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Exogenous carbohydrate oxidation during ultraendurance exercise

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Jeukendrup, Asker E., Luke Moseley, Gareth I. Mainwaring, Spencer Samuels, Samuel Perry, and Christopher H. Mann. Exogenous carbohydrate oxidation during ultraendurance exercise. *J Appl Physiol* 100: 1134–1141, 2006. First published December 1, 2005; doi:10.1152/jappphysiol.00981.2004.—The purposes of this study were: 1) to obtain a measure of exogenous carbohydrate (CHO_{Exo}) oxidation and plasma glucose kinetics during 5 h of exercise; and 2) to compare CHO_{Exo} following the ingestion of a glucose solution (Glu) or a glucose + fructose solution (2:1 ratio, Glu+Fru) during ultraendurance exercise. Eight well-trained subjects exercised three times for 5 h at 58% maximum O₂ consumption while ingesting either Glu or Glu+Fru (both delivering 1.5 g/min CHO) or water. The CHO used had a naturally high ¹³C enrichment, and five subjects received a primed continuous intravenous [6,6-²H₂]glucose infusion. CHO_{Exo} rates following the ingestion of Glu leveled off after 120 min and peaked at 1.24 ± 0.04 g/min. The ingestion of Glu+Fru resulted in a significantly higher peak rate of CHO_{Exo} (1.40 ± 0.08 g/min), a faster rate of increase in CHO_{Exo}, and an increase in the percentage of CHO_{Exo} oxidized (65–77%). However, the rate of appearance and disappearance of Glu continued to increase during exercise, with no differences between trials. These data suggest an important role for gluconeogenesis during the later stages of exercise. Following the ingestion of Glu+Fru, cadence (rpm) was maintained, and the perception of stomach fullness was reduced relative to Glu. The ingestion of Glu+Fru increases CHO_{Exo} compared with the ingestion of Glu alone, potentially through the oxidation of CHO_{Exo} in the liver or through the conversion to, and oxidation of, lactate.

stable isotopes; carbohydrate absorption; glucose; fructose; metabolism

IT IS GENERALLY ACCEPTED THAT carbohydrate (CHO) feedings have the potential to improve exercise performance when exercise duration is greater than ~45 min (8, 20, 23). The mechanisms for this performance enhancement are thought to be via the maintenance of plasma glucose (Glu) concentrations and the high rates of CHO oxidation in the latter stages of exercise (6, 8). Studies indicate that the exogenous CHO (CHO_{Exo}) oxidation from a single CHO source can contribute up to 1.0–1.1 g/min to total CHO oxidation (for review, see Ref. 23). However, most studies have been 2–2.5 h in duration, and, even though oxidation rates generally seem to level off after 90–120 min, it has recently been suggested that CHO_{Exo} oxidation can still continue to rise after that.

Coyle et al. (8) reported data from subjects who rode to fatigue at 71% maximum O₂ uptake ($\dot{V}O_{2\max}$) (242 ± 20 min) while ingesting CHO at a relatively high rate (~1.8 g/min). In the fourth and final hour of exercise, subjects were able to maintain high rates of CHO oxidation, despite already depleted

muscle glycogen stores. It is often assumed that hepatic Glu production is unlikely to have significantly contributed to plasma Glu oxidation, since the exercise was preceded by a 16-h fast (31), and Glu ingestion has been observed to suppress hepatic Glu production during exercise (25, 28). This suggests that the high rates of CHO oxidation observed (~2 g/min) could only have been maintained via the oxidation of CHO_{Exo}. Estimates of hepatic Glu production during ultraendurance exercise when CHO was fed would tend to support this (3). Plasma Glu kinetics during very prolonged exercise were examined recently by Angus et al. (3). In this study, an infusion of [²H]glucose was combined with the ingestion of [³H]glucose. Subjects ingested CHO at a mean rate of 1.3 g/min and exercised to fatigue at an intensity of ~68% peak O₂ uptake ($\dot{V}O_2$) (232 ± 14 min). CHO_{Exo} was estimated by assuming that 95% of the Glu rate of disappearance (R_d) from the circulation was oxidized (24). Under these circumstances, R_d rose almost linearly and showed no sign of leveling off, and peak CHO_{Exo} was estimated to be 1.36 ± 0.08 g/min. Thus there are suggestions that CHO_{Exo} can rise above the 1.0–1.1 g/min during ultraendurance exercise, which is generally believed to be the maximum oxidation rate. If this is true, it could have major implications for the recommendations to ultraendurance athletes. However, it must be noted that CHO_{Exo} oxidation was only estimated in these studies, and no studies have directly measured CHO_{Exo} oxidation in these conditions. Therefore, the first aim of this study is to accurately quantify CHO_{Exo} and Glu kinetics during 5 h of exercise. We hypothesize that CHO_{Exo} will level off after 90–120 min, as observed in previous studies, and will not increase linearly, as suggested by Angus et al. (3).

Recent studies have demonstrated that combining Glu and fructose (Fru) (Glu+Fru) can result in higher CHO_{Exo} rates (40), potentially via the utilization of more than one transport mechanism for intestinal absorption (Glu = sodium glucose cotransporter-1, Fru = GLUT5), causing less competition for transport (for review, see Ref. 21). The findings of Adopo et al. (1) and Jentjens et al. (16–18, 45) reported increased CHO_{Exo} when Glu and Fru are ingested together compared with the ingestion of a single CHO. The oxidation of multiple transportable CHO has only been studied for up to 2.5 h of exercise, and, therefore, the second aim of this study was to investigate the oxidation of a Glu+Fru mixture compared with Glu using a tracer infusion of [²H]glucose to examine Glu kinetics. We hypothesize that the higher CHO_{Exo} oxidation with Glu+Fru will persist even during 5 h of exercise.

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METHODS

Subjects. Eight endurance-trained men volunteered to participate in this study, seven of whom had Ironman distance triathlon personal best times of <10 h 30 min, with the remaining subject holding an elite road racing license (National level). Subjects' mean characteristics are shown in Table 1. The study was approved by the local ethics committee, and all subjects signed a consent form after reading the information and after the procedure was explained to them. All subjects were healthy, as assessed by a general health questionnaire.

General design. Subjects visited the laboratory on a total of four occasions (an incremental test to exhaustion and three experimental conditions). The independent variable was the composition of the beverage given during exercise, which was blinded to the subjects. Each experimental condition consisted of a 5-h steady-state exercise bout performed on a cycle ergometer. Beverages contained either Glu only, Glu+Fru in a 2:1 ratio, or no CHO (water). Five subjects received a primed continuous infusion of [6,6-²H]glucose for the determination of Glu rates of appearance (R_a) and R_d . Blood samples were collected during exercise, which were later analyzed for free fatty acid (FFA), triglyceride (TG), glycerol, lactate, Glu concentrations, and [²H]glucose enrichments. Samples of expired air were collected during exercise for later determination of the ¹³C enrichment.

Preliminary testing. Before the start of the experiments, the subjects were familiarized with the equipment and the procedures. One week before the start of the experiment, subjects were asked to perform a graded exercise test to exhaustion on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). Subjects reported to the laboratory after a 10–12 h overnight fast, and body mass and height were determined. Subjects started cycling at 95 W, and the work rate was increased by 35 W every 3 min until exhaustion. Heart rate (HR) was recorded continuously during the test using radio telemetry (Polar Vantage, Polar Electro, Kempele, Finland). Breath-by-breath measurements were performed throughout exercise using an online automated gas analysis system (Oxycon Pro, Jaeger, Wuerzberg, Germany). The volume and gas analyzers of the system were calibrated using a 3-liter calibration pump and calibration gas (4.95% CO₂–balance N₂). Maximal work rate was calculated from the last completed work rate, plus the fraction of time spent in the final noncompleted work rate, multiplied by the work rate increment. $\dot{V}O_2$ was considered to be maximal when at least two of the following criteria were met: 1) a leveling off of $\dot{V}O_2$ with increasing work rate (increase of no more than 2 ml·kg⁻¹·min⁻¹), 2) a HR within 10 beats/min of the predicted maximum (220 beats/min minus age), and 3) a respiratory exchange ratio > 1.05. $\dot{V}O_{2\max}$ was calculated as the average $\dot{V}O_2$ over the last 60 s of the test.

Subjects were asked to refrain from strenuous exercise and record and repeat their diets on the day before the experimental trials. Due to the relatively high natural occurrence of [¹³C]glucose in C4 plants (33), subjects were asked to undertake an exhaustive exercise bout 5 days before each trial to oxidize any ¹³C-enriched glycogen and to avoid foodstuffs with a naturally high ¹³C abundance for the subsequent 5 days. Subjects were given guidance to do this (43). Additionally, subjects were asked to refrain from strenuous exercise and the use of alcohol for 24 h before trials.

Experimental trials. Subjects arrived in the laboratory after an overnight fast of at least 10 h. Teflon catheters (Quickcath, Baxter, Norfolk, UK) were inserted into antecubital veins in each elbow and kept patent with isotonic saline (Becton Dickinson, Drogheda, UK) containing 1 IU/l heparin (CP Pharmaceuticals, Wrexham, UK). Resting blood and expired gas samples were collected immediately before subjects commenced exercising at 50% of their previously determined maximal work rate (~58% $\dot{V}O_{2\max}$). At the onset of exercise, a [6,6-²H]glucose prime was given (31.5 μM/kg). Thereafter, a continuous sterile, pyrogen-free infusion of [6,6-²H]glucose (Isotech, Miamisburg, OH) dissolved in isotonic saline was administered at a rate of ~0.7 μM·kg⁻¹·min⁻¹. The calibration of the infusion pumps (Asena GS, Alaris Medical Systems, Basingstoke, UK) was checked before and after use. Blood and breath samples were taken every 20 min, along with measures of $\dot{V}O_2$ and CO₂ production ($\dot{V}CO_2$), using an online automated gas analysis system (Oxycon Pro, Jaeger). In addition, measures of gastrointestinal “fullness” were taken using a 10-point scale, ranging from 1 (“not full at all”) to 10 (“the fullest I’ve ever been”) (4), while ratings of perceived exertion (RPE) were taken using the 6–20 point scale of Borg (5). HR was continuously recorded at 15-s intervals and averaged over each hour.

Beverage ingestion. At the onset of exercise, subjects consumed an initial bolus of 600 ml followed by 270 ml at 20-min intervals throughout the trial. The CHO beverages delivered 1.5 g/min CHO and consisted of either 100% Glu (Amylum, London, UK) or Glu+Fru (Krystar 300, A.E. Staley Manufacturing, Decatur, IL) in a 2:1 ratio (Glu+Fru). Both CHO beverages had a naturally high abundance of ¹³C [Glu = -10.49 ‰ vs. Pee Dee Bellemnitella (PDB), Glu+Fru = -10.21 ‰ vs. PDB] and had osmolalities of 665 ± 3 (Glu) and 659 ± 3 (Glu+Fru) mosmol/kgH₂O. The water beverage had an osmolality of 11 ± 1 mosmol/kgH₂O. All three drinks contained 30 mmol/l of sodium in the form of sodium citrate.

Blood analysis. Blood samples were collected in chilled evacuated tubes containing 200 μl 0.2 M K₃ EDTA (Vacutainer, Becton Dickinson). These were then centrifuged at 3,500 rpm (2,300 g) for 5 min at a temperature of 4°C. The plasma was then removed, immediately frozen in liquid nitrogen, and stored at -70°C until analysis. Plasma lactate, Glu, glycerol, TG, and FFA concentrations were determined enzymatically [Glu liquid reagent, lactate reagent, and TG (GPO-Trinder); Sigma Diagnostics, Dorset, UK; NEFA C, Waco Chemicals USA, Richmond, VA]. All analyses were performed on a semiautomatic analyzer (COBAS Bio, Roche, Switzerland). For determination of plasma Glu enrichment, plasma samples were deproteinized with 2 ml methanol-chloroform (2.3:1), 2 ml chloroform, and water with pH 2.0. The samples were then derivatized with 250 μl of butyl boric acid/pyridine (1 mg/10 ml) and 250 μl acetic anhydride. The enrichment was subsequently measured by means of GC-MS. Briefly, 1 μl of the derivative was injected on an Agilent 6890N gas chromatograph equipped with a split/splitless injector and 7683 autosampler (Agilent). Mass spectra were obtained by using an Agilent 5973N mass-selective detector. Data were acquired using selected ion monitoring for masses of 297 and 299 mass-to-charge ratio.

Breath analysis. Breath samples were collected in 10-ml evacuated tubes (Exetainers, Labco, Buckinghamshire, UK) from a 6-liter mixing chamber. Samples were subsequently analyzed for ¹³C/¹²C ratio by continuous-flow isotope ratio mass spectrometry (Europa Scientific, Crewe, UK). The contents of samples and references were flushed and transported by helium carrier gas through a packed column gas chromatograph, held at 75°C. The resultant chromatographic peak then entered the isotope ratio mass spectrometry, where the isotopomers at mass-to-charge ratio of 44, 45, and 46 for CO₂ were measured, and a δ ¹³C value was determined. The reference gas used during analysis was 3.3% CO₂ in a helium balance with δ ¹³C = -29.01 ‰ vs. PDB. The 3.3% CO₂ mixture was prepared from a CO₂ cylinder calibrated against NBS-19 (δ ¹³C value of +1.95 ‰ vs. PDB), an isotope reference standard distributed by the International Atomic Energy Agency, Vienna.

Table 1. Mean subject characteristics

	Age, yr	Height, m	Body Weight, kg	\dot{W}_{\max} , W	$\dot{V}O_{2\max}$, l/min	$\dot{V}O_{2\max}/kg$, ml·kg ⁻¹ ·min ⁻¹
Mean	30	1.78	75.3	367	4.69	62.7
SE	4	0.02	3.2	6	0.16	2.3

$n = 8$. \dot{W}_{\max} , peak power output; $\dot{V}O_{2\max}$, maximum O₂ uptake.

Using indirect calorimetry ($\dot{V}O_2$, $\dot{V}CO_2$) and breath enrichment ($^{12}CO_2/^{13}CO_2$ ratio in expired gas), total fat, total CHO, CHO_{Exo} , and endogenous CHO oxidation rates were calculated.

Calculations. From measures of $\dot{V}CO_2$ and $\dot{V}O_2$, total CHO and fat oxidation rates were calculated using the formulas of Frayn (12), assuming negligible protein oxidation:

$$\text{Total fat oxidation (g/min)} = 1.67 \cdot \dot{V}O_2 - 1.67 \cdot \dot{V}CO_2$$

$$\text{Total CHO oxidation (g/min)} = 4.55 \cdot \dot{V}CO_2 - 3.21 \cdot \dot{V}O_2$$

The isotopic enrichment was expressed as δ per milliliter difference between the $^{12}C/^{13}C$ ratio of the sample and a known laboratory reference standard, according to the formula of Craig (9):

$$\delta^{13}C = \left[\left(\frac{^{12}C/^{13}C \text{ sample}}{^{12}C/^{13}C \text{ standard}} \right) - 1 \right] \times 10^3\text{‰}$$

The $\delta^{13}C$ was then related to an international standard PDB.

Glu CHO_{Exo} was calculated using the formula:

$$CHO_{Exo} = \dot{V}CO_2 \cdot \left(\frac{\delta Exp - Exp_{bkg}}{\delta Ing - Exp_{bkg}} \right) \cdot \left(\frac{1}{k} \right)$$

where δExp is ^{13}C enrichment of expired air during exercise, δIng is ^{13}C enrichment of the ingested beverage, Exp_{bkg} is ^{13}C enrichment of expired air in the water trial (background), and k is 0.7467 [volume of CO_2 (liters) produced by the oxidation of 1 g of Glu].

A methodological consideration when using $^{13}CO_2$ in expired air to calculate CHO_{Exo} is the trapping of $^{13}CO_2$ in the bicarbonate pool, where a proportion of the CO_2 arising from the oxidation of Glu is temporarily trapped each minute (35). However, during exercise, $\dot{V}CO_2$ increases severalfold, so that a physiological steady-state condition will occur, and $^{13}CO_2$ in the expired air will be equilibrated with the CO_2/H_2CO_3 pool. Under exercising conditions, recovery of $^{13}CO_2$ from oxidation of [^{13}C]glucose will approach 100% after 60 min when dilution in the bicarbonate pool becomes negligible (32). Values for CHO_{Exo} are, therefore, only valid after 60 min.

The total R_a and R_d of Glu in the systemic circulation were calculated using the single pool non-steady-state equations of Steele (42), adapted for use with stable isotopes (46). R_a represents the sum of Glu appearing from hepatic glycogenolysis, gluconeogenesis, and Glu absorbed from the gut.

$$R_a = \frac{F - V[(C_2 + C_1/2)][(E_2 - E_1)/(t_2 - t_1)]}{(E_2 + E_1)/2}$$

$$R_d = R_a - V \left(\frac{C_2 - C_1}{t_2 - t_1} \right)$$

where F is the infusion rate ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$); V is volume of distribution (160 ml/kg); C_1 and C_2 are plasma Glu concentrations (mmol/l) at time points t_1 and t_2 , respectively; and E_1 and E_2 are plasma Glu enrichments (%) at time points t_1 and t_2 , respectively.

Statistical analysis. Data for fullness, RPE, HR, and cadence were averaged for each hour. Data were checked to ensure that parametric assumptions were met, and a two-way (time \times trial) ANOVA for repeated measures was applied. All data were checked for sphericity, and, where necessary, a Huynh-Feldt correction was used. In all cases, a Tukey honestly significant difference test was applied in the event of a significant F -ratio. Data analysis was performed using SPSS 10.0 for Windows software (Chicago, IL) or by hand. Statistical significance was set at $P < 0.05$.

RESULTS

Substrate oxidation and Glu kinetics. Ingestion of water resulted in significant increases in fat oxidation and decreases in total CHO oxidation over time, changes that did not occur in

Glu and Glu+Fru (Fig. 1). The ingestion of both CHO beverages resulted in a rapid and significant rise in $\delta^{13}CO_2$ [Fig. 2, main effect of time; $F(15,105) = 145.9$], while there were no significant changes in $^{13}CO_2$ production following the ingestion of water (grand mean = -25.4 ± 0.1 ^{13}C $\delta\text{‰}$ vs. PBD). The breath $^{13}CO_2$ enrichment leveled off after 100 min in Glu and after 80 min in Glu+Fru. Despite exhibiting similar excretion patterns, breath $\delta^{13}CO_2$ enrichment was significantly different between CHO trials at 20–140, 220, 260, and 300 min [significant interaction effect $F(15,105) = 4.5$]. These differences in $\delta^{13}CO_2$ enrichment were reflected in CHO_{Exo} rates [Fig. 3; significant interaction effect $F(15,105) = 2.8$]; Glu+Fru leveled off earlier (100 vs. 120 min, $P < 0.05$), had greater CHO_{Exo} at 20–120, 220, and 300 min ($P < 0.05$), and exhibited a greater mean CHO_{Exo} once the oxidation rate leveled off (Glu = 1.32 ± 0.04 g/min, Glu+Fru = 1.49 ± 0.06 g/min). These differences resulted in a significant difference between the estimated total amounts of CHO_{Exo} oxidized during the exercise period. A minimum of 293 ± 9 g was oxidized in Glu, whereas a minimum of 346 ± 9 g was oxidized in Glu+Fru. This represents 65 and 77% of the ingested CHO, respectively.

The ingestion of CHO resulted in an increase in the R_a of Glu in the circulation [Fig. 4; significant interaction effect $F(16,64) = 7.7$], with significant main effects of time [$F(8,32) = 29.2$] and trial [$F(2,8) = 17.9$]. The pattern of the

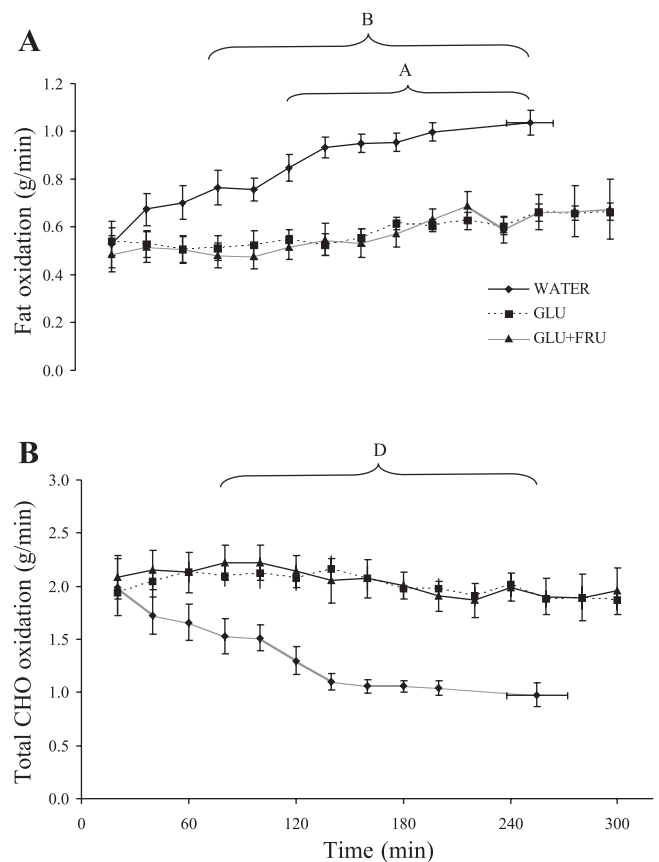


Fig. 1. Total fat (A) and carbohydrate (CHO) oxidation (g/min) (B). Values are means \pm SE. ^A Fat oxidation significantly greater than at 20 and 40 min; ^B fat oxidation in water significantly greater than in either glucose (Glu) or Glu + fructose (Fru); ^D total CHO oxidation in water significantly lower than either Glu or Glu+Fru: $P < 0.05$.

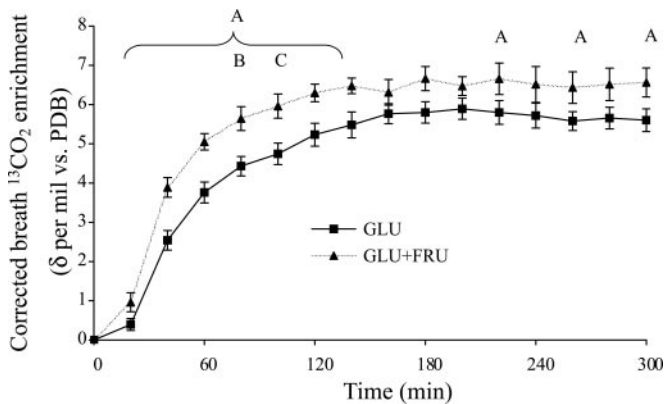


Fig. 2. Increase in corrected breath $^{13}\text{CO}_2$ enrichment [$\delta\%$ vs. Pee Dee Bellemnitella (PDB)] during exercise. Values are means \pm SE, $n = 8$. ^A Significant difference between Glu+FrU and Glu, $P < 0.05$. ^B No further increase in Glu+FrU; ^C no further increase in Glu.

increase over time was similar in both CHO trials, linear in nature with a peak at 300 min of exercise (Glu = 144 ± 18 and Glu+FrU = $137 \pm 30 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). The pattern of Glu R_a was different in water, with no increase over time and R_a at exhaustion lower than either of the CHO trials ($46 \pm 6 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). This pattern was repeated in R_d , with the ingestion of CHO resulting in a linear increase in R_d , with a maximum occurring at 300 min (144 ± 18 and $137 \pm 30 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for Glu and Glu+FrU, respectively).

Plasma measures. The ingestion of CHO resulted in a transient but significant increase in plasma Glu concentration (Fig. 5), with significant main effects of time [$F(15,30) = 12.0$] and trial [$F(2,4) = 18.3$] and a significant interaction effect [$F(30,60) = 4.1$]. At 20 min, plasma Glu concentrations in Glu and Glu+FrU were 6.20 ± 0.34 and 6.04 ± 0.11 mmol/l, respectively, compared with 4.70 ± 0.06 mmol/l in water. The ingestion of water resulted in a steady decline in plasma Glu concentration, with the nadir occurring at fatigue (3.44 ± 0.14 mmol/l). Both CHO beverages were equally as effective at maintaining plasma Glu concentrations.

Plasma lactate at rest was similar in all trials (0.83 ± 0.09 mmol/l), with significant main effects of time [$F(15,30) = 7.972$] and trial [$F(2,4) = 5.511$] and a significant interaction

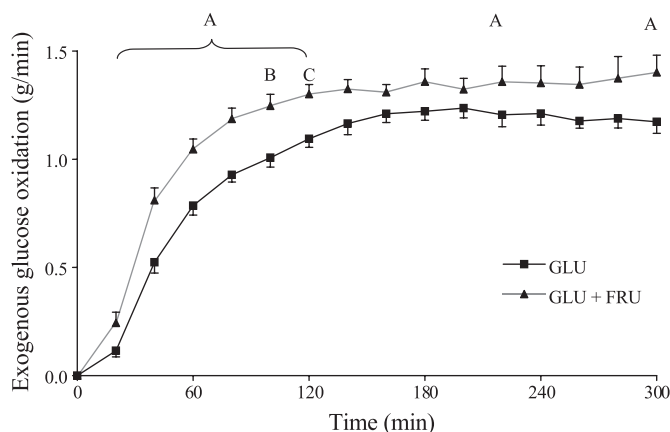


Fig. 3. Exogenous CHO oxidation (g/min). ^A Exogenous CHO oxidation in Glu+FrU significantly greater than in Glu trial, $P < 0.05$. ^B No further increase over time in Glu+FrU; ^C no further increase over time in Glu.

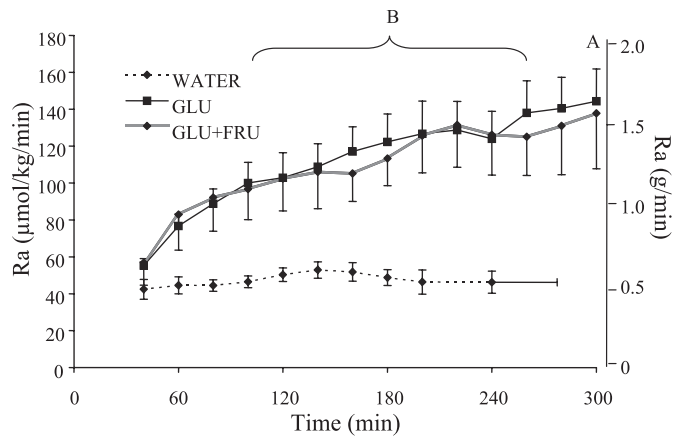


Fig. 4. Glu rate of appearance (R_a ; $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Values are means \pm SE; $n = 5$. ^A Both Glu+FrU and Glu significantly greater than time = 40–120 min; ^B Glu+FrU and Glu significantly greater than water: $P < 0.05$.

effect [$F(30,60) = 3.354$]. While there was no change over time in the Glu trial, the ingestion of Glu+FrU caused a transient elevation in plasma lactate, such that it was higher than Glu at 40 min (1.84 ± 0.16 vs. 1.27 ± 0.09 mmol/l) and higher than in water at 20, 40, and 80 min (Fig. 6). In comparison, plasma lactate concentration rose gradually over time in water, with lactate concentration at exhaustion greater than at 0 min (1.53 ± 0.13 vs. 0.81 ± 0.09 mmol/l).

The ingestion of water resulted in an increase in plasma free glycerol concentration [significant interaction effect, $F(16,112) = 39.6$] to levels significantly higher than those occurring in Glu and Glu+FrU (0.47 ± 0.03 , 0.18 ± 0.01 , and 0.17 ± 0.03 mmol/l at exhaustion for water, Glu, and Glu+FrU, respectively). Plasma FFA concentrations showed a similar pattern [significant interaction effect, $F(16,112) = 23.4$], rising over time in water (from 241 ± 70 to $1,571 \pm 133 \mu\text{mol/l}$) to be significantly greater than both Glu and Glu+FrU during the latter stages of exercise ($1,571 \pm 133$, 655 ± 55 , and $583 \pm 73 \mu\text{mol/l}$, respectively, at exhaustion). There was no effect of drink composition on plasma free TG concentrations. Plasma FFA, free glycerol, and free TG concentrations are shown in Fig. 7.

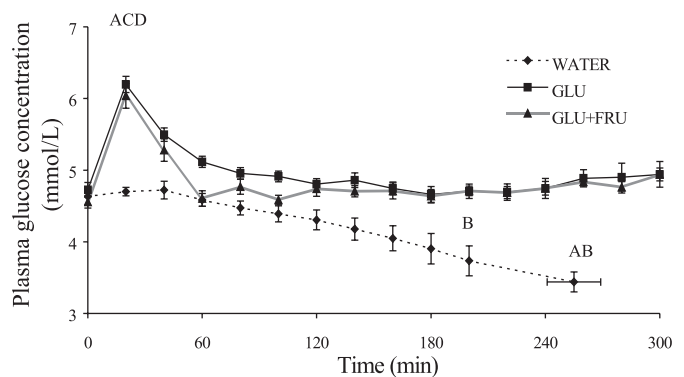


Fig. 5. Plasma Glu concentration (mmol/l) during exercise. Values are means \pm SE; $n = 8$. ^A Glu and Glu+FrU significantly greater than water; ^B water significantly lower than at time = 0, 20, 40, and 60 min; ^C Glu significantly greater than at all other time points; ^D Glu+FrU significantly greater than at time = 0, 60–300 min: $P < 0.05$.

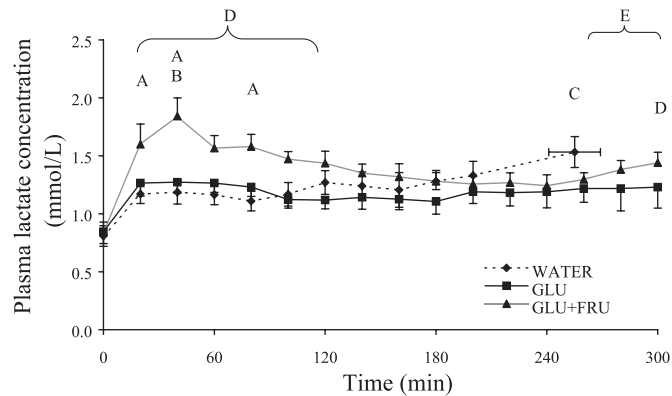


Fig. 6. Plasma lactate concentration (mmol/l). ^AFru significantly different from water; ^BFru significantly greater than Glu; ^Cwater significantly greater than at time = 0; ^DFru significantly greater than at time = 0; ^E $P < 0.05$.

Additional data. HR rose over time in all trials but was lower in water than in both CHO trials in the first hour (115 ± 3 vs. 125 ± 3 and 124 ± 3 beats/min, respectively) and lower than Glu in the third hour (123 ± 3 vs. 133 ± 3 beats/min). During the final hour of exercise, HR in water, Glu, and Glu+Fru was 132 ± 3 , 138 ± 3 , and 134 ± 3 beats/min, respectively. Only with the ingestion of Glu+Fru were subjects able to maintain cadence (92 ± 3 rpm in the first hour and 87 ± 2 rpm in the final hour); a fall over time occurred in both water and Glu (from 88 ± 2 and 89 ± 2 to 81 ± 3 and 78 ± 2 rpm, respectively).

There was a gradual and significant rise in RPE over time in all trials, and, while there were no significant differences between trials, there was a trend in the final hour of exercise for RPE to be lower following the ingestion of Glu+Fru (13.1 ± 0.7) compared with water (14.2 ± 0.7) or Glu (14.2 ± 0.8). The ingestion of Glu had a marked effect of reported fullness (Fig. 8). There was a significant increase in ratings of perceived fullness in Glu over time (first hour = 4.0 ± 0.6 , final hour = 5.7 ± 0.5). In the final hour of exercise, this increase resulted in a difference between Glu and both water and Glu+Fru.

Changes in body mass were similar in all trials (-0.2 ± 0.1 , -0.3 ± 0.4 , and -0.6 ± 0.3 kg for water, Glu, and Glu+Fru, respectively).

DISCUSSION

In this study, we observed that the ingestion of CHO at an average rate of 1.5 g/min of Glu during 300 min of exercise resulted in a leveling off of CHO_{Exo} after 120 min, with a peak rate of 1.24 ± 0.04 g/min. The observation of a leveling off of CHO_{Exo} rate is important for the interpretation of this data. Angus et al. (3) reported similar absolute peak values during ~4 h of exercise, but they suggested that CHO_{Exo} rate increased linearly over time, a suggestion based on the linear increase in R_d and the assumption that 95% of R_d was oxidized. The R_d data presented here agree with that presented by Angus et al. (3), both in terms of the gradual linear rise in R_d continuing to the cessation of exercise and in the R_d values at ~240 min [$118 \pm 7 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (1.59 ± 0.09 g/min) vs. $144 \pm 20 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (1.63 ± 0.19 g/min)]. However, in the present study, the use of the ^{13}C tracer technique allowed direct measurement of CHO_{Exo} , and the pattern observed did not match the pattern of R_d . There was a rapid rise

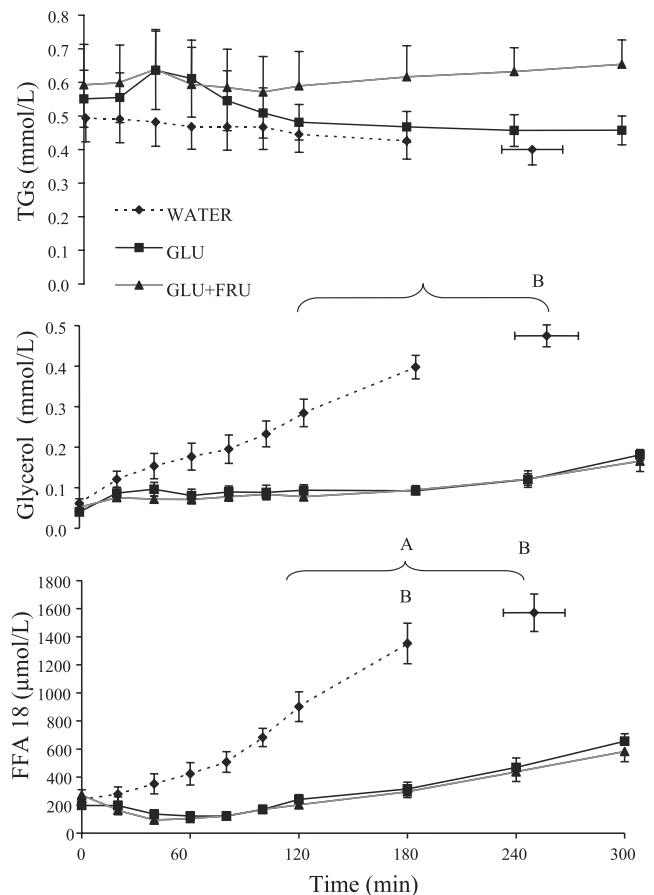


Fig. 7. Circulating free triglyceride (TG), free glycerol, and free fatty acid (FFA) concentrations. Values are means \pm SE. ^AWater significantly greater than Glu and Glu+Fru; ^Bwater significantly greater than at time = 0; $P < 0.05$.

in the first hour of exercise followed by a leveling off after 120 min, similar to that seen previously (19, 22, 26, 38). The rate of rise may even be underestimated due to some trapping of $^{13}\text{CO}_2$ in the bicarbonate pool; nevertheless, these data confirm previous reports that CHO_{Exo} rates do not continue to increase after 120 min of exercise. The fact that R_d continues to increase and CHO_{Exo} leveled off implies that there is an increase in hepatic Glu output during the later stages of exercise (>3 h). During the last hour, R_d Glu is as high as 1.6–2.2 g/min,

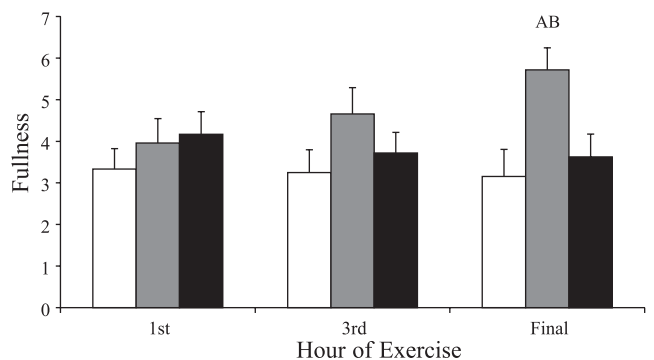


Fig. 8. Mean perceived stomach fullness in the first, third, and final hours of exercise \pm SE; $n = 8$. Open bars, water; shaded bars, Glu; solid bars, Glu+Fru. ^ASignificantly greater than water and Glu+Fru; ^Bsignificantly greater than first hour of exercise; $P < 0.05$.

whereas CHO_{Exo} plateaued at 1.2–1.4 g/min. This suggests that hepatic Glu production contributed ~ 0.4 – 0.8 g/min during the last hour of exercise. Since, after an overnight fast, liver glycogen may have become nearly depleted, it is likely that the source of this Glu is gluconeogenesis (2). With the increase in gluconeogenic substrate availability (especially lactate), it is not unlikely that gluconeogenesis did occur in this situation. It was recently demonstrated that elevating plasma lactate concentration during low-to-moderate-intensity exercise increases gluconeogenesis (29, 36). Unfortunately, the tracer techniques used in this study do not allow quantification of gluconeogenesis.

The values for CHO_{Exo} (and $\delta^{13}\text{CO}_2$ breath enrichment) seen here were higher than those normally observed in the literature (23, 26, 27, 44). To exclude any possibility of measurement error, duplicates of the breath samples and the ingested CHO were analyzed at two different laboratories. There was no difference between the two sets of results. In addition, the validity and reliability of the gas analysis system used to measure $\dot{V}\text{CO}_2$ has been investigated (7), and, during the course of this data collection, the system was again checked using an automated calibrator. To correct for shift in background $^{13}\text{CO}_2$ breath enrichment, we used a water trial. It would have been more appropriate to use a background trial with Glu of low natural $^{13}\text{CO}_2$ abundance. However, since the same background correction was made in the Glu and Glu+Fru trials, this would not have affected the conclusions.

Following ingestion of Glu, a peak CHO_{Exo} rate of 1.24 g/min was observed, a value greater than the often suggested maximum CHO_{Exo} rate (1.1 g/min) (23). High CHO_{Exo} rates were also suggested by the study of Coyle et al. (8), who employed ultraendurance exercise to exhaustion (242 ± 20 min) at $71 \pm 1\%$ peak $\dot{V}\text{O}_2$ while consuming CHO at ~ 1.5 g/min. In this study, CHO_{Exo} was estimated by assuming that maintenance of CHO oxidation (2 g/min), in the absence of further glycogen depletion, was largely a result of CHO_{Exo} . The present study suggests that these high rates of plasma Glu oxidation are achieved by both hepatic Glu output from gluconeogenesis (and/or liver glycogenolysis) and CHO_{Exo} .

While the CHO_{Exo} rates estimated by Coyle et al. (8) are substantially higher than those measured in this study, taken together, the data would seem to suggest that the maximum CHO_{Exo} rates suggested in the literature (1.1 g/min) (23) may be exceeded under certain conditions. One possible explanation for these findings may be interindividual variations in the ability of a person to uptake CHO. The subjects in this study were all endurance-trained athletes used to consuming a high-CHO diet and relatively large volumes of CHO drink on a daily basis. It is known that dietary Glu supplementation can accelerate the gastric-emptying (GE) time of a Glu solution, through a downregulation of Glu receptors or an increase in intestinal Glu uptake (10, 14). It is possible that the subjects in this study exhibited such an adaptation to their high-dietary CHO intake and had an increased capacity to empty or absorb CHO.

Another important finding of this study is that, also during very prolonged exercise (5 h), ingestion of Glu and Fru (in a 2:1 ratio) results in greater CHO_{Exo} rates than the ingestion of an isoenergetic amount of Glu alone. However, this happens in the absence of an increase in R_d Glu, since R_d Glu was similar in the Glu and Glu+Fru trials. One possible explanation is that a larger proportion of the CHO from Glu+Fru is transported to

the muscle in another form than Glu, for example, lactate. Indeed, we have consistently found increased plasma lactate concentrations when Fru is coingested with Glu (16–18, 45).

The difference in CHO_{Exo} between Glu and Glu+Fru becomes apparent after 20 min of exercise, exists throughout exercise, and results in a significantly greater contribution of CHO_{Exo} to total CHO oxidation (65 vs. 77%). Very high CHO_{Exo} rates following the ingestion of a Glu+Fru mixture have been previously observed in this laboratory (16–18, 45). It has been suggested that this is due to an increase in either intestinal absorption and/or the rate of hepatic Glu release (23). The higher CHO_{Exo} rates with Glu+Fru occurred without a concurrent higher Glu R_a , suggesting that CHO_{Exo} was oxidized in the liver and/or non-Glu energy substrates were formed and oxidized in the muscle during exercise.

Interestingly, there is some evidence that ingestion of Glu+Fru increases intestinal water absorption by 65% compared with Glu alone (40), for which the most likely explanation is increased solute absorption. It may be that, when more than one CHO is ingested, there is an increased capacity for intestinal CHO absorption, potentially by utilizing more than one transporter to cross the intestinal membrane (Glu = sodium glucose cotransporter-1 and Fru = GLUT-5). An alternative explanation could be that the presence of Fru is enhancing the absorption of Glu (13, 37). Certainly, Fru has been observed to result in faster GE than Glu (11, 14, 15, 30, 41), which may explain the reduced perception of gastric fullness late in exercise seen in Glu+Fru. However, since the GE of a 6% combined Glu+Fru beverage over 30 min has been reported not to be different to either Glu alone or Fru alone (39), further investigations into the effect of Glu+Fru on GE rate are warranted.

The $[^2\text{H}]$ glucose tracer technique used in this study cannot differentiate between Glu appearing from the intestine and hepatic Glu release. Therefore, it is not possible to isolate the mechanism responsible for the higher CHO_{Exo} following the ingestion of Glu+Fru. However, despite the difference between conditions in CHO_{Exo} , there was no difference in Glu R_a or R_d . There are two possible explanations for an increase in CHO_{Exo} without an increased R_a or R_d : 1) CHO is being oxidized in the liver, or 2) CHO is being converted to lactate and oxidized in the liver or in the other tissues. Release of $[^{13}\text{C}]$ lactate and subsequent oxidation by the muscle would not affect R_a , as measured by $[^2\text{H}]$ glucose, but would result in increased expired $^{13}\text{CO}_2$ and thus would contribute to calculations of CHO_{Exo} . Rauch et al. (34) suggested a similar mechanism to explain their findings. Their estimation of total muscle glycogen oxidation during 6 h of cycling at 55% $\dot{V}\text{O}_{2\text{max}}$ (~ 530 g) was greater than the predicted initial muscle glycogen content (~ 250 g). The protocol (employing U- ^{14}C -labeled Glu and U- ^{14}C -labeled lactate) enabled the authors to speculate that this was due to “considerable diffusion of unlabeled lactate from glycogen breakdown in inactive muscle fibres to adjacent active muscle fibres.” Interestingly, we observed that ingestion of Glu+Fru resulted in raised plasma lactate concentrations (Fig. 5). Any suggestion that increases in CHO_{Exo} rate may be beneficial during exercise would need to be questioned, if indeed the source of the increased $^{13}\text{CO}_2$ excretion is the oxidation of labeled Glu or lactate in the liver.

Examination of the measures of substrate oxidation and circulating substrate levels illustrates that both CHO beverages

maintained CHO oxidation to the same degree and attenuated the rises in plasma FFA and free glycerol concentrations that occurred in the water trial. Endogenous CHO oxidation was not directly measured, but the maintenance of total CHO and fat oxidation, combined with the significant difference between trials in CHO_{Exo}, would suggest a reduction in endogenous CHO oxidation. Indeed, estimated endogenous CHO use over the exercise period was significantly reduced in Glu+Fru compared with Glu.

In addition to effects on Glu kinetics, the ingestion of Glu+Fru resulted in changes in subjects' behavior and sensations. In the Glu+Fru trial, subjects were able to maintain their self-selected cadence throughout exercise, while there were significant reductions in cadence following both water and Glu ingestion. In addition, the ingestion of Glu led to a significant increase in the subjects' perception of gastric volume ("fullness"), an increase that did not occur following water and Glu+Fru ingestion (Fig. 7). Finally, there was a trend for RPE to be reduced in the final hour of exercise following the ingestion of Glu+Fru.

In summary, these data suggest that, during ultraendurance exercise, gluconeogenesis may play an important role after 3 h of exercise, even when CHO is ingested at high rates. CHO_{Exo} oxidation during exercise reached high rates (well over 1 g/min), but did level off after ~2 h of exercise, in contrast to the linear increase over time suggested by authors who had previously estimated CHO_{Exo} under similar conditions (3). In addition, the ingestion of a solution containing both Glu and Fru (in a 2:1 ratio) resulted in an increase in CHO_{Exo} compared with the Glu-only solution, even after 5 h of exercise. This increase occurred without a concurrent increase in Glu R_a, suggesting CHO_{Exo} occurring in the liver and/or the formation and oxidation of non-Glu energy substrates during exercise.

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