsequent exercise bouts (Fig. 1). Horowitz *et al.* (1997) have previously reported that pre-exercise CHO ingestion which results in a small (about 20 μ U/ml) elevation in plasma insulin concentration reduces FA oxidation during subsequent exercise.

Contrary to our expectations, there was no additive effect of co-ingesting nicotinic acid (about 1400 mg) with the CHO meal with regard to suppression of plasma free FA concentration. This finding contrasts with the results of Murray *et al.* (1995) who reported that the ingestion of a carbohydrate–electrolyte solution plus nicotinic acid (about 330 mg), or water plus nicotinic acid both resulted in lower circulating plasma free FA concentrations during submaximal (about 70 % VO_{2max}) cycling, compared with the ingestion of carbohydrate alone. Such a discrepancy is difficult to explain in that inhibition of lipolysis by nicotinic acid has been reported to be maximal at a plasma concentration of about 1 μ mol/l (Marcus *et al.* 1989), a level far below the plasma nicotinic acid concentration

observed in the subjects of Murray *et al.* (1995). Indeed, based solely on the results of the investigation by Murray *et al.* (1995) we decided not to include a nicotinic acid plus placebo (water) treatment in the current study.

We specifically wished to determine the effects of altering FA availability in competitive athletes exercising at the highest work rates they could sustain for about 1 h, as most (Okano *et al.* 1996, 1998; Pitsiladis *et al.* 1999) but not all (Whitley *et al.* 1998) previous investigations that have utilised a variety of pre-exercise meals to increase FA availability and oxidation have used moderately-trained subjects cycling for prolonged (>90 min) periods at moderate intensities (60–70 % VO_{2max}). Accordingly, we chose a 20 min experimental period during which pulmonary gas exchange measures were taken, followed immediately by a time trial lasting about 30 min in which the average power output sustained by subjects was similar to the first 20 min of the ride (about 310–320 W). Hence, the total exercise time was 50–55 min at about 80 % VO_{2max} . More to the point, most of the glycogen ‘sparing’ has been shown to occur early in exercise (Dyck *et al.* 1993, 1996; Vukovich *et al.* 1993) yet still result in a performance enhancement during the later stages of intense work (Spriet *et al.* 1992). We found a significant reduction in carbohydrate oxidation during intense exercise when plasma free FA concentration was elevated almost four-fold by HIFAT (Table 1). However, an RER value of 0.94 during HIFAT still represents an 80 % contribution from carbohydrate to total energy. On the assumption that the majority of the carbohydrate ‘sparing’ in the HIFAT trial compared with either CHO or NA was from muscle glycogen, it would only represent a 10 % reduction in the contribution to total energy from carbohydrate. Our data are in excellent agreement with the 12 % reduction in muscle glycogen utilisation estimated by Romijn *et al.* (1995) in well-trained cyclists exercising for 20 min at about 83 % VO_{2max} after a lipid–heparin infusion that elevated plasma FA concentration to >1.5 mM. It is difficult to extrapolate the magnitude of a glycogen ‘sparing’ effect into a meaningful performance change. However, using the original data of Spriet *et al.* (1992) it can be calculated that it would take a 20 % reduction in glycogenolysis (about 20 mmol/kg wet mass) to increase sustainable power output during cycling at 80 % VO_{2max} by about 1.5 % (see Hopkins *et al.* 1999).

Our results showing a dominance of exercise intensity over dietary manipulation and substrate availability in determining the choice of fuel by the working muscle are consistent with those of others that have used moderate-intensity exercise (Whitley *et al.* 1998; Bergman & Brooks, 1999). Whitley *et al.* (1998) studied well-trained cyclists during prolonged moderate intensity exercise 4 h after ingesting either high-fat or high-carbohydrate meals. Although blood glucose, insulin and free FA concentrations differed before exercise as a result of the different meals, substrate selection during exercise was similar and was dominated by carbohydrate oxidation. Others have also reported that substrate utilisation was not affected by prior diet during moderate intensity exercise in either untrained or trained subjects (Bergman & Brooks, 1999; Pitsiladis *et al.* 1999). Taken collectively, these results suggest that at

the exercise intensities at which most athletes train and compete, they are dependent on carbohydrate oxidation for energy, regardless of prior dietary patterns. Accordingly, the finding that high-intensity cycling performance was similar between all dietary treatments in the current investigation was, perhaps, not surprising as the work rates (>310–320 W, 80–85 % $\text{VO}_{2\text{max}}$) sustained by our subjects for almost 1 h resulted in high rates of total carbohydrate oxidation and glycolytic flux. Indeed, the only study to find an increase in exercise capacity with fat feeding was that of Pitsiladis *et al.* (1999). These workers reported that cycling time to exhaustion was prolonged (from 118 to 128 min) when their trained subjects ingested a high-fat (90 % energy) *v.* a high-carbohydrate (70 % energy) meal 4 h prior to exercise. As there were no differences in total carbohydrate oxidation for the first 90 min of exercise between trials (383 *v.* 362 g for the carbohydrate and fat meals respectively), it is difficult to explain a metabolic basis for the prolonged cycling time.

The effects of nicotinic acid administration *per se* on exercise performance have been investigated previously, with conflicting results. For example, Bergstrom *et al.* (1969) found no difference in cycling time to exhaustion after nicotinic acid administration even though muscle glycogen utilisation was 33 % greater, arterial lactate concentrations elevated, and plasma free FA concentration lower compared with a control trial. Furthermore, Murray *et al.* (1995) found no differences in performance during high-intensity cycling (which followed 2 h submaximal exercise) after ingestion of carbohydrate, carbohydrate plus nicotinic acid, or water plus nicotinic acid. Interestingly, these workers hypothesised that feeding a combination of carbohydrate plus nicotinic acid would result in a greater reliance and oxidation of carbohydrate during intense exercise which might result in a concomitant improvement in performance. Such a hypothesis was based on previous observations that there is a strong relationship (r 0.94) between the rate of carbohydrate oxidation and power production during intense cycling (Neufer *et al.* 1987).

A third finding of the present study was that the co-ingestion of 20 mg nicotinic acid/kg BM with the CHO meal resulted in significantly higher plasma lactate concentrations and rates of ventilation than when either carbohydrate or fat meals were ingested alone. Some (Bergstrom *et al.* 1969) but not all (Carlson *et al.* 1963) studies have reported elevations in lactate concentration with nicotinic acid administration in response to a standard submaximal exercise task when compared with either a glucose feedings or a fasted control group. However, to the best of our knowledge the present study is the first to find an elevation in ventilation during intense exercise in response to nicotinic acid feedings. Such a finding is difficult to explain in light of the similar values measured for both the rate of O_2 consumption and, more importantly, the rate of CO_2 production during all three experimental rides.

In conclusion, the results of the current investigation show that compared with the consumption of an iso-energetic carbohydrate meal, the ingestion of a high-fat meal (plus heparin administration) significantly increased subsequent FA availability and altered the patterns of

substrate utilisation during intense exercise. However, the reduction in the rate of carbohydrate oxidation in the presence of high circulating plasma free FA had little effect on the performance of a 30 min time trial, which followed 20 min intense exercise. At the exercise intensities sustained by competitive athletes during training and competition, it is likely that the decrease in carbohydrate utilisation after a fat feeding is inadequate to contribute to a performance enhancement, as substrate selection during such intense exercise is dominated by carbohydrate oxidation.

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