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Glucose kinetics during prolonged exercise in euglycaemic and hyperglycaemic subjects

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Abstract. To determine the limits to oxidation of exogenous glucose by skeletal muscle, the effects of euglycaemia (plasma glucose 5 mM, ET) and hyperglycaemia (plasma glucose 10 mM, HT) on fuel substrate kinetics were evaluated in 12 trained subjects cycling at 70% of maximal oxygen uptake ($\dot{V}O_{2,\,max}$) for 2 h. During exercise, subjects ingested water labelled with traces of U-^{14}C-glucose so that the rates of plasma glucose oxidation (R_{ox}) could be determined from plasma ^{14}C-glucose and expired $^{14}CO_2$ radioactivities, and respiratory gas exchange. Simultaneously, 2-^{3}H-glucose was infused at a constant rate to estimate rates of endogenous glucose turnover (R_a), while unlabelled glucose (25% dextrose) was infused to maintain plasma glucose concentration at either 5 or 10 mM. During ET, endogenous liver glucose R_a (total R_a minus the rate of infusion) declined from 22.4 ± 4.9 to 6.5 ± 1.4 μmol/min per kg fat-free mass [FFM] ($P < 0.05$) and during HT it was completely suppressed. In contrast, R_{ox} increased to 152 ± 21 and 61 ± 10 μmol/min per kg FFM at the end of HT and ET respectively ($P < 0.05$). HT (i. e., plasma glucose 10 mM) and hyperinsulinaemia (24.5 ± 0.9 μU/ml) also increased total carbohydrate oxidation from 203 ± 7 (ET) to 310 ± 3 μmol/min per kg FFM ($P < 0.0001$) and suppressed fat oxidation from 51 ± 3 (ET) to 18 ± 2 μmol/min per kg FFM ($P < 0.0001$). As the rates of oxidation at more physiological euglycaemic concentrations of glucose were limited to 92 ± 9 μmol/min per kg FFM, and were similar to those reported when carbohydrate is ingested, the results of the current study suggest that the concentrations of glucose and insulin normally present during prolonged, intense exercise may limit the rate of muscle glucose uptake and oxidation.

Key words: Glucose oxidation – Glucose infusion – Insulin

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Introduction

Several factors may determine the rate at which ingested carbohydrate (CHO) solutions are ultimately utilised by the working muscles during prolonged, intense (70% of maximal oxygen uptake [$\dot{V}O_{2,\,max}$]) exercise. These include the rate of gastric emptying of the drink, its rate of digestion, absorption and passage via the liver into the systemic blood supply and the rate of glucose oxidation by the working muscle.

The idea that gastric emptying may limit the ultimate rates of ingested CHO oxidation during the early (up to 90 min) stages of exercise has been clearly refuted by the results of several recent investigations which have shown that the amount of CHO leaving the stomach after the repeated ingestion of solutions containing a variety of mono-, di- and polysaccharides is more than double the amount oxidised [19, 22, 24]. Irrespective of the ingestion regimen, peak rates of ingested CHO oxidation rise to about 1 g/min during the later stages of prolonged exercise [18]. Rates of ingested CHO oxidation are, therefore, either limited by the release of the CHO into the systemic circulation or by the rate of glucose oxidation by the working muscle. Thus, the present study was designed to bypass both intestinal absorption and liver glucose uptake by employing intravenous glucose infusion and to determine if a higher delivery of glucose into the systemic circulation would increase the rates of plasma glucose oxidation by the working muscles.

While estimates of the rate of glucose oxidation by the active muscles vary widely [7], Coyle et al. [9] have suggested that highly trained individuals can oxidise plasma glucose at rates approaching 2 g/min during the latter stages of prolonged exhaustive exercise. In support of this postulate, Coyle et al. [10] have recently infused glucose into well-trained subjects to maintain plasma glucose concentration at approximately 10 mM, and found that the rate of CHO oxidation (CHO_{ox}, estimated from respiratory gas exchange data) was markedly ele-

vated above control values (4−5 mM plasma glucose concentration) during 2 h of intense (73% of $\dot{V}O_{2,max}$) cycling. Under hyperglycaemic conditions, the rate of glucose infusion needed to maintain plasma glucose concentration at about 10 mM increased from 1.6 g/min early in exercise to more than 2.6 g/min during the last 20 min of the exercise [10]. As rates of glucose infusion provide an estimate of plasma glucose disposal (R_d) [11, 12], the results of Coyle et al. [10] suggest that trained individuals may be able to oxidise plasma glucose at rates considerably greater that the peak rates of about 1 g/min found when subjects ingest CHO [18]. However, without the use of isotopic tracers, Coyle et al. [10] were unable to determine the proportion of infused glucose that was oxidised. Further, the conditions of hyperglycaemia are artificial, and could be detrimental to endurance performance due to inhibition of fat metabolism with an accelerated CHO_{ox} the opposite effects of endurance training, and those believed to aid performance during prolonged exercise [9].

The purpose of this study was to quantify fuel substrate kinetics during prolonged, steady-state exercise in highly trained subjects when plasma glucose concentration was maintained at either 5 mM (euglycaemic clamp, ET) or raised to 10 mM (hyperglycaemic clamp, HT) by continuous, variable-rate, intravenous glucose infusions. Specifically, this study aimed to determine whether the rate of muscle glucose oxidation is only increased when plasma glucose and plasma insulin concentrations are raised above physiological values, or whether bypassing both intestinal absorption and liver glucose uptake allows muscle to oxidise glucose at rates greater than the approximately 1 g/min peak values found when subjects ingest CHO.

Materials and methods

Experimental protocol

Subjects and preliminary testing. Twelve male 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. Subjects were fully aware of the nature and risks of the testing procedures, which were explained in detail to each individual prior to obtaining his written informed consent. To minimise the exposure to radioactivity in these investigations, subjects were randomly divided into two groups ($n = 6$ per group) and each performed only one experimental trial. Subject characteristics are summarised in Table 1. There were no significant differences in any of the variables measured in the two groups.

All subjects were tested for $\dot{V}O_{2,max}$ on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands) as described in detail previously [17]. The results of the initial maximal test were used to determine the work rate corresponding to 70% of each subject's $\dot{V}O_{2,max}$. The maximal test and experimental trial were separated by a period of 7−10 days, during which time subjects maintained their usual training and dietary regimens.

The subjects' fat free mass (FFM) and percentage body fat were calculated from their total mass and the sum of skinfold measurements using conventional formulae [13]. Such values were used to correct the measurements of glucose turnover and oxidation during the experimental trials for differences in FFM.

Experimental trial. Each cyclist completed one 125-min ride at an intensity of 70% of $\dot{V}O_{2,max}$. On the day of the trial, subjects reported to the laboratory between 0800−1000 h, a minimum of 2 h after a standardised breakfast which contained approximately 85 g CHO. After the subject had urinated, a flexible 18-gauge cannula (Jelco, Johnson and Johnson, Halfway House, Tvl., South Africa) attached to a three-way stop-cock (Industrias Palex, Barcelona, Spain) was positioned in an antecubital vein of the right forearm. This cannula was used for blood sampling during the experimental trial. After a resting sample was taken, the cannula was immediately flushed with 2−3 ml of sterile saline (containing 5 U/ml heparin), a procedure which was repeated after the withdrawal of each sample. A 20-gauge cannula attached to a three-way stop-cock was then inserted into an antecubital vein of the left forearm. This cannula was used for both a continuous constant infusion of 2-^{3}H-glucose tracer (Amersham, Buckinghamshire, UK) and for the variable-rate infusion of glucose (25% dextrose, Adco Pharmaceutica, Adcock Ingram, Johannesburg, South Africa), at a rate necessary to maintain plasma glucose concentration at either 5 mM (ET) or 10 mM (HT) for the duration of the trial. Euglycaemia was maintained by means of intravenous glucose infusion rather than by CHO ingestion as previous studies have shown that net hepatic uptake of glucose is much greater after an oral glucose load than with comparable loading by peripheral intravenous glucose infusion, even with similar insulin concentrations [4, 11].

Table 1. Characteristics of subjects

	ET	HT
Age (yr)	23.5 ± 1.0	26.0 ± 1.1
Mass (kg)	79.8 ± 1.8	72.8 ± 4.9
FFM (kg)	68.6 ± 1.4	64.6 ± 4.7
W_{peak} (W)	405.0 ± 21.5	388.5 ± 13.4
Workrate at ~70% $VO_{2,max}$	255.4 ± 13.5	244.8 ± 8.4
$VO_{2,max}$ (L/min)	4.86 ± 0.17	4.81 ± 0.14

ET, euglycaemic clamp; HT, hyperglycaemic clamp; FFM, fat free mass; $VO_{2,max}$, maximal oxygen uptake; all values are mean ± SEM

The 2-^{3}H-labelled glucose infusion was used to determine rates of glucose appearance (R_a). A ^{3}H-label at the C2 position on the glucose was selected to prevent re-cycling of the label because we wished to determine liver glucose output from both hepatic glycogenolysis and gluconeogenesis from unlabelled three-carbon products.

Glucose labelled with ^{3}H in the C2 position is lost in the glucose phosphate isomerase reaction of the glycolytic and gluconeogenic pathway [5]. Loss of the label in the hepatic glucose-6-P/fructose-6-P equilibrium also limits the incorporation of ^{3}H into liver glycogen during pre-exercise infusion of 2-^{3}H-glucose [5]. Such incorporation and subsequent hydrolysis of the labelled glycosyl residues during the exercise period would result in an underestimation of R_a. Avoiding this potential error was considered to be more important than the possible small (i. e. 9.4%) overestimation of R_a that is thought to occur when 2-^{3}H-glucose tracers are used [23].

Subjects remained seated for some 75 min prior to commencing exercise while pyrogen-free, sterile 2-^{3}H-glucose was infused at a constant rate of 0.29 ml/min (900 KBq/h; 25 μCi/h) using a calibrated auto-syringe (Travenol, Hooksett, N. H., USA). A previous study from this laboratory has shown that tracer equilibration, as evidenced by a constant plasma glucose specific activity (*SA*), is achieved after a 60−70 min infusion [5]. After tracer equilibration had been attained, a 1-ml blood sample was taken and rapidly analysed for glucose concentration (Reflolux II, Haemo-Glukotest 20-800R, Boehringer Mannheim, Mannheim, Germany). Subjects then began a 3- to 5-min warm-up on the cycle ergometer. During this time subjects ingested a 400-ml loading bolus of artificially flavoured water labelled with tracer amounts of U-^{14}C-glucose (Amersham) for the determination of R_{ox} from the plasma

^{14}C-glucose *SA* and expired $^{14}CO_2$ *SA*. The *SA* of the U-^{14}C-glucose solution was 6.3 Bq/g (0.17 μCi/g) and a total of 40 μCi (148×10^4 Bq) was ingested during the trial.

At the same time, an initial priming dose of unlabelled glucose was infused to achieve the desired plasma glucose concentration for that trial. The rate at which the syringe pump administered glucose was constantly adjusted throughout the duration of the ride via the negative feedback principle 'clamp' technique described by DeFronzo et al. [12]. This was achieved by drawing a 1-ml blood sample every 5 min from the cannula in the non-infused arm, promptly analysing for glucose concentration, and then adjusting the infusion rate to maintain the desired plasma glucose concentration.

After the warm-up subjects commenced the 125 min of exercise. To minimise any possible dehydration, subjects ingested a further 100 ml of water containing the U-^{14}C-glucose every 10 min, so that by the end of exercise they had ingested a total of 1.6 l. During exercise subjects were cooled with an electric fan while the laboratory was maintained at a constant temperature of 22°C.

Sample collection and analyses

Gas exchange measurements. Steady-state gas exchange ($\dot{V}O_2$, CO_2 production [$\dot{V}CO_2$] and the respiratory exchange ratio [RER, $\dot{V}CO_2/\dot{V}O_2$]) was measured for 5−6 min after 5 min exercise and every 20 min thereafter until the end of the trial. In addition, expired $^{14}CO_2$ was trapped by passing air from the gas analyser vent through a solution containing 1 ml 1N hyamine hydroxide in methanol (United Technologies, Packard, Ill., USA), 1 ml of 96% ethanol (Saarchem, Krugersdorp, South Africa) and 1−2 drops of phenolphthalein (Saarchem), as described previously [28]. Liquid scintillation cocktail (10 ml, Ready Gel, Beckman, Fullerton, Calif., USA) was then added to the solution and $^{14}CO_2$ specific activity (dpm/mmol) measured in an Insorb 460C Automatic Liquid Scintillation Counter (United Technologies). All $^{14}CO_2$ counts were corrected for the differences in quench and background counts.

Plasma glucose and insulin concentrations. Blood samples were obtained at rest and at 5, 25, 45, 65, 85, 105 min and 1 min prior to the termination of exercise. Approximately 10 ml of blood was drawn each time. Half the sample was placed into pre-chilled tubes containing lithium heparin, for subsequent glucose and insulin determinations, and the rest of the sample was added to tubes containing potassium oxalate and sodium fluoride. Both sets of samples were kept on ice until centrifuged at $2000 \times g$ for 10 min at 4°C upon completion of the trial. Supernatant (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 Analyser, Analox, London, UK). Plasma insulin concentrations were determined by radioimmunoassay (Phadeseph Insulin RIA package, Pharmacia, Uppsala, Sweden) according to the techniques of Goetz and Greenberg [16]. The intra-assay variation was less than 3.0%.

Plasma glucose and plasma lactate specific activity. Plasma samples (1 ml) for the separation of glucose and lactate were adjusted to a pH of 4.0 with 60 μl 2 M H_2PO_4, placed in sealed tubes and deproteinised by heating for 10 min at 70°C in a shaking water bath. After cooling on ice for a further 10 min, the seals of the tube were removed and the inside of the tube rinsed with 1 ml H_2O (pH raised to 8.0 by the addition of traces of NaOH) 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 40 μl 3 M K_2CO_3 before centrifugation at 5,000 rpm for 10 min. Following centrifugation, the supernatant was stored, the pellet resuspended in 0.75 ml H_2O, recentrifuged, and the supernatant added to that previously saved. This process of washing with 0.75 ml H_2O and recentrifuging was repeated a further three times.

Separation of glucose from lactate in the plasma samples was achieved by passing the combined supernatants through anion exchange columns containing Sephadex (Bakerbond SAX, Cape Town, South Africa) that had been conditioned with several void volumes of distilled H_2O, the pH of which had also been raised to 8.0 by the addition of a trace of NaOH. Glucose appeared in the void volume and was fully eluted with 3 ml H_2O. Lactate was subsequently eluted into a second vial with 2 ml 1 M $CaCl_2$ (adjusted to a pH 2.0 with HCl). Passage of the solutions through the exchange column was accelerated to 1 ml/min with a vacuum processor. The eluates were collected into scintillation vials and evaporated to near dryness at 70°C for 20 h in order to reduce the presence of ^3H_2O from the metabolism of 2-^{3}H glucose in the glycolytic pathway to less than 2% and to reduce the water/liquid scintillation cocktail ratio during subsequent counting. Each time plasma glucose and lactate samples were separated, a non-labelled plasma sample was 'spiked' with measured quantities of 2-^{3}H- and U-^{14}C-glucose and run simultaneously to enable correction of the experimental counts for the percentage recovery. Recoveries were greater than 90% for the U-^{14}C glucose samples and greater than 97% for 2-^{3}H-glucose samples. All counts were corrected for background and quench.

Plasma glucose oxidation. After the separation of plasma glucose and lactate, it was found that the plasma lactate counts were not significantly different from background counts. R_{ox}, therefore, did not need to be corrected for ^{14}C-lactate oxidation and were determined from the following formula:

$$R_{ox} = (SA_{CO_2}/SA_{glu}) \cdot \dot{V}CO_2 \quad (1)$$

where R_{ox} is the rate of plasma glucose oxidation (micromole per minute per kilogram FFM); $SACO_2$ is the specific activity of expired $^{14}CO_2$ (disintegration per minute per millimole); SA_{glu} is the corresponding specific activity of the plasma glucose; and $\dot{V}CO_2$ is the CO_2 production rate (micromole per minute per kilogram FFM) calculated from the $\dot{V}CO_2$, (litre per minute) the subject's FFM and the molar gas volume (22.4 l). Since the complete conversion of one molecule of U-^{14}C-glucose to six molecules of $^{14}CO_2$ decreases the *SA* by a factor of 6, the CO_2 does not need to be divided by 6 to allow for the six CO_2 molecules arising from the oxidation of one glucose molecule.

Exogenous glucose turnover. R_a and R_d were determined from the non-steady state equations of Steele [29] which have been validated by Radziuk et al. [23]:

$$R_a = [I-(p \cdot V \cdot Glu \cdot dSA/dt)]/SA \quad (2)$$

$$R_d = R_a-(p \cdot V \cdot dGlu/dt) \quad (3)$$

where R_a and R_d are the rate of liver (plus infused) glucose appearance and disappearance (millimole per minute per kilogram FFM); *I* is the 2-^{3}H glucose infusion rate in disintegrations per minute per kilogram FFM, *p* is the pool fraction, 0.75, recommended for near steady-state studies where changes in blood glucose concentration are relatively slow [21]; *V* is the glucose distribution volume (19.6% of body mass) in litre; *Glu* is the mean of successive plasma glucose concentrations in millimole per litre; d*SA*/d*t* is the change in plasma 2-^{3}H *SA* (disintegrations per minute per millimole) over the sample interval (minutes); *SA* is the average glucose *SA* in disintegrations per minute per millimole in successive samples; and d*Glu*/d*t* is the rate of change of glucose concentration (millimole per litre per minute).

Liver glucose output. Liver glucose production was estimated from the differences between the total glucose R_a data (described above) and the corresponding average glucose infusion rates over the first 25 and then over successive 20 min intervals until the completion of the ride.

Total carbohydrate oxidation. Overall CHO_{ox} (grams per minute) was calculated from the formula of Consolazio et al. [8], total

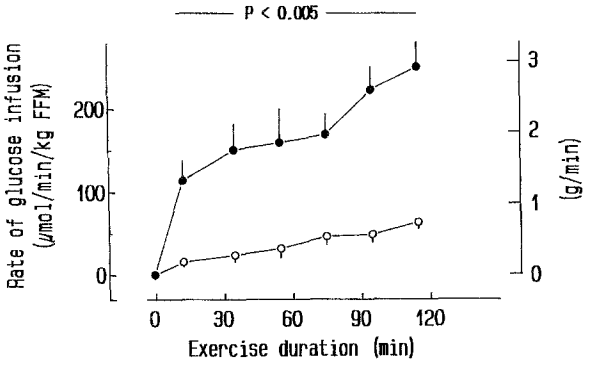

Fig. 1. The rate of intravenous infusion required to maintain plasma glucose concentration at 5 mM (euglycaemic clamp; *ET*, ○) and 10 mM (hyperglycaemic clamp; *HT*, ●) during 125 min cycling

CHO utilisation during exercise was estimated from the area under the CHO_{ox}/time curve for each subject. CHO_{ox} in grams per minute was converted to micromoles per minute per kilogram FFM by dividing by the molecular weight of glucose (180) in micrograms and the subject's FFM. Rates of fat oxidation in grams per minute were converted to micromoles per minute per kilogram FFM by dividing by the molecular weight of palmitate (237) in micrograms and the subject's FFM.

Statistical analyses. All results are expressed as means ± standard error of the mean (SEM) of a number ($n = 6$) of experiments. Statistical significance of changes over time was assessed by one-way analysis of variance (ANOVA) for repeated measures. Significance of differences between means with respect to time were tested by Schèffe's post-hoc test. Between-treatment means were compared using an unpaired *t*-test. A value of $P < 0.05$ was regarded as significant.

Results

Rates of glucose infusion

The rates of glucose infusion required to maintain plasma glucose at either 5 mM (ET) or 10 mM (HT) increased progressively during exercise (Fig. 1). For the first 25 min of the ET, the rate of glucose infusion averaged 15.8 ± 5.3 μmol/min/kg FFM and increased to 63.7 ± 7.6 μmol/min/kg FFM during the 105- to 125-min period ($P < 0.005$). In the HT the corresponding glucose infusion rates increased from 114.1 ± 23.9 μmol/min/kg FFM in the first 25 min of exercise to 251.5 ± 29.1 μmol/min/kg FFM in the last 20 min ($P < 0.005$). As expected, a significantly greater amount of glucose was infused over 125 min to maintain plasma glucose at 10 mM than at 5 mM (21.9 ± 2.9 versus 4.4 ± 0.8 mmol/kg FFM, $P < 0.005$).

Plasma glucose concentration

As a result of the increasing rate of glucose infusion (Fig.1), the plasma glucose concentration during the ET was maintained at a mean value of 4.8 ± 0.1 mM (range 4.4−5.3 mM) during the 125 min ride (Fig. 2). During

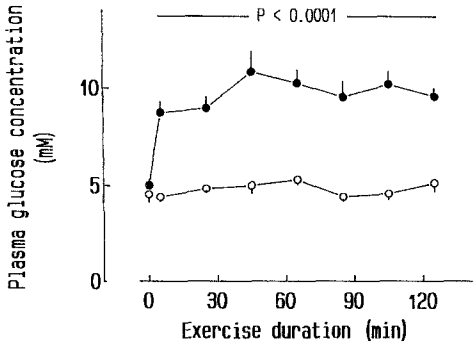

Fig. 2. Plasma glucose concentration during 125 min cycling under ET (○) and HT (●) conditions

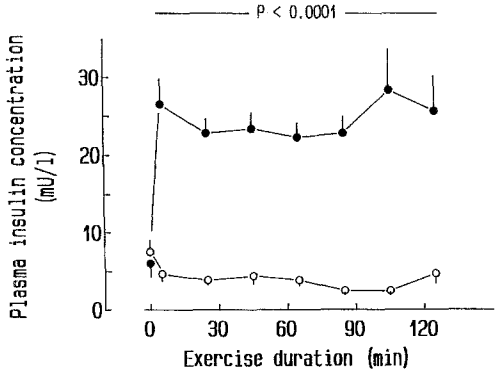

Fig. 3. Plasma insulin concentration during 125 min cycling under ET (○) and HT (●) conditions

the HT, plasma glucose concentration increased significantly from 5.0 ± 0.3 mM at rest to 8.7 ± 0.6 mM after 5 min exercise ($P < 0.05$; Fig. 2), and thereafter remained relatively constant at 9.7 ± 0.3 mM for the duration of the ride. As intended, differences in glucose concentration between the ET and HT were highly significant ($P < 0.0001$).

Plasma insulin concentrations

With the high rates of glucose infusion (Fig. 1) and the resultant elevation of plasma glucose concentration in the HT (Fig. 2), plasma insulin concentration in the HT was also increased (Fig. 3; $P < 0.0001$). Whereas in the ET plasma insulin concentration declined from 7.5 ± 1.4 to 4.8 ± 1.3 μU/ml at the end of exercise (Fig. 3), in the HT plasma insulin concentration rose from 5.9 ± 1.7 to 26.5 ± 3.3 μU/ml after 5 min and remained elevated thereafter ($P < 0.0001$).

Glucose turnover

During the ET liver glucose output (endogenous R_a), calculated from the difference between total R_a and the rate of glucose infusion, declined from 22.4 ± 4.9 μmol/min/kg FFM in the first 25 min of exercise to 6.5 ± 1.4 μmol/min/kg FFM in the last 20 min ($P < 0.05$). During the HT trial, R_a values were not significantly dif-

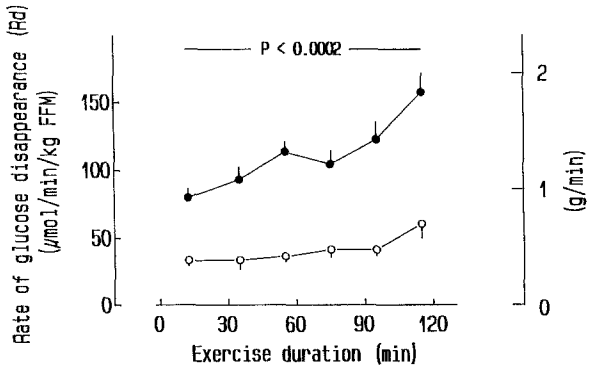

Fig. 4. The rate of glucose disappearance (R_d) during 125 min cycling under ET (○) or HT (●) conditions

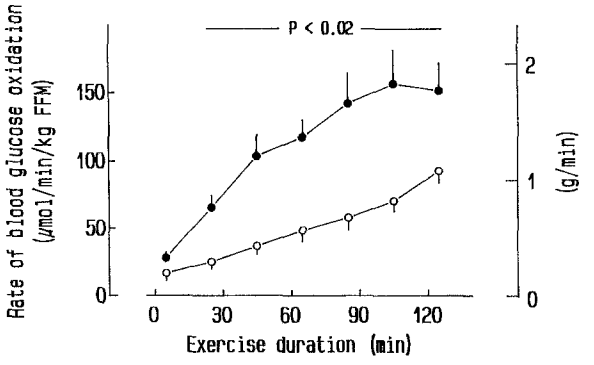

Fig. 5. The rate of plasma glucose oxidation (R_{ox}) during 125 min cycling under ET (○) and HT (●) conditions

ferent from zero, which suggests that endogenous R_a may have been completely suppressed by the high plasma glucose and insulin concentrations. In contrast, the mean R_d values were significantly higher throughout exercise in the HT than in the ET (111.9 ± 11.1 versus 45.7 ± 3.2 μmol/min/kg FFM; $P < 0.0002$, Fig. 4).

During the ET, R_d increased from 33.7 ± 3.8 μmol/min/kg FFM in the first 25 min of exercise to 60.8 ± 10.4 μmol/min/kg FFM in the last 20 min ($P < 0.05$; Fig. 4). Corresponding values measured during the HT were 79.8 ± 6.4 and 157.8 ± 14.4 μmol/min/kg FFM ($P < 0.005$ over time Fig. 4).

Rates of plasma glucose oxidation

Figure 5 shows R_{ox} as a function of time for the two experimental rides. During the ET, R_{ox} increased from 16.5 ± 6.1 μmol/min/kg FFM after 5 min to 92.3 ± 8.6 μmol/min/kg FFM at the end of the ride ($P < 0.0001$). During the HT the corresponding values were 30.2 ± 4.4 and 151.8 ± 20.8 μmol/min/kg FFM.

Total carbohydrate and fat oxidation

Tables 2 and 3 show the steady-state gas exchange data and the total calculated CHO_{ox} and fat oxidation rates during the two experimental rides. As the absolute metabolic demand of the slightly heavier subjects in the ET was greater than that of the subjects in the HT (average $\dot{V}O_2$ 3.55 ± 0.03 versus 3.20 ± 0.02 l/min, $P < 0.05$), the rates of CHO_{ox} and fat oxidation have been expressed relative to each subject's FFM (Table 3).

During the ET, the CHO_{ox} declined from 228.3 ± 27.4 μmol/min/kg FFM at the start of exercise to 178.8 ± 28.3 μmol/min/kg FFM at the end (Table 3). Associated with the fall in CHO_{ox} in the ET was a rise in the rate of fat oxidation from 45.1 ± 12.9 to 62.1 ± 11.4 μmol/min/kg FFM. In contrast, CHO_{ox} in the HT was maintained at an average of 310.1 ± 3.1 μmol/min/kg FFM and rates of fat oxidation only rose from 9.0 ± 2.9 FFM to 19.8 ± 1.7 μmol/min/kg FFM. The higher CHO_{ox} in the HT than the ET (Table 3) reduced the percentage contribution to total energy production from fat oxidation from between 35−55% in the ET to between 12−18% in the HT (Fig. 6). Instead, more energy was derived from plasma glucose oxidation in the HT. With hyperglycaemia, the percentage contribution to total energy production from plasma glucose oxidation increased from 8.2 ± 1.6% at the beginning of exercise to 40.8 ± 12.2% at the end ($P < 0.001$, Fig. 6). Corresponding values for the ET were 4.7 ± 1.7% versus 26.3 ± 3.1%, ($P < 0.001$, Fig. 6).

Areas under the total CHO_{ox} and plasma glucose oxidation versus time curves during the HT and ET are shown in Fig. 7. The total CHO_{ox} in the HT was significantly higher than in the ET (37.9 ± 2.3 versus 25.5 ± 3.5 mmol/kg FFM, $P < 0.05$). Total R_{ox} was also significantly higher in the HT than in the ET (13.3 ± 1.5 versus 5.8 ± 0.6 mmol/kg FFM, $P < 0.005$). On the other hand, muscle glycogen (plus lactate) oxidation, estimated from the difference between the total CHO_{ox} and total R_{ox} was not significantly different between the HT and ET (24.7 ± 2.1 versus 19.7 ± 4.0 mmol/kg FFM respectively, Fig. 7).

Glucose oxidation versus glucose infusion

Figure 8 compares the amounts of glucose infused to the amounts oxidised during the two experimental rides. Whereas in the ET the amount infused was similar to the total amount of plasma glucose oxidised (4.4 ± 0.8 versus 5.8 ± 0.6 mmol/kg FFM), during the HT the amount of glucose infused (21.9 ± 2.9 mmol/kg FFM) was significantly greater than the amount oxidised (13.3 ± 1.4 mmol/kg FFM, $P < 0.05$).

Discussion

The first finding of the present study was that when glucose was infused (Fig. 1) to elevate plasma glucose concentration to approximately 10 mM in the HT (Fig. 2), the rate of glucose infusion required to maintain plasma glucose concentration rose from 1.6 ± 0.2 g/min during the first 60 min of exercise to 2.9 ± 0.3 g/min during the last 20 min. These rates are almost identical to the 1.6 to 2.6 g/min infusion rates reported by Coyle et al.

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

	Time (min)						
	5	25	45	65	85	105	125
VO_2 (l/min)							
ET	3.50 ± 0.12	3.52 ± 0.13	3.51 ± 0.11	3.51 ± 0.13	3.53 ± 0.13	3.61 ± 0.13	3.70 ± 0.14
HT	3.11 ± 0.16	3.20 ± 0.13	3.17 ± 0.13	3.19 ± 0.10	3.21 ± 0.10	3.24 ± 0.12	3.31 ± 0.14
RER							
ET	0.89 ± 0.03	0.88 ± 0.03	0.87 ± 0.03	0.87 ± 0.02	0.86 ± 0.03	0.85 ± 0.03	0.84 ± 0.03
HT	0.97 ± 0.01 *	0.95 ± 0.01 *	0.95 ± 0.01 *	0.94 ± 0.01 *	0.94 ± 0.01 *	0.94 ± 0.01 *	0.94 ± 0.01 *

ET, euglycaemic clamp; HT, hyperglycaemic clamp; VO_2, oxygen consumption (l/min); RER, respiratory exchange ratio; all values are mean ± SEM. * HT significantly greater than ET, $P < 0.05$

Table 3. Carbohydrate and fat energy during two experimental rides

	Time (min)						
	5	25	45	65	85	105	125
CHO oxidation (g/min)							
ET	2.82 ± 0.33	2.72 ± 0.40	2.62 ± 0.39	2.52 ± 0.30	2.40 ± 0.36	2.28 ± 0.34	2.20 ± 0.33
HT	3.69 ± 0.28	3.59 ± 0.21	3.57 ± 0.28	3.46 ± 0.22	3.42 ± 0.23	3.48 ± 0.28	3.60 ± 0.23
CHO oxidation (μmol/min/kg FFM)							
ET	228.3 ± 27.4	220.1 ± 32.2	212.2 ± 31.5	204.2 ± 23.3	195.0 ± 30.7	185.3 ± 28.0	178.8 ± 28.3
HT	321.6 ± 24.3 *	314.2 ± 20.7 *	313.7 ± 27.1 *	303.2 ± 21.8 *	299.6 ± 22.2 *	302.9 ± 23.1 *	315.8 ± 23.2 *
Fat oxidation (g/min)							
ET	0.68 ± 0.18 #	0.73 ± 0.21	0.77 ± 0.19#	0.80 ± 0.16#	0.85 ± 0.19#	0.94 ± 0.18#	1.01 ± 0.18#
HT	0.18 ± 0.07	0.25 ± 0.06	0.25 ± 0.06	0.30 ± 0.06	0.32 ± 0.05	0.32 ± 0.07	0.30 ± 0.03
Fat oxidation (μmol/min/kg FFM)							
ET	45.1 ± 12.9#	44.7 ± 12.6	47.4 ± 11.8#	49.4 ± 10.4#	52.7 ± 12.3#	57.9 ± 11.6#	62.1 ± 11.4#
HT	9.0 ± 2.9	16.6 ± 4.0	14.3 ± 4.3	19.8 ± 4.1	21.6 ± 3.9	21.3 ± 5.3	19.8 ± 1.7

ET, euglycaemic clamp; HT, hyperglycaemic clamp; carbohydrate and fat oxidation calculated from gas exchange data; all values are mean ± SEM. * HT significantly greater than ET, $P < 0.05$. # ET significantly greater than HT, $P < 0.05$

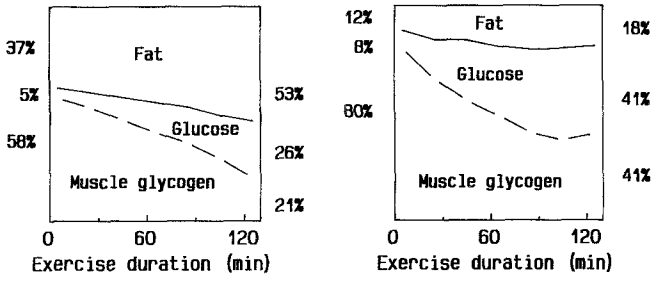

Fig. 6. The relative contribution of fuel substrates to total energy production during 125 min cycling under ET (*left hand panel*) and HT (*right-hand panel*) conditions. Rates of carbohydrate and fat oxidation were calculated from steady-state gas exchange data measured after the first 5 min of exercise and thereafter at successive 20-min intervals. Muscle glycogen (plus lactate) oxidation was estimated from the difference between the total carbohydrate oxidation and R_{ox} at the same time (—— = fat; - - - - = glucose)

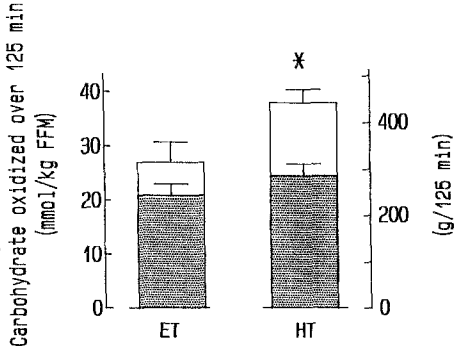

Fig. 7. Carbohydrate oxidation during 125 min cycling under ET and HT conditions. Muscle glycogen (plus lactate) oxidation (▨) has been estimated from the difference between total carbohydrate oxidation and R_{ox} (□). * Total carbohydrate oxidation significantly greater during HT than ET ($P < 0.05$)

[10] in their studies of hyperglycaemia in highly trained cyclists during 2 h intense exercise. Such high rates of infusion confirm the exceptional ability of well-trained subjects to dispose of large amounts of infused glucose. Associated with the elevation of plasma glucose concentration in the HT (Fig. 2), was a modest (24.5 ± 0.9 μU/ml) hyperinsulinaemia (Fig. 3) comparable to the 16–24 μU/ml plasma insulin concentrations reported by Coyle et. al. [10]. This combination of hyperglycaemia (Fig. 2) and hyperinsulinaemia (Fig. 3) totally suppressed endogenous liver glucose R_a throughout the ride

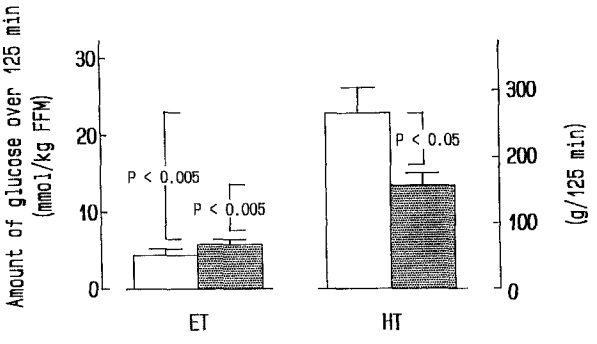

Fig. 8. Amounts of glucose infused (□) and oxidised (▩) during 125 min cycling under ET and HT conditions

and increased R_d (Fig. 4) and R_{ox} (Fig. 5) during the final 30 min of exercise to approximately 1.8 g/min.

It should be noted that during the 2nd h of exercise in both the HT and ET, R_d values were often less than estimated values of R_{ox}. The tracer-dilution technique has previously been reported to underestimate the true rates of R_d during euglycaemic clamps [3, 15, 26, 27, 32, 33]. Several possible explanations have been proposed for this paradox including recycling of the tracer [26, 27, 33], incorporation of the tracer into glycogen followed by release of labelled glucose during glycogenolysis [3], methodological error in the processing and or measurement of the specific activity of tritiated glucose [3] and, finally, inappropriate application of Steele's [29] steady-state calculations during non steady-state exercise [2, 3, 15, 23]. These assumptions and limitations do not invalidate the isotopic tracer method, but suggest that the contribution of plasma glucose oxidation to overall substrate metabolism during prolonged exercise may be underestimated in those investigations which have used the isotopic tracer approach [7].

In this investigation a 2-^{3}H label was used for the determination of glucose turnover because there is no recycling of this label [5] and little incorporation of the label into glycogen during exercise. As the presence of ^3H_2O from the metabolism of 2-^{3}H glucose in the glycolytic pathway was less than 2%, it is most unlikely that underestimates of glucose disposal could have been due to methodological processing of ^{3}H-glucose samples. Further, although there were rapid perturbations in the plasma 2-^{3}H *SA* during the early (i.e. 30–45 min) stages of the infusions, steady-state conditions were attained during the final 60–75 min of exercise when changes in plasma 2-^{3}H *SA* were small. Thus, the most likely explanation for the current underestimation of tracer-derived glucose disposal rates is inappropriate application of the one-compartment, fixed-pool volume model of glucose kinetics, as described by Steele [29]. Although others have previously proposed that a variable-pool volume approach might yield more accurate estimates of glucose turnover than the single-compartment model [15, 33], it is worth noting that the equations of Steele [29] have been validated and found to yield similar results when either single or multiple glucose pools are employed [23].

In the current study, the high values of R_{ox} in the HT maintained the total rates of CHO_{ox} at 3.4–3.7 g/min throughout the 2 h of exercise (Tables 2 and 3) and limited the percentage contribution to total energy production from fat oxidation to between 12 and 18% (Fig. 6). Since Coyle et al. [10] found that hyperglycaemia and hyperinsulinaemia suppressed the rise in plasma free fatty acid (FFA) concentrations during 2 h exercise when compared to a euglycaemic (4–5 mM glucose) control group, the low rates of fat oxidation may have been due to an insulin-induced acceleration of adipocyte triglyceride resynthesis. Low concentrations of circulating FFA, together with the high plasma glucose and insulin concentrations facilitate a high rate of glucose disposal by exercising muscle [25].

Despite the high (1.8 g/min) peak R_{ox} in the HT there was no sparing of muscle glycogen (Fig. 7). Muscle glycogen (plus lactate) oxidation, as estimated from the difference between the total CHO_{ox} and total R_{ox} was similar in the HT and in the ET (286 ± 25 versus 243 ± 50 g/125 min respectively). As has previously been found by Coyle et al. [10], the lack of glycogen sparing, together with the higher values of R_{ox} (Fig. 5) elevated the total CHO_{ox} by 40% from 325 ± 43 g/125 min in the ET trial to 441 ± 28 g/125 min in the HT trial (Fig. 7, $P < 0.05$).

Higher values of R_{ox} in the HT, however, did not account for all of the glucose infused (Fig. 8). In agreement with investigations that have measured both the rates of gastric emptying and oxidation of ingested CHO during prolonged exercise, and which have found that the amount of CHO oxidised is far less than the amount delivered to the intestine [19, 22, 24], there was a large disparity between the amount of glucose infused (255 ± 35 g/125 min) and the calculated amount oxidised (155 ± 17 g/125 min) in the HT (Fig. 8). Of the remaining approximately 100 g of infused glucose, about 10 g would be required to provide the 55 mmols of glucose needed to raise the plasma glucose concentration in the approximately 11 l glucose distribution volume (used in Steele's [29] equations) by 5 mM. The remaining 90 g was presumably incorporated into non-working muscle glycogen stores, as hepatic tissue retains very little glucose directly, even in the presence of supra-physiological concentrations of glucose and insulin [11]. Hyperinsulinaemia accelerates glucose transport and hexokinase activity and elevated intracellular glucose 6-P concentrations promote glycogen synthesis.

In contrast, under more physiological ET conditions, the rate of glucose infusion required to maintain plasma glucose at 5 mM was significantly less than that needed to maintain hyperglycaemia ($P < 0.005$, Fig. 1), such that during the last 20 min of the ET, the rate of glucose infusion (0.7 ± 0.1 g/min) was still lower than during the first 25 min of the HT (1.3 ± 0.3 g/min). The lower rate of glucose infusion in the ET than in the HT (Fig. 1) resulted in a modest hypoinsulinaemia (Fig. 3). By the end of the ride, plasma insulin concentration had declined from 7.5 ± 1.4 to 4.8 ± 1.3 μU/ml. The latter insulin concentrations were almost fivefold less than the corresponding values measured during the HT. Lower plasma glucose and insulin concentrations during the ET failed to suppress completely endogenous (liver) glucose

R_a. With the progressively increasing rate of glucose infusion (Fig . 1), endogenous R_a was gradually reduced from 0.27 ± 0.06 to 0.08 ± 0.01 g/min by the end of the 2-h ride. Glucose infusion during exercise is known to elevate plasma FFA concentration and inhibit hepatic glycogenolysis [14, 20] such that the exercise-induced R_a is usually precisely regulated to maintain euglycaemia [21, 23, 31].

Despite the progressive inhibition of liver glycogen-to-glucose conversion, the R_d (Fig. 4) and R_{ox} (Fig. 5) increased throughout the ET, although both rates were significantly lower than those measured in the HT. In agreement with previous arteriovenous balance and isotopic tracer studies of glucose metabolism during prolonged exercise, which show that the peak R_{ox} is 0.9–1.3 g/min [5, 6, 30], euglycaemic plasma glucose was only oxidised at rates of 1.1 ± 0.1 g/min after 2 h exercise (Fig. 5). Although the R_{ox} was still rising, these values closely match R_{ox} in glycogen-depleted subjects at the end of 3 h cycling at 70% $\dot{V}O_{2,max}$ [5]. Interestingly, the highest values of R_{ox} in euglycaemic exercising subjects [5, 6, 30] are similar to the maximum rates of oxidation from ingested CHO found in individuals consuming a variety of CHO solutions during prolonged exercise [18]. These rates are also comparable to the maximum reported rates of glucose absorption from the small bowel [1].

Without hyperglycaemia and hyperinsulinaemia to maintain high rates of CHO_{ox} throughout the 2-h exercise, there was a progressive reduction in CHO_{ox} during the ET (Tables 2 and 3). CHO_{ox} declined from 2.8 ± 0.3 g/min at the start of exercise to 2.2 ± 0.3 g/min at the end of the ET (Table 3). Associated with the lower CHO_{ox} throughout the ET (Fig. 7) was a significantly greater contribution to energy requirements from fat metabolism compared with the HT (Fig. 6). Fat oxidation increased from 0.7 ± 0.2 g/min at the start of exercise to 1.0 ± 0.2 g/min during the latter stages of the ride. Related to the higher rate of fat oxidation during the ET, was a significant reduction in the contribution to energy production from plasma glucose oxidation compared with the HT (14.2 ± 2.8% versus 29.9 ± 4.8% for ET and HT respectively, $P < 0.05$). Thus, unlike the HT, where the amount of plasma glucose oxidised was significantly less than the amount infused, the amount oxidised during the ET (72 ± 8 g/125 min) was not significantly different from the amount infused (55 ± 10 g/125 min, Fig. 8).

In summary, the results of the current study show that in well-trained subjects, hyperglycaemia (i. e. a plasma glucose concentration of 10 mM) and the associated hyperinsulinaemia (i. e. a plasma insulin concentration of 25 μU/ml), caused a complete suppression of endogenous R_a, maintained high rates of CHO_{ox} and inhibited fat oxidation throughout 2 h of intense exercise. Hyperglycaemia was also associated with an increasing R_d throughout the 2-h ride, such that during the latter stages of exercise, well-trained cyclists were capable of utilising plasma glucose at rates approaching Coyle et al.'s [9, 10] predicted values of approximately 2 g/min. Such a high R_{ox} confirms the exceptional capacity of skeletal muscle to oxidise exogenous glucose when hyperglycaemic. Whether such a high R_{ox} occurs under the physiological conditions typically encountered during prolonged exercise, however, remains to be verified experimentally. The results of the current investigation suggest that even when glucose is infused to bypass any possible limitation in the rates of digestion, absorption and liver glucose output, the oxidation of euglycaemic concentrations of glucose are restricted to approximately 1 g/min at the end of 2 h of exercise. It is hypothesised that glucose oxidation by skeletal muscle is precisely regulated by the prevailing plasma glucose concentration which, in turn, regulates hepatic glucose uptake and release, at least in the current experimental model. The practical implication of this postulate is that it is the physiological concentration of plasma glucose normally present during prolonged, intense exercise which may ultimately limit the rate of muscle glucose uptake and its subsequent oxidation.

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