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Effects of glucose ingestion or glucose infusion on fuel substrate kinetics during prolonged exercise

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Abstract. To determine if bypassing both intestinal absorption and hepatic glucose uptake by intravenous glucose infusion might increase the rate of muscle glucose oxidation above 1 $g \cdot min^{-1}$, ten endurance-trained subjects were studied during 125 min of cycling at 70% of peak oxygen uptake (VO_{2peak}). During exercise the subjects ingested either a 15 $g \cdot 100\ ml^{-1}$ U-^{14}C labelled glucose solution or H_2O labelled with a U-^{14}C glucose tracer for the determination of the rates of plasma glucose oxidation (Rox) and exogenous carbohydrate (CHO) oxidation from plasma ^{14}C glucose and $^{14}CO_2$ specific activities, and respiratory gas exchange. Simultaneously, 2-^{3}H glucose was infused at a constant rate to measure glucose turnover, while unlabelled glucose (25% dextrose) was infused into those subjects not ingesting glucose to maintain plasma glucose concentration at 5 $mmol \cdot l^{-1}$. Despite similar plasma glucose concentrations [ingestion 5.3 (SEM 0.13) $mmol \cdot l^{-1}$; infusion 5.0 (0.09) $mmol \cdot l^{-1}$], compared to glucose infusion, CHO ingestion significantly increased plasma insulin concentrations [12.9 (1.0) vs 4.8 (0.5) $mU \cdot l^{-1}$; $P<0.05$], raised total Rox values [9.5 (1.2) vs 6.2 (0.7) $mmol \cdot 125\ min^{-1}$ kg fat free $mass^{-1}$ (FFM); $P<0.05$] and rates of CHO oxidation [37.2 (2.8) vs 24.1 (3.9) $mmol \cdot 125\ min^{-1}$ kg FFM^{-1}; $P<0.05$]. An increased reliance on CHO metabolism with CHO ingestion was associated with a decrease in fat oxidation. Whereas the contribution from fat oxidation to energy production increased to 51 (10)% with glucose infusion, it only reached 18 (4)% with glucose ingestion ($P<0.05$). Despite these differences in plasma insulin concentration and rates of fat oxidation, the rates of glucose oxidation by muscle were similar after 125 min of exercise for both trials [ingestion 93 (8); infusion 85 (5) $\mu mol \cdot min^{-1}$ kg FFM^{-1}], suggesting that peak rates of muscle glucose oxidation were primarily dependent on blood glucose concentration which, in turn, regulated the hepatic appearance of ingested CHO.

Key words: Fat – Glucose oxidation – U-^{14}C glucose

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Introduction

Previous studies utilising arteriovenous balance methods and isotopic tracer techniques have shown that the peak rates of oxidation of both ingested carbohydrate (CHO) (for review see Hawley et al. 1992a) and plasma glucose (Bosch et al. 1993; Broberg and Sahlin 1989; Coggan et al. 1991; Stein et al. 1989) are limited to approximately 1 $g \cdot min^{-1}$ at the end of prolonged (>90 min), moderate-intensity [65%–75% of maximal oxygen uptake ($\dot{V}O_{2max}$)] exercise. Whether the rate of oxidation of ingested CHO is limited by the rate of digestion, absorption and subsequent transport of ingested glucose into the systemic blood supply, or by the rate of glucose uptake and oxidation by the exercising muscles, has yet to be determined. Therefore, the first aim of the current study was to determine if intravenous glucose infusion, which bypasses both intestinal absorption and hepatic glucose uptake, might lead to rates of muscle glucose oxidation greater than the peak rates of 1 $g \cdot min^{-1}$ measured after oral CHO ingestion.

As hepatic glucose appearance has been shown to be greater after administration of an oral glucose load than with peripheral intravenous glucose infusion (Bergman et al. 1982; DeFronzo et al. 1978), an additional purpose of this study was to evaluate the effect of euglycaemic (i.e. 5 $mmol \cdot l^{-1}$) plasma glucose concentrations, maintained by either CHO ingestion or intravenous glucose infusion, on the rates of liver glucose turnover during exercise.

Methods

Subjects and preliminary testing

Ten experienced endurance-trained cyclists participated in this investigation, which was approved by the Research and Ethics Committee of the Faculty of Medicine of the University of Cape Town. The tests and associated risks were explained in detail to each individual before his written informed consent was obtained. To minimise the subject's exposure to radio-activity in

Table 1. Characteristics of subjects

	Ingestion		Infusion	
	Mean	SEM	Mean	SEM
Age (years)	25.8	0.7	23.8	0.9
Mass (kg)	80.4	2.2	78.9	1.9
FFM (kg)	67.7	1.9	67.4	0.7
PPO (W)	411.6	26.6	388.9	15.2
Work rate at 63% PPO (W)	260.4	14.4	246.2	8.0
VO_{2peak} ($l \cdot min^{-1}$)	4.98	0.21	4.84	0.15

Ingestion, the subjects ingested carbohydrate (15 $g \cdot 100\ ml^{-1}$ of a U-^{14}C labelled glucose solution) throughout exercise; Infusion, the subjects received a variable-rate glucose infusion throughout exercise; FFM, fat free mass; PPO, peak sustained power output attained during the maximal test; VO_{2max}, maximal oxygen uptake

these investigations, they were divided into two groups (five per group) and any one subject performed only one experiment. The characteristics of the subjects are given in Table 1. There were no significant differences for any of the variables measured.

All subjects were tested for peak oxygen consumption (VO_{2peak}) and peak sustained power output (PPO) on an electronically braked cycle ergometer (Lode, Groningen, Holland) modified with low-profile bars and clip-on pedals. The incremental cycle test to exhaustion, the calculation of PPO and the accompanying gas collection procedures have been described in detail previously (Hawley and Noakes 1992). Briefly, the test protocol commenced at an exercise intensity of 3.33 $W \cdot kg^{-1}$ body mass for 150 s and thereafter was increased by 50 W for a further 150 s. After the second stage the exercise intensity was increased by 25 $W \cdot 150\ s^{-1}$ until the subject claimed fatigue, which always coincided with either a decrease in pedal rate greater than 10 $rev \cdot min^{-1}$, a respiratory exchange ratio (R) greater than 1.10, or both. VO_{2peak} was taken as the highest rate of oxygen consumption measured during any 60 s, while PPO was defined as the highest exercise intensity the subject completed for 150 s. If an exercise intensity for the prescribed period was not completed, PPO was calculated as described by Hawley and Noakes (1992).

The results of the initial maximal test were used to determine the exercise intensity, which corresponded to 63% of each subject's PPO (approximately 70% of VO_{2peak}). This exercise intensity was used during the experimental rides and subsequently elicited a mean VO_2 of 68.8 (0.02)% of VO_{2peak}. The maximal test and experimental trial were separated by 8–10 days, during which time the subjects maintained their usual dietary and training regimens.

Fat free masses (FFM) and the percentage body fat of each subject was assessed from their total mass, and the sum of four skinfold measurements (Durnin and Wormersley 1974). These values were used to adjust measurements of glucose turnover and oxidation during the experimental trials for differences in FFM which are described later.

Experimental trial

On the day of the trial, the subjects reported to the laboratory between 0800 hours and 1000 hours, a minimum of 3 h after a standardised breakfast similar in size and composition to that which they would normally ingest before training or competition (approximately 85 g of CHO). After the subject had been weighed, a flexible 18-gauge Jelco (Johnson and Johnson, Halfway House, Transvaal, Republic of South Africa) cannula attached to a three-way stopcock (Industrias Palex Plc, Barcelona, Spain) was positioned in an antecubital vein of the right forearm. This cannula was used for the sampling of blood during the experiments. After a resting blood sample had been taken, the cannula was immediately flushed with 2–3 ml of sterile saline (plus 5 $IU \cdot ml^{-1}$ of heparin), a procedure which was repeated after the withdrawal of each sample. A 20-gauge cannula attached to a three-way stopcock was then inserted into an antecubital vein of the left forearm. This cannula was used for a constant infusion of 2-^{3}H-glucose tracer (Amersham International, Amersham, Bucks, UK) to allow for the determination of hepatic glucose output and for the variable rate of glucose infusion (25% Dextrose, Adco Pharmaceutica, Adcock Ingram Laboratories, Johannesburg, Republic of South Africa) in the euglycaemic clamp trials. The 2-^{3}H glucose was chosen as the tracer because a large proportion of the label is lost through cycling between hepatic glucose 6-phosphate and fructose 6-phosphate (Katz and Dunn 1967), thus minimising ^{3}H incorporation into hepatic glycogen. Pyrogen-free, sterile 2-^{3}H-glucose was infused at a constant rate of 0.29 $ml \cdot min^{-1}$ [900 $kBq \cdot h^{-1}$; 25 $\mu Ci \cdot h^{-1}$] via a calibrated auto-syringe (Travenol Laboratories Inc., Hooksett, NH, USA) 75 min prior to and throughout the trial.

Immediately before the CHO ingestion trial, the subjects drank a 400-ml loading bolus of a 15 $g \cdot 100\ ml^{-1}$ glucose solution (D-glucose anhydrous, SAARCHEM Pty Ltd, Krugersdorp, Republic of South Africa) which was labelled with U-^{14}C glucose (Amersham International). The specific activity of the drink was 6.3 $kBq \cdot g^{-1}$ (0.17 $\mu Ci \cdot g^{-1}$). Thereafter, a further 100 ml of the labelled drink was ingested every 10 min so that by the end of the ride, subjects had ingested a total of 1600 ml. During the glucose infusion trial the subjects followed the same drinking pattern except that the drink was artificially sweetened coloured water labelled with the same amount of U-^{14}C glucose tracer. A U-^{14}C glucose label was added to the drinks so that rates of plasma and ingested glucose oxidation could be calculated from drink and plasma ^{14}C-glucose specific activities and expired $^{14}CO_2$ specific activity. Simultaneously, an initial priming dose of unlabelled glucose was infused to maintain a plasma glucose concentration of 5 $mmol \cdot l^{-1}$ throughout the ride. The rate at which the syringe pump administered glucose was constantly adjusted via the negative feedback principle "clamp" technique described by DeFronzo et al. (1979). Briefly, this was achieved by obtaining blood samples (1 ml) every 5 min from the cannula in the non-infused arm, promptly analysing for glucose concentration (Reflolux II, Haemo-Glukotest 20-800R, Boehringer Mannheim, Germany), and then adjusting the infusion rate to maintain euglycaemia.

For the subsequent determinations of the rates of plasma glucose oxidation and liver glucose production from the differences between the total rates of glucose turnover (determined by isotope dilution) and the glucose infusion rates. The infusion rates during the euglycaemic glucose infusion was averaged for the first 25 min and then over successive 20-min intervals until the completion of the 125-min ride. During both rides the subjects were cooled with an electric fan while the laboratory was maintained at a constant temperature of 22–23° C.

Sample collection and analyses

VO_2, carbon dioxide production and $^{14}CO_2$ measurements. Steady-state gas exchange (VO_2, VCO_2, R) was measured for 5 min after 5 min of exercise, and then every 20 min until the completion of the trial. Expired $^{14}CO_2$ was trapped by passing air from the gas analyser vent through a solution containing 1 ml of 1 N hyamine hydroxide in methanol (United Technologies, Packard, Ill., USA), 1 ml of 96% ethanol (SAARCHEM) and 1–2 drops of phenolphthalein (SAARCHEM), as previously described (Scherrer et al. 1978).

Plasma glucose and insulin concentrations. Blood samples were obtained at rest and at 5, 25, 45, 65, 85, 105 and 1 min prior to the termination of exercise. A sample of 10 ml of blood was drawn,

of which half was placed into pre-chilled tubes containing lithium heparin for subsequent plasma glucose and insulin determinations, and the rest was added to tubes containing potassium oxalate and sodium fluoride for separation of glucose and lactate counts. All samples were kept on ice until centrifuged at 2500 rpm at 5° C upon completion of the trial. Plasma samples were then stored at −20° C for later analyses of glucose and insulin concentrations. Plasma glucose concentrations were determined, in duplicate, by an automated glucose analyser (LM3 Glucose Analyser, Analox Instruments, London, UK). Plasma insulin concentrations were determined by radio-immunoassay (Phadeseph Insulin RIA package, Pharmacia Diagnostics AB, Uppsala, Sweden) according to the methods described by Goetz and Greenburg (1961).

Plasma glucose and lactate specific activity. Plasma samples (1 ml) for the separation of glucose and lactate were adjusted to a pH of approximately 4.0 with 60 µl of 2 M H_3PO_4, placed in sealed tubes and deproteinised by heating for 10 min at 70–75° C in a shaking water bath. After being cooled on ice, the seals of the tube were removed and the inside of the tube rinsed with 1 ml of H_2O (raised to pH 8.0 by the addition of traces of ammonion hydroxide) to wash down any lactate which may have condensed onto the side of the tube. The pH of the samples was then adjusted to 7.0 with approximately 40 µl of 3 M K_2CO_3 before centrifugation at 5000 rpm for 10 min. After centrifugation, the supernatant was stored, the pellet resuspended in 0.75 ml of H_2O, recentrifuged, and the supernatant was added to that previously saved, a process that was repeated a further three times.

Separation of plasma glucose from plasma lactate was achieved by passing the combined supernatants through anion exchange columns containing Sephadex (Bakerbond SAX, Cape Town, Republic of South Africa) that had been conditioned with several void volumes of distilled water. Glucose that appeared in the void volume was fully eluted with 3 ml of water. Lactate was subsequently eluted into a second vial with 2 ml of 1 M $CaCl_2$ (adjusted to a pH of 2.0 with HCl). The eluants were collected into scintillation vials and evaporated to near dryness at 70–80° C for approximatley 20 h to (1) minimise the presence of ^3H_2O from the metabolism of 2-^{3}H glucose in the glycolytic pathway to less than 2%, and (2) reduce the water/liquid scintillation mixture ratio during subsequent counting. Each time plasma glucose and lactate samples were separated, a non-labelled plasma sample was "spiked" with a measured quantity of 2-^{3}H and U-^{14}C glucose and run simultaneously to correct the experimental dpm values for the percentage recovery. Such recoveries were 88% for the U-^{14}C glucose samples and 96% for 2-^{3}H glucose samples, and by correcting for the recoveries we were able to calculate plasma glucose specific radioactivities in dpm·mmol^{-1}.

Plasma glucose oxidation. As the plasma lactate counts were subsequently found not to be significantly different from background counts, Rox did not need to be corrected for ^{14}C lactate oxidation and could be determined from the following equation:

$$\text{Rox} = (\text{SACO}_2/\text{SA}_{\text{glu}}) \cdot V\text{CO}_2 \quad (1)$$

where Rox is the rate of plasma glucose oxidation in µmol·min·kg^{-1} FFM^{-1}; SACO_2 is the specific activity of expired $^{14}CO_2$ in dpm·mmol^{-1}; SA_{glu} is the corresponding specific activity of the plasma glucose in dpm·mmol^{-1}; and $V\text{CO}_2$ is the volume of expired CO_2 in µmol·min−kg^{-1} FFM^{-1} calculated from the l·min^{-1} $V\text{CO}_2$, the subject's FFM and the 22.4 l·mol^{-1} gas volume. Since the complete conversion of one molecule of U-^{14}C glucose to 6 molecules of $^{14}CO_2$ decreases the dpm·mmol^{-1} specific activity by a factor of 6, the CO_2 does not need to be divided by 6 to allow for six CO_2 molecules arising from the oxidation of one glucose molecule.

Whole body glucose turnover. Glucose appearance (Ra) and disappearance (Rd) were determined from the non-steady state equations of Steele (1959), validated by Radzuik et al. (1978):

$$\text{Ra} = (I - (\text{p}V \cdot \text{Glu} \cdot \text{dSA}/\text{d}t)/\text{SA} \quad (2)$$

$$\text{Rd} = \text{Ra} - (\text{p}V \cdot \text{dGlu}/\text{d}t) \quad (3)$$

where Ra and Rd are the rate of liver (plus ingested or infused) glucose appearance and disappearance in mmol·min^{-1} kg^{-1}; I is the 2-^{3}H glucose infusion rate in dpm·min^{-1} kg^{-1}; p is the pool fraction, 0.75 (Jenkins et al. 1985); V is the 19.6% of body mass glucose distribution volume in litres; Glu is the mean of successive plasma glucose concentrations in mmol·l^{-1}; dSA/dt is the rate of change in plasma 2-^{3}H specific activity in dpm·mmol^{-1} over the sample interval in min; SA is the average dpm·mmol^{-1} glucose specific activity in successive samples; and dGlu is the mmol·l^{-1} min^{-1} rate of change in glucose concentration.

Exogenous carbohydrate oxidation. The rates of exogenous glucose oxidation were determined from the following equation (Hawley et al. 1992b):

$$\text{Ing CHOox} = ((14\text{CO}_2 \cdot 6/[\text{SACHO}/\text{CHOd}) \cdot 180]) \cdot V\text{CO}_2 \cdot 1.35 \quad (4)$$

where Ing CHOox is the ingested carbohydrate oxidised in g·min^{-1}; $^{14}CO_2 \cdot 6$ is the $^{14}CO_2$ dpm·mmol^{-1} value multiplied by 6, as there are six carbon atoms per molecule of glucose absorbed into the systemic circulation; SACHO is the specific activity of the ingested (glucose) solution in dpm·ml^{-1}; CHOd is the carbohydrate concentration of the drink in g·l^{-1} and 180 is the molecular mass of glucose; $V\text{CO}_2$ is the volume of expired carbon dioxide in l·min^{-1}; and 1.35 is the number of grams of glucose oxidised to produce 1 l of CO_2.

This equation does not take into account the $^{14}CO_2$ retained by the bicarbonate pool, which has been estimated to be approximately 17% of the total CO_2 turnover (Costill et al. 1973). It has been estimated that up to 80 min are required to equilibrate $^{14}CO_2$ with the plasma CO_2/HCO^{3-} pool during steady-state, moderate-intensity (i.e. 60%–70% of $V\text{O}_{2\text{max}}$) exercise (Coggan et al. 1990, 1991). However, the exercise duration in the current experiments was over 2 h and any underestimation of the amount of plasma glucose oxidised would have been similar from trial to trial.

Total carbohydrate oxidation. Overall CHO oxidation in g·min^{-1} was calculated from the formula of Consolazio et al. (1963) given below, and total CHO during exercise was estimated from the area under the CHO oxidation versus time curve for each subject.

$$\text{Total CHO oxidation} = 4.55\ V\text{CO}_2 - 3.21\ V\text{O}_2 \quad (5)$$

where $V\text{CO}_2$ is the volume of CO_2 in the expired air measured in l·min^{-1}, and $V\text{O}_2$ is the oxygen uptake in l·min^{-1} measured during the same period. Rates of CHO oxidation were converted from g·min^{-1} values to µmol·min^{-1} kg FFM^{-1} units by dividing the values by the molecular weight of glucose (180) in µg and the subject's FFM. Rates of fat oxidation were converted from g·min^{-1} values to µmol·min^{-1} kg FF^{-1} units by dividing the values by the molecular weight of palmitate (237) in µg and the subject's FFM.

The total energy demands of exercise (i.e. the sum of CHO and fat oxidation in kilocalories·min^{-1}) was derived from the g·min^{-1} rates of CHO and fat oxidation multiplied by 4.1 and 9.3 kcal·g^{-1} respectively.

Statistical analyses. Statistical significance was assessed by a one-way analysis of variance for repeated measures. Significant differences between means over time were located by Schéffe's post-hoc test. Between treatment means were compared using an unpaired Student's t-test. A value of $P < 0.05$ was regarded as significant. All results are expressed as means (SEM).

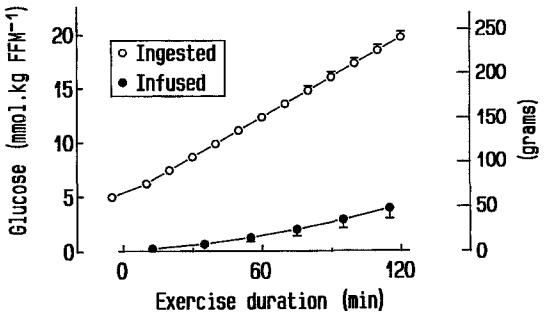

Fig. 1. The rates of carbohydrate ingestion and intravenous glucose infusion required to maintain euglycaemia (i.e. a plasma glucose concentration of 5 mmol·l^{-1}) during 125 min of cycling. *FFM,* fat free mass

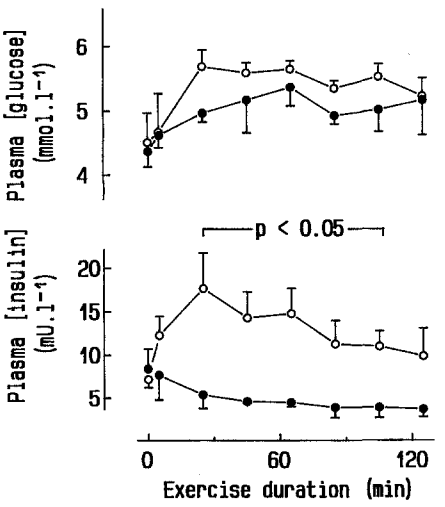

Fig. 2. Plasma glucose and plasma insulin concentrations during 125 min of cycling when the subjects either ingested carbohydrate (*open circles*) or received a variable-rate intravenous glucose infusion (*filled circles*)

Results

Rates of glucose infusion and ingestion

The rate of intravenous glucose infusion required to maintain the plasma glucose concentration at 5 mmol·l^{-1} increased progressively during exercise (Fig. 1). For the first 25 min of exercise, the rate of glucose infusion averaged 18.0 (4.9) μmol·min kg^{-1} FFM^{-1} and increased to 60.8 (8.7) μmol·min^{-1} kg FFM^{-1} during the 105–125 min period of exercise ($P<0.01$). The total amount of glucose infused to maintain euglycaemia during the 125 min of exercise was 3.92 (0.92) mmol·kg FFM^{-1} [47.8 (11.6) g]. In contrast, far more glucose was consumed during the CHO ingestion trial [19.7 μmol·min^{-1} kg FFM^{-1} (240 g; Fig. 1)].

Plasma glucose and plasma insulin concentrations

Figure 2 shows the plasma glucose and plasma insulin concentrations measured during the two periods of exercise. Glucose ingestion and glucose infusion both maintained euglycaemia during the 125-min rides. With CHO ingestion, plasma glucose concentration averaged 5.3 (0.13) mmol·l^{-1} [range 4.6 (0.60) to 5.7 (0.26) mmol·l^{-1}], and with glucose infusion it averaged 5.0 (0.09) [range 4.6 (0.34) to 5.4 (0.29) mmol·l^{-1}]. Despite the similar plasma glucose concentrations in the two experimental trials, CHO ingestion resulted in significantly higher average plasma insulin concentrations throughout exercise than did intravenous glucose infusion [12.9 (1.0) vs 4.74 (0.5) mU·l^{-1} respectively, $P<0.001$]. During the CHO ingestion trial, plasma insulin concentration increased significantly from 7.1 (0.95) mU·l^{-1} at rest to 12.2 (2.3) mU·l^{-1} after only 5 min of exercise ($P<0.05$). Thereafter plasma insulin concentration continued to climb to 17.6 (4.2) mU·l^{-1} after 25 min ($P<0.05$ compared to rest) and then decreased to 9.7 (3.3) mU·l^{-1} at the end of exercise. In contrast, with intravenous glucose infusion, plasma insulin concentrations decreased and were significantly lower after 25 min of exercise than at the start of exercise [5.3 (1.6) vs 8.3 (2.3) mU·l^{-1} respectively; $P<0.05$]. Values decreased further to 3.9 (0.9) mU·l^{-1} at the end of exercise.

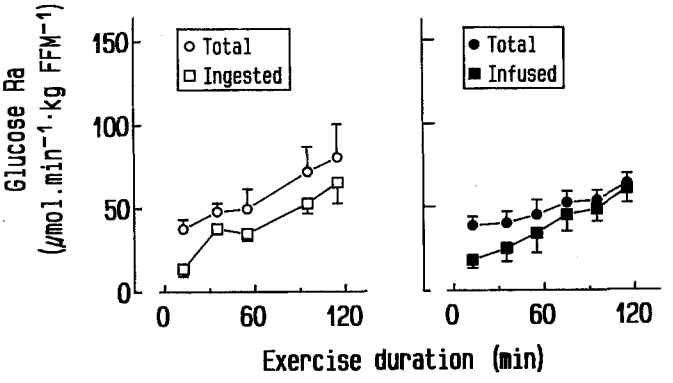

Fig. 3. The rate of whole-body glucose appearance (Ra) during 125 min of cycling when subjects either ingested carbohydrate (*open circles*) or received a variable-rate intravenous glucose infusion (*filled circles*). *FFM,* fat free mass

Glucose turnover

During the CHO ingestion trial, total (endogenous plus ingested) glucose Ra and Rd (not shown) increased significantly throughout exercise to 81 (21) μmol·min^{-1} kg FFM^{-1} during the last 20 min of the ride ($P<0.05$ compared to first 20 min; Fig. 3, left panel). At that stage, the contribution of ingested glucose to the total Ra was 65 (13) μmol·min^{-1} kg FFM^{-1} or 81 (7)%.

In the glucose infusion trial, total glucose (endogenous plus infused) Ra increased to 64 (6) μmol·min^{-1} kg FFM^{-1} during the last 20 min of the ride ($P<0.05$ compared to first 20 min; Fig. 3, right panel). Here the

contribution from infused glucose to total Ra was 93 (5)%. Although glucose Ra values tended to be lower with intravenous glucose infusion than with CHO ingestion, these differences were not statistically significant.

Rates of plasma glucose oxidation

Although CHO ingestion significantly increased Rox values from 25 to 105 min ($P<0.05$; Fig. 4), there were no differences in Rox for glucose ingestion or glucose infusion at the end of exercise [93 (8) vs 85 (5) $\mu mol \cdot min^{-1}$ kg FFM^{-1}].

Total carbohydrate and fat oxidation

Tables 2 and 3 show the steady-state gas exchange data and the total calculated CHO and fat oxidation rates during the two experimental rides. Because the absolute oxygen cost of riding at 63% of PPO was significantly greater for the subjects who received the glucose infusion compared to glucose ingestion [average VO_2 3.56 (0.03) $l \cdot min^{-1}$ vs 3.18 (0.01) $l \cdot min^{-1}$; $P<0.05$], the rates of CHO and fat oxidation are expressed both relative to FFM and in absolute ($g \cdot min^{-1}$) terms (Table 3).

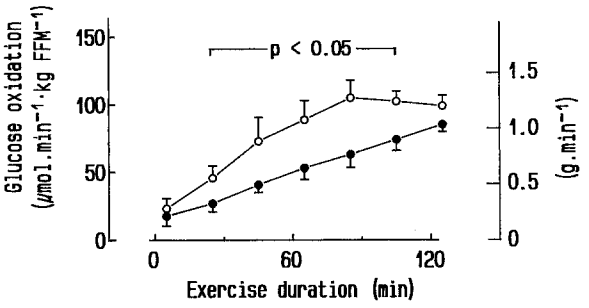

Fig. 4. The rate of plasma glucose oxidation during 125 min of cycling when subjects either ingested carbohydrate (*open circles*) or received a variable-rate intravenous glucose infusion (*filled circles*). *FFM,* fat free mass

During the glucose ingestion trial the rate of CHO oxidation declined from 306 (19) $\mu mol \cdot min^{-1}$ kg FFM^{-1} after the first 5 min of exercise to 282 (22) $\mu mol \cdot min^{-1}$ kg FFM^{-1} at the end of the ride (Table 3) and the rate of fat oxidation rose from 10.6 (2.7) $\mu mol \cdot min^{-1}$ kg FFM^{-1} to 20.5 (4.6) $\mu mol \cdot min^{-1}$ kg FFM^{-1}. As a result, the contribution from fat oxidation to the total energy demands of exercise increased from 10.9 (4.7)% after 5 min of exercise to 18.2 (4.5)% at the end of the 125 min ride (Fig. 5).

In contrast, rates of CHO oxidation were significantly lower throughout the glucose infusion trial with rates declining from 223 (32) $\mu nmol \cdot min^{-1}$ kg FFM^{-1} after 5 min of exercise to 178 (35) $\mu mol \cdot min^{-1}$ kg FFM^{-1} at the end of the ride (Table 3). Hence the rates of fat oxidation in the glucose infusion trial were significantly higher than in the glucose ingestion trial ($P<0.05$; Table 3) and contributed 51 (10)% of the total energy demands of exercise at the end of the ride (Fig. 5).

Ingested CHO oxidation peaked at 80 (10) $\mu mol \cdot min^{-1}$ kg FFM^{-1} at the end of the 125 min ride and throughout the ride accounted for 83 (11) g or 17.5 (1.4)% of the total CHO oxidation. The contribution of ingested CHO oxidation to total CHO oxidation was 28 (2)% at the end of the ride.

Areas under the total CHO oxidation and plasma glucose oxidation versus time curves during the two rides are shown in Fig. 6. Both total CHO oxidation [37.2 (2.8) $mmol \cdot 15\ min^{-1}$ kg FFM^{-1} vs 24.1 (3.9) $mmol \cdot 125\ min^{-1}$ kg FFM^{-1}; $P<0.05$] and total plasma glucose oxidation [9.5 (1.2) $\mu mol \cdot 125\ min^{-1}$ kg FFM^{-1} vs 6.2 (0.74) $\mu mol \cdot 125\ min^{-1}$ kg FFM^{-1}; $P<0.05$] were significantly higher with CHO ingestion

Table 2. Steady-state gas exchange data during the two experimental trials

	Time (min)													
	5		25		45		65		85		105		125	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
VO_2 ($l \cdot min^{-1}$)														
Ingestion	3.14	0.04	3.13	0.05	3.19	0.08	3.16	0.10	3.22	0.10	3.23	0.13	3.23	0.13
Infusion	3.15[a]	0.15	3.53[a]	0.16	3.50[a]	0.14	3.50	0.16	3.54	0.16	3.63[a]	0.15	3.72[a]	0.17
VCO_2 ($l \cdot min^{-1}$)														
Ingestion	3.03	0.07	3.04	0.08	3.06	0.10	3.00	0.12	3.09	0.14	3.05	0.14	3.04	0.15
Infusion	3.06	0.04	3.05	0.04	3.00	0.04	2.98	0.07	3.01	0.04	3.05	0.06	3.10	0.07
R														
Ingestion	0.97[b]	0.02	0.97[b]	0.02	0.96[b]	0.01	0.95[b]	0.02	0.95[b]	0.02	0.94[b]	0.01	0.94[b]	0.01
Infusion	0.88	0.03	0.87	0.04	0.86	0.03	0.86	0.03	0.85	0.03	0.84	0.03	0.84	0.03

Ingestion, the subjects ingested carbohydrate (a 15 $g \cdot 100\ ml^{-1}$ U-^{14}C labelled glucose solution) throughout exercise; Infusion, the subjects received a variable-rate glucose infusion throughout exercise; VO_2, oxygen consumption ($l \cdot min^{-1}$); VCO_2, carbon dioxide production ($l \cdot min^{-1}$); *R,* respiratory exchange ratio; FFM, fat free mass

[a] Infusion significantly greater than Ingestion trial, $P<0.05$

[b] Ingestion trial significantly greater than Infusion trial, $P<0.05$

Table 3. Carbohydrate (CHO) and fat energy during two experimental trials

Time (min)	5		25		45		65		85		105		125	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
CHO oxidation ($g \cdot min^{-1}$)														
Ingestion	3.73[b]	0.26	3.78[b]	0.29	3.69[b]	0.23	3.52[b]	0.25	3.74[b]	0.37	3.49[b]	0.26	3.45[b]	0.30
Infusion	2.69	0.37	2.56	0.21	2.40	0.40	2.34	0.29	2.31	0.43	2.21	0.40	0.40	0.40
CHO oxidation ($\mu mol \cdot min^{-1}$ kg FFM^{-1})														
Ingestion	306.2[b]	19.9	310.b[b]	21.9	303.0[b]	16.7	288.3[b]	17.5	306.6[b]	28.4	286.8[b]	18.1	282.2[b]	21.6
Infusion	222.8	32.3	212.6	38.6	207.6	39.9	193.4	25.6	191.7	36.9	183.5	34.6	177.8	34.7
Fat oxidation ($g \cdot min^{-1}$)														
Ingestion	0.17	0.09	0.15	0.10	0.21	0.07	0.26	0.06	0.21	0.11	0.31	0.06	0.32	0.06
Infusion	0.74[a]	0.21[a]	0.79[a]	0.24	0.84[a]	0.21	0.87[a]	0.18	0.90[a]	0.23	0.98[a]	0.22	1.04[a]	0.22
Fat oxidation ($\mu mol \cdot min^{-1}$ kg FFM^{-1})														
Ingestion	10.6	2.7	13.5	4.6	13.7	4.2	16.6	4.1	17.7	4.8	19.4	3.9	20.5	4.6
Infused	45.7[a]	12.6	45.7[a]	16.6	52.4[a]	12.8	53.9[a]	10.8	55.8[a]	13.3	60.9[a]	13.2	64.9[a]	13.6

Ingestion, the subjects ingested carbohydrate (15 $g \cdot 100\ ml^{-1}$ U-^{14}C glucose solution) throughout exercise; Infusion, the subjects received a variable-rate glucose infusion throughout exercise; FFM, fat free mass

[a] Infusion trial significantly greater than Ingestion trial, $P<0.05$
[b] Ingestion trial significantly greater than Infusion trial, $P<0.05$

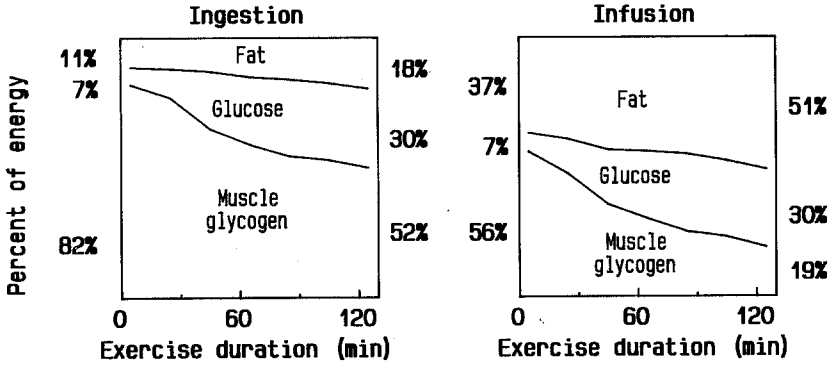

Fig. 5. The relative contribution of fuel substrates to total energy production during 125 min of cycling when the subjects either ingested carbohydrate or received a variable-rate intravenous glucose infusion

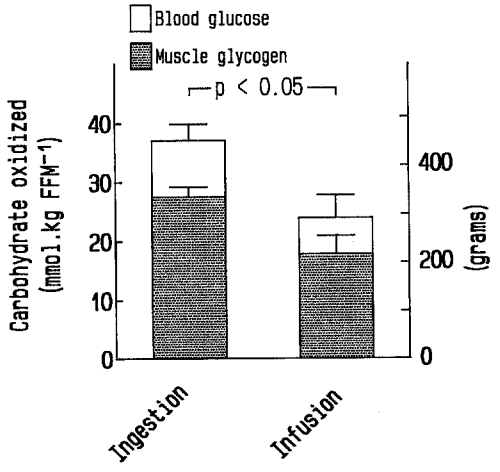

Fig. 6. Carbohydrate oxidation during 125 min of cycling when the subjects either ingested glucose or received a variable-rate intravenus glucose infusion. *FFM*, fat free mass

compared to glucose infusion (Fig. 6). The contribution from muscle glycogen (plus lactate) utilisation (as estimated from the difference between the total CHO oxidation and total plasma glucose oxidation) was also greater with CHO ingestion versus intravenous glucose infusion [ingestion 27.6 (4.6) $mmol \cdot 125\ min^{-1}$ kg FFM^{-1} vs 17.8 (4.6) $mmol \cdot 125\ min^{-1}$ kg FFM^{-1}], but these differences were only significant at 125 min ($P<0.05$).

Discusssion

The major finding of the current study was that intravenous glucose infusion, which could provide a more or less infinite rate of glucose delivery into the systemic circulation, did not result in higher rates of glucose oxidation by muscle than when glucose was ingested.

When CHO was ingested to maintain plasma glucose concentration at 5 $mmol \cdot l^{-1}$, plasma insulin concentrations were significantly greater throughout most

of the 125-min ride than when euglycaemia was maintained via intravenous glucose infusion (Figs. 1, 2). Higher plasma insulin concentrations with CHO ingestion could probably have resulted from the large differences in the amounts of glucose administered (Fig. 1). Whereas 240 g of glucose was ingested during the 125 min of exercise, only 48 (12) g was required to maintain euglycaemia during the intravenous glucose infusion.

Alternatively, the differences in plasma insulin concentrations may have been due to the routes of glucose administration. Hepatic glucose appearance has been shown to be greater after administration of an oral glucose load than with peripheral intravenous glucose infusion, despite the maintenance of similar insulin and glucagon concentrations (Bergman et al. 1982; DeFronzo et al. 1978).

Despite the differences in plasma insulin concentrations (Fig. 2), there were no measurable differences in Ra (Fig. 3). With glucose ingestion, Ra tended to be higher towards the end of exercise but the differences were neither significant nor perhaps that reliable, particularly duirng the early (less than 75 min) stages of exercise. Comparisons between the final Ra values in Fig. 3, and the final Rox values in Fig. 4 indicated that glucose Ra determinations probably underestimated actual rates of glucose appearance during both experiments by up to 15% with CHO ingestion and 25% with glucose infusion.

While tracer techniques have been shown to provide reasonable estimates of glucose turnover under steady-state conditions (Allsop et al. 1978), they have frequently underestimated glucose turnover during non-steady-state conditions typical of the intravenous glucose clamp method (Argoud et al. 1987; Finegood et al. 1987, 1988). Reasons why the isotope tracer method consistently underestimates glucose turnover have been addressed in detail elsewhere (Allsop et al. 1978; Argoud et al. 1987; Coggan 1991; Finegood et al. 1987, 1988). The most likely explanation is the use of Steele's (1959) one-compartment, fixed pool volume model of glucose kinetics which may be inappropriate for the variable rates of glucose appearance with this method (Finegood et al. 1987), and also with repeated glucose ingestion. The glucose Ra data, therefore, should be regarded as more of an indication of a tendency than an absolute value. They show that, towards the end of exercise, most of the glucose Ra was from ingested or infused glucose. As recently noted by Wolfe and George (1993) "recognizing the limitations in quantifying the oxidation of labelled substrates does not eliminate the use of such compounds to make qualitative or comparative observations."

The more reliable measurements of plasma glucose oxidation, which do not rely on assumed distribution volumes, showed that between 25 and 105 min of exercise, rates of glucose oxidation and plasma insulin concentrations increased together in the CHO ingestion trials (Fig. 4; $P<0.05$). Whereas with CHO ingestion, Rox increased to 93 (8) $\mu mol \cdot min^{-1}$ kg FFM^{-1} within 90 min of exercise, the final Rox value after 125 min of intravenous glucose infusion was 85 (5) $\mu mol \cdot min^{-1}$ kg FFM^{-1} and still rising, despite the falling plasma insulin concentrations. Wolfe et al. (1986) have also reported that despite markedly different plasma insulin (19.8 vs 9.2 $\mu U \cdot min^{-1}$) and plasma FFA (0.18 vs 0.70 $\mu mol \cdot ml^{-1}$) concentrations, glucose oxidation increased to a similar extent in subjects who exercise for 60 min at 40% of VO_{2max}. The rates of plasma glucose oxidation found in the current study are in accord with the 92 $\mu mol \cdot min^{-1}$ kg FFM^{-1} rates of plasma glucose disposal estimated by Coggan and Coyle (1987) at the end of 215 min of exhausting cycling in subjects who received an intravenous glucose infusion in the latter stages of exercise to maintain plasma glucose concentration at approximately 5.0 $mmol \cdot l^{-1}$.

The peak (about 1 $g \cdot min^{-1}$) rates of plasma glucose oxidation found in the current study are also similar to the values reported by Bosch et al. (1993) in moderately trained subjects who ingested 170 ml of a 10 $g \cdot 100^{-1}$ ml glucose beverage every 20 min during 3 h of cycling at 70% of VO_{2max}. Although such rates of plasma glucose uptake and oxidation are considerably higher than some previous reports (Ahlborg and Felig 1976, 1982; Wahren 1977), they are in close agreement with findings from recent studies using either arteriovenous sampling or isotope tracers during dynamic exercise (Broberg and Sahlin 1989; Coggan et al. 1991; Hawley et al. 1992a, b; Katz et al. 1986; Stein et al. 1989).

As rates of glucose oxidation by muscle eventually increased to similar values with both intravenous glucose infusion and CHO ingestion (Fig. 4), these findings suggest that when sufficient CHO is ingested, either the appearance of glucose in the systemic blood supply, or the prevailing plasma glucose concentration, regulates the rate of glucose oxidation by working muscles, at least after 90 min of moderate-intensity exercise. Thus, the data in Fig. 4, and the results of other studies cited previously, would suggest that the rate of oxidation of glucose by muscle is probably limited to approximately 1 $g \cdot min^{-1}$, at least when plasma glucose concentrations are 5 $mmol \cdot l^{-1}$ and plasma insulin concentrations are not greatly increased. The precise mechanism for this regulation is, at present, unknown.

In this regard, the estimated 2 $g \cdot min^{-1}$ rates of blood CHO oxidation found by Coyle et al. (1986) from gas exchange measurements at the end of continuous, exhausting moderate-intensity cycling when muscle glycogen was depleted and plasma glucose concentrations were 4–5 $mmol \cdot l^{-1}$ are of interest. Since rates of muscle glucose oxidation appear to be limited to 1 $g \cdot min^{-1}$ (Broberg and Sahlin 1989; Coggan et al. 1991; Hawley et al. 1992a, b; Katz et al. 1986; Stein et al. 1989), the data of Coyle et al. (1986) suggest that the final rates of blood CHO oxidation in exhausting exercise were probably due to a combination of glucose and lactate oxidation. A breakdown of glycogen in non-working muscles to provide lactate to glycogen-depleted muscles for oxidation has been proposed as an important mechanism for redistributing CHO dur-

ing exercise (Bosch et al. 1993; Brooks 1986; Walsh and Bannister 1988).

The reduced plasma insulin concentrations with intravenous glucose infusion were associated with lower *R* values (Table 2) and hence higher calculated rates of fat oxidation (Table 3). Whereas the contribution to energy production from fat oxidation increased from 11 (4)% at the beginning of exercise to only 18 (4)% after 125 min with CHO ingestion, it rose from 37 (10)% to 51 (10)% with glucose infusion (Fig. 5). These high percentage contributions to energy production from fat oxidation with intravenous glucose infusion are similar to those found in subjects ingesting water during exercise of similar intensity and duration (Coyle et al. 1986, 1991). Costill et al. (1977) have reported that oxidation of fat is significantly greater throughout 30 min of treadmill running at 68% of $\dot{V}O_{2max}$ when plasma FFA concentrations are elevated above those found when either water or 75 g of glucose is ingested.

An elevated rate of fat oxidation with hypo-insulinaemia probably arises from a decreased re-esterification of the FFA released from adipocyte triglyceride lipolysis, and supports the concept that the utilisation of plasma FFA during moderate to high intensity exerice may be limited by the release into the bloodstream of FFA from adipocyte triglyceride breakdown (Hargreaves et al. 1991; Romijn et al. 1992). Elevated plasma insulin concentrations are known to reduce circulating plasma FFA concentrations (Wolfe et al. 1986), to facilitate a high rate of glucose disposal, and to inhibit fat oxidation (Costill et al. 1977; Rennie and Holloszy 1977).

Conversely, artificially raised plasma FFA concentrations have been shown to increase the rates of FFA oxidation and inhibit glucose utilisation in some (Balasse and Neef 1974; Ferrannini et al. 1983), but not all (Wolfe et al. 1988) investigations. Studies in animals (Rennie and Holloszy 1976) and humans (Costill et al. 1977) have shown that an elevation of plasma FFA concentrations reduces the utilisation of working muscle glycogen by up to 45% during submaximal exercise, although more recent experiments have reported that CHO oxidation (Ravussin et al. 1986) and glycogen metabolism (Hargreaves et al. 1991) are unaffected by physiological elevations in plasma FFA concentrations. In the current study we found that the higher rates of fat oxidation in the glucose infusion trial, than with CHO ingestion, were associated with decreased muscle glycogen (plus lactate) utilisation (estimated from the difference between the rate of total CHO oxidation minus the plasma glucose oxidation) after 125 min of exercise, and reduced plasma glucose oxidation and overall CHO oxidation throughout exercise (Fig. 6).

In conclusion, these results show that compared to repetitive CHO ingestion, intravenous glucose infusion and the associated hypo-insulinaemia result in reduced initial rates of muscle glucose oxidation, decreased rates of CHO oxidation, and increased rates of fat oxidation during 2 h of moderate-intensity exercise. However, despite lower plasma insulin concentrations with glucose infusion, the rates of muscle glucose oxidation were the same at the end of 125 min of exercise as they were with CHO ingestion, which caused significantly higher plasma insulin concentrations. These findings would indicate that the rate of exogenous glucose oxidation by the muscle is a function of either the prevailing plasma glucose concentration or the rate of hepatic glucose appearance, rather than plasma insulin concentrations or the rate of fat and CHO oxidation. The finding that intravenous glucose infusion, which could theoretically produce an infinite rate of glucose delivery, did not elicit higher rates of muscle glucose oxidation than CHO ingestion indicates that the rate of muscle glucose uptake, or the prevailing plasma glucose concentration, regulates the rate of exogenous glucose oxidation, at least in this model. Either would explain why the ingestion of a variety of mono-, di-, and oligosaccharides, all of which elicit similar (i.e. 5 $mmol \cdot l^{-1}$) plasma glucose concentrations are limited to approximately 1 $g \cdot min^{-1}$ (Hawley et al. 1992a), while raising plasma glucose concentration to 10 $mmol \cdot l^{-1}$ (i.e. hyperglycaemia) increases the rate of muscle glucose oxidation to nearly 2 $g \cdot min^{-1}$ (Hawley et al. 1994).

Of practical significance was the finding that any condition which elevates plasma insulin concentration during the first 90–120 min of prolonged (i.e. 3–4 h) exhausting exercise *may* have a detrimental effect on endurance performance due to the inhibition of fat metabolism with an accelerated rate of CHO metabolism, the opposite effect of that which is believed to aid performance during prolonged exercise (for review see Coggan and Coyle 1991). In this regard, Gisolfi and Duchman (1992) have recommended that during the early stages of athletic events lasting between 1–3 h, "fat metabolism should be promoted" and "the inclusion of carbohydrate in beverages (early in these events) should be avoided" as it would "turn on carbohydrate metabolism and promote glycogen utilization ... which could lead to premature fatigue."

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