Exogenous carbohydrate oxidation rates are elevated following combined ingestion of glucose and fructose during exercise in the heat

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ABSTRACT

The first purpose of this study was to investigate whether a GLU+FRUC beverage would result in a higher exogenous CHO oxidation rate and a higher fluid availability during exercise in the heat compared with an isoenergetic GLU beverage. A second aim of the study was to examine whether ingestion of GLU at a rate of 1.5 g/min during exercise in the heat would lead to a reduced muscle glycogen oxidation rate when compared with ingestion of water (WAT). Eight trained male cyclists (VO_{2max}: 64±1 ml·kg·min^{-1}) cycled on three different occasions for 120 min at 50% maximum power output at an ambient temperature of 31.9±0.1 °C. Subjects received, in random order, a solution providing either 1.5 g/min of GLU, 1.0 g/min of GLU + 0.5 g/min of FRUC, or WAT. Exogenous CHO oxidation during the last hour of exercise was ~36% higher (P<0.05) in GLU+FRUC compared with GLU and peak oxidation rates were 1.14±0.05 and 0.77±0.08 g/min, respectively. Endogenous CHO oxidation was significantly lower (P<0.05) in GLU+FRUC compared with WAT. Muscle glycogen oxidation was not different following ingestion of GLU or WAT. Plasma deuterium enrichments were significantly higher (P<0.05) in WAT and GLU+FRUC compared with GLU. Furthermore, at 60 and 75 min of exercise, plasma deuterium enrichments were higher (P<0.05) in WAT compared with GLU+FRUC. Ingestion of GLU+FRUC during exercise in the heat resulted in higher exogenous CHO oxidation rates and fluid availability when compared with ingestion of GLU and reduced endogenous CHO oxidation when compared with ingestion of WAT.

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INTRODUCTION

Exercise in the heat results in a shift in substrate utilization toward a greater reliance on carbohydrate (CHO) metabolism (13, 17). This effect is attributed to an increased muscle glycogen utilization (13, 17), which is associated with higher blood lactate concentrations. In addition, the combination of exercise and heat stress also results in an increased hepatic glucose production (HGP) with no alteration in glucose disappearance, leading to elevated blood glucose concentrations (2, 17).

Little is known about the effects of heat stress on exogenous CHO oxidation during exercise (20). We have recently shown that the oxidation rate of ingested CHO is reduced in the heat compared with a cool environment and this might be due to a reduced CHO absorptive capacity of the intestine as a result of a decreased intestinal blood flow (20). These results also support the hypothesis that intestinal CHO absorption is a limiting factor for exogenous CHO oxidation (23, 25, 29). Interestingly, a study by Adopo et al. (1) and studies from our laboratory (22, 23, 26) have shown that during 120 to 150 min of cycling exercise, ingestion of glucose in combination with fructose resulted in 20-55% higher exogenous CHO oxidation rates compared with ingestion of an isoenergetic amount of glucose only. It is likely that this is caused by the fact that glucose and fructose are absorbed by different intestinal transport mechanisms (SGLT1 and GLUT-5 transporters, respectively) (50) and hence there may be less competition for absorption. The effect of a glucose+fructose mixture on exogenous CHO oxidation during exercise in the heat remains to be investigated.

It has been suggested that CHO availability is not a limiting factor for exercise in the heat (12) when the heat stress is uncompensable, since muscle glycogen concentrations are relatively
high at the point of fatigue (36, 39). However, CHO supplementation may be important during prolonged exercise in the heat when the heat stress is compensable. Furthermore, because the rate of muscle glycogen utilisation is increased during exercise in the heat, glycogen stores may rapidly decrease in athletes who train twice daily for several days or who compete on consecutive days in hot environments. Low or reduced muscle glycogen stores at the start of exercise have the potential to impair exercise performance. Interestingly, CHO feedings (~1.2 g/min) during exercise in the heat may reduce muscle glycogen utilisation (51). In contrast, the results of a study by Angus et al. (2) suggest that glycogen oxidation in the heat is not reduced when glucose is ingested at rate of ~1 g/min. These seemingly contradictory findings may be due to differences in research designs, including variations in exercise duration, type of CHO consumed, and method used to measure glycogen utilisation (muscle biopsies vs. stable isotope methodology). Of note, in the study of Yaspelkis et al. (51) and Angus et al. (2), exogenous CHO oxidation was not measured. In the present study, naturally $^{13}$C-enriched glucose and a $[6,6^{-2}H_2]$-glucose tracer were used simultaneously, which enabled us to measure the oxidation rate of ingested glucose and muscle glycogen (non-invasively).

Exercise in the heat is also accompanied by significant losses of sweat as the body attempts to delay the rise in body temperature (hyperthermia). When fluid lost is not replaced, dehydration will occur. Even low levels of dehydration (2% of body mass) may impair endurance performance (for review see references (5, 6)). Thus, maintenance of fluid balance is a major concern for athletes who exercise in hot environments. A CHO beverage that leads to high fluid (and CHO) delivery rates may have important implications for exercise performed in hot environments. Interestingly, we have recently shown, using a deuterium oxide tracer, that ingestion of a ~9% glucose+fructose solution during exercise in a thermoneutral environment
resulted in a higher fluid availability than ingestion of a ~9% glucose solution (26). Furthermore, it has been demonstrated that fluid availability at rest and during exercise is not different following ingestion of a glucose+fructose drink compared with ingestion of water (9, 26). Therefore, it seems that ingestion of a glucose+fructose drink can result in high exogenous CHO oxidation rates (> 1.0 g/min) (23, 26) without compromising fluid delivery (26). However, there are no studies available that have directly compared the effect of a glucose and glucose+fructose solution on fluid availability and exogenous CHO oxidation during exercise in a hot environment.

The first purpose of the present study was to examine whether combined ingestion of glucose+fructose in the heat would result in higher rates of exogenous CHO oxidation when compared with ingestion of glucose only. A second purpose of this study was to examine whether ingestion of glucose at a rate of 1.5 g/min during prolonged exercise in the heat would lead to a reduced rate of muscle glycogen oxidation when compared with the ingestion of water. The third aim was to investigate whether a glucose+fructose beverage would result in a higher fluid availability during exercise in the heat compared with a beverage containing an isoenergetic amount of glucose only.
METHODS

Subjects

Eight trained non-acclimated male cyclists or triathletes aged 27.8±2.9 y and with a body mass of 74.5±2.8 kg took part in this study. Subjects trained at least 3 times a wk for more than 2 h a day and had been involved in endurance training for at least 3 y. Prior to participation, each of the subjects was fully informed of the purpose and risks associated with the procedures and a written informed consent was obtained. All subjects were healthy as assessed by a general health questionnaire. The study was approved by the South Birmingham Local Research Ethics Committee, UK.

Preliminary testing

At least 1 wk before the start of the experimental trials an incremental cycle exercise test to volitional exhaustion was performed in order to determine the individual maximum power output (Wmax) and maximal oxygen consumption (VO2max). This test was performed on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands), modified to the configuration of a racing bicycle with adjustable saddle height and handlebar position. After reporting to the laboratory body mass (Seca Alpha, Hamburg, Germany) and height were recorded. Subjects then started cycling at 95 W for 3 min, followed by incremental steps of 35 W every 3 min until exhaustion. Heart rate (HR) was recorded continuously by a radiotelemetry heart rate monitor (Polar Vantage NV, Kempele, Finland). Wmax was calculated from the last completed work rate, plus the fraction of time spent in the
final non-completed work rate multiplied by the work rate increment. The results were used to determine the work rate corresponding to 50% Wmax, which was later employed in the experimental exercise trials. Breath-by-breath measurements were performed throughout exercise using an online automated gas analysis system (Oxycon Pro, Jaeger, Hoechberg, Germany). The volume sensor was calibrated using a 3-liter calibration syringe and the gas analyzers were calibrated using a 5.03% CO₂: 94.97% N₂ gas mixture. Oxygen uptake (VO₂) was considered to be maximal (VO₂max) when at least two of the three following criteria were met: 1) a leveling off of VO₂ with increasing workload (increase of no more than 2 ml·kg⁻¹·min⁻¹), 2) a HR within 10 beats/min of predicted maximum (HR 220 minus age), 3) a respiratory exchange ratio (RER) >1.05. VO₂max was calculated as the average oxygen uptake over the last 60 s of the test. The VO₂max and Wmax achieved during the incremental exercise test were 64±1 ml·kg⁻¹·min⁻¹ and 361±8 W, respectively.

**Experimental design**

Each subject performed 3 exercise trials, which were randomly assigned and separated by at least 7 days. All exercise trials were performed during the winter season (October until January). Each trial consisted of 120-min of cycling at 50% Wmax at an ambient dry bulb temperature of 31.9±0.1 °C and a relative humidity of 30±2%, while subjects ingested a 9% glucose+fructose drink (GLU+FRUC)(the ingested glucose-to-fructose ratio was 2:1), 9% glucose drink (GLU), or plain filtered water (WAT). In order to quantify exogenous glucose oxidation, corn-derived glucose monohydrate (Cerestar, Manchester, UK) and crystalline fructose (Krystal 300, A.E. Staley Manufacturing Company, Illinois, USA) were used which have a high
natural abundance of $^{13}$C (-10.8 and -10.7 $\delta^{\text{PDB}}$ vs. Pee Dee Bellemnitella (PDB), respectively). The $^{13}$C-enrichment of the ingested glucose and fructose was determined by elemental analyzer-isotope ratio mass spectrometry (IRMS; Europa Scientific GEO 20-20, Crewe, UK). To both CHO drinks 20 mmol/L of sodium chloride (Sigma-Aldrich, Dorset, UK) was added.

**Diet and activity prior to testing**

Subjects were asked to record their food intake and activity pattern 2 days prior to the first exercise trial and were then instructed to follow the same diet and exercise activities before the other 3 trials. In addition, 5 to 7 days prior to each experimental testing day, they were asked to perform an intense training session, which consisted of 2 to 3 hours of cycling at 80 to 90% of maximum HR (‘Glycogen depleting’ exercise bout), in an attempt to empty any $^{13}$C-enriched glycogen stores. Subjects were further instructed not to consume any food products with a high natural abundance of $^{13}$C (carbohydrates derived from C4 plants: maize, cane sugar) at least 1 week before and during the entire experimental period in order to reduce the background shift (change in $^{13}$CO$_2$) from endogenous substrate stores.

**Protocol**

Subjects reported to the Human Performance Laboratory in the morning (between 7:00-9:00 A.M.) after an overnight fast (10-12 h) and having refrained from any strenuous activity or drinking any alcohol in the previous 24 h. On the day of the experimental trial subjects were instructed to consume approximately 500 ml of tap water on waking to ensure adequate hydration. Before the water was consumed, subjects were asked to collect their first urine of the
day. Urine volume was measured and urine samples were analyzed for osmolality by freezing point depression (Advanced Micro Osmometer 3300, Advanced Instruments Inc., Norwood, USA). For a given subject, all trials were conducted at the same time of the day to avoid any influence of circadian variance. On arrival in the laboratory, subjects were asked to empty their bladder before nude body mass was recorded to the nearest 0.010 kg using a digital industrial bench scale (Champ II, Ohaus UK Ltd, Leicester, UK). Thereafter, a flexible 21-gauge Teflon catheter (Quickcath, Baxter BV, Norfolk, UK) was inserted in an antecubital vein of an arm and attached to a three-way stopcock (Sims Portex, Kingsmead, UK) to allow for repeated blood sampling during exercise. The catheter was kept patent by flushing with 1.5-2.0 ml of isotonic saline (Sodium Chloride (0.9%) saline, Baxter, Norfolk, UK) after each blood sample collection. During the GLU and WAT trials, a second catheter was inserted in the contralateral antecubital vein and used to infuse a sterile solution containing trace amounts of [6,6-\(^2\)H\(_2\)]-glucose. It should be noted that there was no [6,6-\(^2\)H\(_2\)]-glucose infusion in the GLU+FRUC trial and hence no data is presented in Table 2 and Figure 3 for GLU+FRUC. Fructose ingestion may result in elevated lactate concentrations (present study and (22, 23, 26) and this may lead to increased lactate oxidation (33, 41). Increased oxidation of lactate derived from fructose would not affect the rate of disappearance of glucose but would result in increased expired \(^{13}\)CO\(_2\). Therefore, calculations of liver-derived glucose and muscle glycogen oxidation from indirect calorimetry and stable isotope methodology (\(^{13}\)CO\(_2\) and plasma \(^2\)H-glucose enrichments) cannot be done when glucose and fructose are ingested simultaneously.

The subjects then inserted a rectal thermistor (Grant Instruments, Cambridge, UK) 10-15 cm beyond the anal sphincter and rectal temperature (\(T\(_{rec}\)) was monitored continuously. Subjects were rested in a seated position for 20-30 min during which time thermistors (Grant Instruments,
Cambridge, UK) were attached to the skin of the forehead, lower back, dorsal surface of the right hand and calf muscle of the right leg for measurements of skin temperature. Weighted mean skin temperature ($T_{sk}$) was calculated using the equation of Nielsen and Nielsen (37). Next, a resting breath sample was collected in 10-mL Exetainer tubes (Labco Ltd. Brow Works, High Wycombe, UK), which were filled directly from a mixing chamber in duplicate in order to determine the $^{13}$C/$^{12}$C ratio in the expired air. A resting blood sample (10 mL) was taken and stored on ice and later centrifuged. Additional blood samples were drawn at 15-min intervals during exercise. HR was recorded at rest (and during exercise) in 15-s intervals using a radiotelemetry HR monitor (Polar Vantage NV, Kempele, Finland).

Immediately after collection of the first resting blood sample, subjects transferred to a climatic chamber and mounted the cycle ergometer. Before the start of exercise in GLU and WAT, a $[6,6^{-2}\text{H}_2]$-glucose prime was given equal to the amount of isotope infused during 45 min of exercise ($29\pm1$ µmol/kg). After the glucose pool was primed, a continuous infusion of sterile pyrogen-free $[6,6^{-2}\text{H}_2]$-glucose was started at the onset of exercise using a calibrated syringe pump (Asena GS syringe pump, ALARIS Medical System, Basingstoke, UK). The concentration of isotope in the infusate was determined for all GLU and WAT trials to calculate the exact glucose infusion rate. The infusion rates were $0.658\pm0.012$ and $0.647\pm0.018$ µmol/kg/min in GLU and WAT, respectively. Subjects then started the 120-min exercise bout. Expiratory breath samples were collected every 15 min until the end of exercise. VO$_2$, VCO$_2$ (carbon dioxide production) and RER were measured every 15 min for periods of 4 min using an online automated gas analysis system as previously described. $T_{rec}$ and $T_{sk}$ were recorded at the onset of exercise and every 5-min interval during exercise using an automatic data logger (Squirrel...
meter/logger, 1000 series, Grant Instruments, Cambridge, UK). A standing floor fan was placed in front of the subjects to circulate air.

During the first 3 min of exercise subjects drank an initial bolus (600 mL) of one of the three experimental drinks; GLU, GLU+FRUC or WAT. Thereafter, every 15 min a beverage volume of 200 mL was provided. The total fluid provided during the 120-min exercise bout was 2 L. The average rate of glucose intake in the GLU and GLU+FRUC trial was 1.5 and 1.0 g/min, respectively. Furthermore, in the GLU+FRUC trial subjects ingested on average 0.5 g/min of fructose which brought the total CHO intake rate in the GLU+FRUC trial at 1.5 g/min. During all trials, the drink administered after 60 min of exercise also contained 5.0 g of $^2\text{H}_2\text{O}$ (99%, Sigma, Diagnostics, Dorset, UK). Subjects were instructed to consume the $^2\text{H}_2\text{O}$ dose within 30 s, after which the dose bottle was rinsed out with $\sim$10 mL of the experimental drink volume. The rinse volume was also consumed to ensure that all $^2\text{H}_2\text{O}$ was ingested. The appearance of $^2\text{H}_2\text{O}$ in plasma was used as a qualitative marker of fluid availability (9, 10, 26, 42).

Subjects were asked to rate their perceived exertion (RPE) for whole body and legs every 30 min on a scale from 6 to 20 using the Borg category scale (3). In addition, subjects were asked every 30 min to fill in a questionnaire in order to rate (possible) gastrointestinal (GI) problems. Subjects were also asked to fill out a questionnaire every 30 min during the exercise trials. While subjects were on the bike and continued their exercise each question was answered by simply ticking a box on the questionnaire that corresponded to the severity of the GI problem addressed. The items were scored on a 10-point scale (1 = not at all, 10 = very, very much). The severity of the GI symptoms was divided into two categories; severe and nonsevere symptoms, as was previously described by Jentjens et al. (28). Immediately after exercise, the catheter(s) and thermisters were removed and subjects were towel dried before nude body mass was measured.
again. Body mass loss, corrected for fluid intake, isotope solution infused, blood collected, urine produced, respiratory water loss (34) and metabolic changes (45), was taken representing sweat loss. All urine produced during the 120-min exercise bout was collected. A final urine sample was obtained post-exercise and pooled with the urine produced during exercise. The volume and osmolality of the pooled urine samples were determined as previously described.

**Analyses**

Blood samples (9 ml) were collected into pre-chilled K$_3$EDTA containing tubes (Beckton Dickinson, Plymouth, UK) and centrifuged at 2300 g and 4°C for 10 min. Aliquots of plasma were immediately frozen in liquid nitrogen and stored at −25°C until analyses for glucose and lactate. Glucose (Glucose HK 125, ABX diagnostics, Shefford, UK) and lactate (Lactic Acid 10, ABX diagnostics, Shefford, UK) were analyzed on a COBAS MIRA semi-automatic analyzer (ABX Diagnostics, Montpellier, France). Approximately, 1 ml of the EDTA-treated blood was used for measurements of hematocrit and hemoglobin so that changes in plasma volume (PV) from rest could be calculated as previously described by Dill and Costill (11). Hematocrit was determined in triplicate by microcentrifugation, and hemoglobin (duplicate) was analyzed on an automatic analyzer (Coulter Ac·T diff analyzer, Beckman Coulter UK Ltd, Buckinghamshire, UK). Blood samples (1 ml) were also collected in Lithium-heparinized tubes (Beckton Dickinson, Plymouth, UK) and centrifuged at 2300 g and 4°C for 10 min. Aliquots of plasma were subsequently analyzed for osmolality by freezing point depression ((Advanced Micro Osmometer 3300, Advanced Instruments Inc., Norwood, USA). Plasma deuterium enrichment (ppm) was determined in duplicate by the equilibration technique where a sample volume of 0.25
ml was pipetted into evacuated tubes (Exetainers, Labco Ltd. Brow Works, High Wycombe, UK) containing 5% platinum on alumina catalyst (Sigma Aldrich, Dorset, UK) added. The tubes were then sealed, filled with pure hydrogen gas and left for a period of not less than 3 days to allow complete equilibration of the water with the hydrogen gas. Analysis was undertaken by continuous-flow IRMS using a Europa Scientific ANCA-GSL and GEO 20-20 IRMS (Europa Scientific, Crewe, UK).

Breath samples were analyzed for $^{13}$C/$^{12}$C ratio by gas chromatography continuous flow isotope ratio mass spectrometry (GC-IRMS)(Europa Scientific, Crewe, UK). For determination of plasma [$^2$H]-glucose enrichment, plasma samples were deproteinized and derivatized with heptafluorobutyric acid (HFB). Briefly, 100 µL plasma was deproteinized with 1 ml acetone. The samples were then derivatized with 160 µL of a HFB/ethylacetate mixture (1:1 ratio). Thereafter, the enrichment of the derivative as measured by gas chromatography-mass spectrometry (GC-MS) by injecting 1 µL of the derivative into an Agilent 6890N gas chromatograph equipped with a split/splitless injector and 7683 autosampler (Agilent, UK). Mass spectra were obtained using an Agilent 5973N mass-selective detector. Data were acquired using selected ion monitoring for masses m/z 519 and 521. From indirect calorimetry (VO$_2$ and VCO$_2$) and stable isotope measurements (breath $^{13}$CO$_2$ enrichment and plasma [$^2$H]-glucose enrichment), oxidation rates of total fat, total CHO, muscle glycogen, liver-derived glucose, plasma glucose and exogenous glucose were calculated.
Calculations

From $\text{VCO}_2$ and $\text{VO}_2$ (L/min), total CHO and fat oxidation rates (g/min) were calculated using stoichiometric equations of Frayn (14) with the assumption that protein oxidation during exercise was negligible:

\[
\text{CHO oxidation} = 4.55 \, \text{VCO}_2 - 3.21 \, \text{VO}_2 \quad \text{(1)}
\]

\[
\text{Fat oxidation} = 1.67 \, \text{VO}_2 - 1.67 \, \text{VCO}_2 \quad \text{(2)}
\]

The isotopic enrichment was expressed as $\delta$ per mil difference between the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and a known laboratory reference standard according to the formula of Craig (8):

\[
\delta^{13}\text{C} = \left( \frac{^{13}\text{C}/^{12}\text{C} \text{ sample}}{^{13}\text{C}/^{12}\text{C} \text{ standard}} - 1 \right) \cdot 10^3 \text{ per mil} \quad \text{(3)}
\]

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

In the GLU and GLU+FRUC trials, the rate of exogenous glucose oxidation (EGO) was calculated using the following formula (40):

\[
\text{Exogenous glucose oxidation} = \text{VCO}_2 \cdot \left( \frac{\delta \text{ Exp} - \delta \text{ Exp bkg}}{\delta \text{ Ing} - \delta \text{ Exp bkg}} \right) \left( \frac{1}{k} \right) \quad \text{(4)}
\]
In which $\delta^{13}\text{Exp}$ is the $^{13}$C enrichment of expired air during exercise at different time points, $\delta^{13}\text{Ing}$ is the $^{13}$C enrichment of the ingested CHO solution, $\delta^{13}\text{Exp}_{\text{bg}}$ is the $^{13}$C enrichment of expired air in the WAT trial (background) at different time points and $k$ is the amount of CO$_2$ (in liters) produced by the oxidation of 1 g of glucose ($k = 0.7467$ liter of CO$_2$ per g of glucose).

A methodological consideration when using $^{13}$CO$_2$ in expired air to calculate exogenous substrate oxidation is the trapping of $^{13}$CO$_2$ in the bicarbonate pool, in which an amount of CO$_2$ arising from decarboxylation of energy substrates is temporarily trapped (43). However, during exercise the CO$_2$ production increases severalfold so that a physiological steady state condition will occur relatively rapidly, and $^{13}$CO$_2$ in the expired air will be equilibrated with the $^{13}$CO$_2$/H$^{13}$CO$_3^-$ pool, respectively. Recovery of $^{13}$CO$_2$ from oxidation will approach 100% after 60 min of exercise when dilution in the bicarbonate pool becomes negligible (38, 43). As a consequence of this, all calculations on substrate oxidation were performed over the last 90 min of exercise (60-150 min).

The total rate of appearance (i.e. total R$_a$) and disappearance (i.e. total R$_d$) of glucose in the systemic circulation were calculated using the single pool non-steady state equations of Steele (47) adapted for use with stable isotopes (49). Total R$_a$ represents the sum of glucose appearing from hepatic glycogenolysis, gluconeogenesis and glucose absorbed from the gut.

$$\text{Total } R_a \text{ glucose} = \frac{F - V [(C_2 + C_1)/2][(E_2 - E_1)/(t_2 - t_1)]}{(E_2 + E_1)/2}, \quad (5)$$

$$\text{total } R_d \text{ glucose} = R_a - V \left( \frac{C_2 - C_1}{t_2 - t_1} \right), \quad (6)$$

$$\text{total } R_d \text{ glucose} = R_a - V \left( \frac{C_2 - C_1}{t_2 - t_1} \right), \quad (6)$$
where $F$ is the infusion rate ($\mu$mol/kg/min), $V$ is the volume of distribution (=160 ml/kg), $C_2$ and $C_1$ are the glucose concentrations at times 2 and 1 (i.e. $t_2$, $t_1$), respectively, and $E_2$ and $E_1$ are the plasma glucose enrichments at times 2 and 1 (i.e. $t_2$, $t_1$), respectively.

In a recent study by Jeukendrup et al. (27), it was shown that $R_d$ glucose (measured with a [6,6-$^2$H$_2$]-glucose tracer) was almost identical (96-100%) to the plasma glucose oxidation rate (measured with a [U-$^{13}$C]-glucose tracer).

Therefore, plasma glucose oxidation in the present study was calculated as:

\[
\text{Plasma glucose oxidation} = \text{total } R_d \text{ glucose}
\]  
(7)

Because plasma glucose oxidation represents the oxidation of both glucose coming from the gut (exogenous glucose) and the contribution of the liver (glycogenolysis/gluconeogenesis), liver derived glucose oxidation could be calculated by the following formula:

\[
\text{Liver derived glucose oxidation} = \text{plasma glucose oxidation} - \text{exogenous glucose oxidation}
\]  
(8)

Muscle glycogen oxidation was estimated by using the formula:

\[
\text{Muscle glycogen oxidation} = \text{total CHO oxidation} - \text{plasma glucose oxidation}
\]  
(9)

Metabolic clearance rate (MCR) was calculated as the $R_d$ glucose divided by the mean glucose concentration over that time period.

\[
\text{MCR (ml/kg/min)} = \frac{R_d}{(C_1+C_2)/2}
\]  
(10)
Statistical analyses

Two-way analysis of variance (ANOVA) for repeated measures was used to compare differences in substrate utilization and in blood related parameters over time between the trials. A Tukey *post hoc* was applied in the event of a significant $F$-ratio. Where appropriate, comparison of variables between two conditions was conducted by using a Student’s $t$-test for paired samples. Data evaluation was performed using SPSS for Windows version 10.0 software package (Chicago, United States). All data are reported as means ± SEM. Statistical significance was set at $P<0.05$. 
RESULTS

Breath $^{13}$CO$_2$ enrichments,

The mean $^{13}$CO$_2$ enrichment of the resting breath samples was $-26.24\pm0.21$ $\delta^{\circ}$ vs. PDB, respectively. Changes in isotopic composition of expired CO$_2$ in response to exercise with ingestion of water (WAT), glucose (GLU) or glucose+fructose (GLU+FRUC) are shown in Figure 1A. In the CHO trials there was a significant increase ($P<0.05$) in the $^{13}$CO$_2$ enrichment of expired breath, reaching an enrichment difference of $\sim4-5$ $\delta^{\circ}$ vs. PDB towards the end of the 120-min exercise (compared with corresponding resting breath sample). From the 30-min time point onwards, breath $^{13}$CO$_2$ enrichment in GLU+FRUC was significantly ($P<0.05$) higher compared with the GLU trial. During the WAT trial, there was a small but significant increase in $^{13}$CO$_2$ enrichment of the expired air ($P<0.05$). Although the background shift was relatively small in the present study, a background correction was made for the calculation of exogenous glucose oxidation in the CHO trials by using the data from the WAT trial.

Exogenous and endogenous CHO oxidation

In the GLU and GLU+FRUC trials, the rate of exogenous CHO oxidation increased significantly ($P<0.05$) during exercise. Peak exogenous CHO oxidation rates were reached towards the end of exercise (105-120 min) and were significantly higher ($P<0.05$) in the GLU+FRUC trial ($1.14\pm0.05$ g/min) compared with the GLU trial ($0.77\pm0.08$ g/min) (Figure 1B). During final 60 min of exercise, exogenous CHO oxidation rates were $\sim36\%$ higher ($P<0.05$) in GLU+FRUC compared with GLU (Table 1).
No difference was found in endogenous CHO oxidation rates between the WAT and the GLU trial. However, the high exogenous CHO oxidation rates in the GLU+FRUC trial resulted in significantly lower (\(P<0.05\)) endogenous CHO oxidation rates compared with the WAT trial (Table 1 and Figure 2). Endogenous CHO oxidation rates were not different between the GLU and GLU+FRUC trial. It should be noted that during the last 60 min of exercise the contribution of endogenous CHO to total energy expenditure in GLU+FRUC when compared with GLU was lower in 6 out of 8 subjects, but this failed to reach statistical significance (\(P=0.061\)). Endogenous CHO oxidation represented 39±4, 38±4 and 29±2% of total energy expenditure in WAT, GLU and GLU+FRUC, respectively (WAT>GLU+FRUC; \(P<0.05\))(Figure 2).

**\(\text{VO}_2, \text{RER, total CHO and fat oxidation}\)**

Data for \(\text{VO}_2\), RER, total CHO (CHO\text{tot}) and fat oxidation over the 60- to 120-min exercise period are shown in Table 1. There was no significant difference in \(\text{VO}_2\) between the three experimental trials (3.04±0.05 L/min). Ingestion of CHO (GLU and GLU+FRUC) resulted in significantly (\(P<0.05\)) higher RER values compared with ingestion of water. The average CHO\text{tot} oxidation rates over the 60- to 120-min exercise period in GLU (2.11±0.14 g/min) and GLU+FRUC (2.19±0.07 g/min) were significantly (\(P<0.05\)) higher compared with WAT. No difference in total CHO oxidation was found between the two CHO trials. Total fat oxidation was markedly suppressed with CHO ingestion (\(P<0.05\)). The average fat oxidation rates during the final 60-min of exercise were 0.96±0.07 g/min (WAT), 0.73±0.04 g/min (GLU) and 0.72±0.04 g/min (GLU+FRUC), respectively. Fat oxidation represented 61±4, 48±3 and 45±1% of total
energy expenditure in WAT, GLU and GLU+FRUC, respectively (WAT>GLU and GLU+FRUC; \( P<0.05 \))(Figure 2).

**Ra and Rd of plasma glucose, MCR, Liver-derived glucose and muscle glycogen oxidation**

Due to technical problems in the \([6,6^-2H_2]\)-glucose infusion trials, data of only 6 subjects is reported here. Both \( R_a \) and \( R_d \) glucose increased during exercise and were significantly higher \( (P<0.05) \) during the last 60 min of exercise in the GLU trial compared with the WAT trial (Figure 3A). MCR during exercise was significantly \( (P<0.05) \) higher with ingestion of GLU compared with ingestion WAT (Figure 3B).

The average liver-derived glucose, muscle glycogen, CHOtot and exogenous CHO oxidation rates \((n=6)\) during the last 60 min of exercise following ingestion of GLU or WAT are shown in Table 2. No differences were found in liver-derived glucose and muscle glycogen oxidation between the GLU and WAT trial. CHOtot during the second hour of exercise was significantly higher in GLU compared with WAT \((159\pm9 \text{ vs. } 110\pm11 \mu\text{mol/kg/min, respectively}; P<0.05)\).

**Plasma metabolites**

Plasma glucose and lactate concentrations at rest and during exercise are shown in Figure 4A and B. There were no differences in fasting plasma glucose concentrations between trials (Figure 4A). Plasma glucose concentrations in the GLU and GLU+FRUC trials peaked within the first 15 min of exercise at a value of \(~5.8\ \text{mmol/L} \). Plasma glucose concentrations then fell and were maintained at values of 4.8 to 5.1 mmol/L for the entire duration of exercise. Plasma
glucose concentrations during exercise with WAT decreased gradually, reaching a nadir of 3.8.0±0.2 mmol/L at the end of exercise. At 15 min of exercise and during the final 45 min of exercise, plasma glucose concentrations were significantly higher ($P<0.05$) in the CHO trials compared with the WAT trial. Furthermore, plasma glucose concentrations at 60 min of exercise were higher ($P<0.05$) in GLU compared with WAT. No differences in plasma glucose concentrations were found between the GLU and GLU+FRUC trials.

Fasting plasma lactate concentrations were similar between trials (on average 0.8±0.1 mmol/L)(Figure 4B). Plasma lactate concentrations between $t=30$ min and $t=60$ min were significantly higher ($P<0.05$) in the GLU+FRUC trial compared with the WAT and GLU trials. No differences in plasma lactate concentrations were found between the GLU and WAT trials.

$T_{rec}$ and $T_{skin}$

$T_{rec}$ at rest and during exercise were similar between experimental trials (Figure 5A). $T_{rec}$ gradually increased from ~36.6 °C at rest to ~38.5 °C by the end of exercise. $T_{skin}$ responses were also similar for the 3 experimental trials (Figure 5B). $T_{skin}$ increased during the first 30–45 min of exercise and reached a steady-state value of around 34 °C. No differences in $T_{rec}$ and $T_{skin}$ were found between the three experimental trials.

Plasma deuterium enrichment

Plasma deuterium enrichments increased significantly ($P<0.05$) over time in all trials (Figure 6). The plasma deuterium enrichments were significantly higher ($P<0.05$) in WAT and GLU+FRUC compared with GLU. At 60 and 75 min of exercise, plasma deuterium enrichments
were higher \((P<0.05)\) in WAT trial compared with GLU+FRUC. No significant differences were observed in plasma deuterium enrichments between WAT and GLU+FRUC during the final 15 min of exercise.

**PV changes, HR, sweat rate,**

PV declined by about 7-9% within the first 45 min of exercise \((P<0.05; \text{main effect of time})\) and remained relatively stable thereafter for the remaining 75 min of exercise in all trials. There were no differences in PV changes during exercise between trials. HR at rest was similar for the three experimental trials (on average 54±2 beats/min). However, the average HR value during exercise was significantly higher \((P<0.05)\) in GLU (146±2 beats/min) compared with WAT (141±3 beats/min) and GLU+FRUC (141±2 beats/min). Pre- and post-exercise body mass was not significantly different between trials (WAT, 73.7±2.7 and 72.8±2.7 kg; GLU, 73.5±2.7 and 72.7±2.6 kg and GLU+FRUC, 73.5±2.7 and 72.8±2.6 kg). The average sweat rates during exercise were similar among experimental trials (WAT, 1.13±0.06 L/h; GLU, 1.12±0.09 L/h and GLU+FRUC, 1.15±0.08 L/h).

**Plasma osmolality and urine production and osmolality**

Plasma osmolality at rest was similar between trials (mean and range values for WAT: 287 and 279-293 mosmol/kg, GLU: 289 and 287-296 mosmol/kg and GLU+FRUC: 288 and 285-291 mosmol/kg). Plasma osmolality increased during exercise in all trials \((P<0.05; \text{main effect of time})\). Plasma osmolality during exercise was lower \((P<0.05; \text{main effect of trial})\) in WAT compared with the two CHO trials. No differences in plasma osmolality were found between the
GLU and the GLU+FRUC trials. There were no differences between trials in the first urine produced (WAT, 508±76 ml; GLU, 732±102 ml and GLU+FRUC, 675±95 ml) and the urine produced during exercise (WAT, 486±106 ml; GLU, 414±84 ml and GLU+FRUC, 362±89 ml; n=7). In addition, there were no differences between trials in the osmolality of the first urine produced (WAT, 545±63 mosmol/kg; GLU, 495±62 mosmol/kg and GLU+FRUC, 438±57 mosmol/kg).

**RPE and GI discomfort**

No significant differences in RPElegs were observed between the three experimental trials. However, RPEoverall at the end of exercise was significantly higher (P<0.05) in GLU (12.8±0.8) compared with GLU+FRUC (11.5±0.4). The mean values for RPE overall and RPE legs during 120-min of exercise were 11.7±0.2 and 12.0±0.3, respectively. The most frequently reported (nonsevere) complaints were urge to urinate and belching. Two subjects reported severe GI discomfort (nausea, bloated feeling and urge to vomit) in the GLU, while there were no severe complaints in the GLU+FRUC and the WAT trials.
DISCUSSION

In the present study, ingestion of glucose+fructose in non-acclimated athletes exercising in heat resulted in ~36% higher oxidation rates compared with the ingestion of glucose only, which is in agreement with earlier findings (1, 22, 23, 26). Although direct evidence is lacking, it is has been suggested that the higher exogenous CHO oxidation rates following combined ingestion of glucose+fructose (23) (and other combination of CHO’s (21, 24)) is due to a higher intestinal CHO transport rate, which might increase the availability of exogenous CHO for oxidation. Furthermore, a high peak exogenous CHO oxidation rate was reached when a mixture of glucose+fructose was ingested (1.15±0.05 g/min). Although it may be difficult to compare results between different studies, the peak oxidation rate of the ingested glucose+fructose in the present study was ~18% lower than the peak oxidation rate observed when a similar rate of glucose+fructose was ingested during exercise performed at a similar work rate (50% Wmax) in a thermoneutral environment (1.40±0.08 g/min; (26), which supports the results of our previous study (20). Interestingly, the oxidation rate of the glucose+fructose beverage in the present study is the highest exogenous CHO oxidation rate ever reported in the literature when exercise is performed in the heat.

The high exogenous CHO oxidation rates following ingestion of GLU+FRUC resulted in lower endogenous CHO oxidation rates when compared with the ingestion of WAT. Whether the lower endogenous CHO oxidation rate was due to a reduced muscle glycogen oxidation or a reduced liver glycogen oxidation or both could not be determined in the present study. Calculations of liver-derived glucose and muscle glycogen oxidation from indirect calorimetry and stable isotope methodology ($^{13}$CO$_2$ and plasma [$^{2}$H]-glucose enrichments) can not be done.
when glucose and fructose are ingested simultaneously. Ingestion of fructose in combination with other carbohydrates (present study and (22, 23, 26) or when ingested alone (30, 31) has been shown to result in elevated lactate concentrations. In a study by van Hall et al. (48) it was shown that active skeletal muscle is able to take up and oxidize large amounts of lactate produced during exercise. It is possible that increased lactate concentrations in the GLU+FRUC trial have contributed to increased lactate oxidation (33, 41). Increased oxidation of lactate derived from fructose would not affect $R_d$ glucose but would result in increased expired $^{13}$CO$_2$. More research is needed to investigate the effect of GLU+FRUC ingestion on lactate metabolism during prolonged exercise in the heat.

In the present study, the rate of muscle glycogen oxidation was not different between the GLU and the WAT trial. This finding is in agreement with the results of a study by Angus et al. (2) and the majority of studies performed in cool or thermoneutral environments (4, 7, 18, 29). Interestingly, in a study by Yaspelkis et al. (51) it was shown that ingestion of a maltodextrin+fructose mixture (2.1:1) at a rate of ~1.2 g/min resulted in a reduced muscle glycogen breakdown during prolonged exercise in the heat. It should be noted that the rate of exogenous CHO oxidation was not measured in the study of Yaspelkis et al. (51). In the present study, GLU+FRUC ingestion resulted in lower endogenous CHO oxidation rates, which supports the data of Yaspelkis et al. (51). Although speculative, the present results and those of Yaspelkis et al. (51) suggest that the ingestion of sufficient amounts (1.2-1.5 g/min) of fructose +glucose/maltodextrin during exercise in the heat may reduce endogenous CHO oxidation (muscle and/or liver glycogen) most likely as a result of an increased availability of exogenous CHO for oxidation.
Studies have shown that glucose ingestion during exercise in thermoneutral environments, suppresses or even completely blocks HGP (4, 29) and reduces or inhibits liver-derived glucose oxidation (29). In the present study, there was no difference in liver-derived glucose oxidation rates between GLU and WAT. This finding seems to support the data of Angus et al. (2), who found similar rates of HGP when either water or glucose (~1 g/min) was ingested during 60 min of exercise in the heat. Angus et al. (2) suggested that during exercise in the heat there is a marked feed-forward stimulation of liver glucose production, which is less sensitive to inhibition by increased blood glucose availability. Although the exact mechanism remains to be investigated, the present data and those of Angus et al. (2) suggest that glucose ingestion at moderate intake rates (1.0-1.5 g/min) does not reduce HGP and liver-derived glucose oxidation during exercise in the heat (2). Because liver-derived glucose and muscle glycogen oxidation rates were not different between GLU and WAT, the higher CHO oxidation rate in GLU could be fully attributed to the oxidation of ingested glucose.

Glucose ingestion in the heat increased glucose uptake (Rd glucose) and MCR when compared with the ingestion of water. These findings are in agreement with several other studies performed in thermoneutral ambient conditions (27, 29). The increased glucose concentrations may have been partly responsible for the increased glucose uptake in GLU (mass action may drive glucose into the cell (27, 29). When glucose is ingested during exercise there may be more GLUT-4 translocation and a higher activity of oxidative enzymes (i.e. phosphofructokinase and/or pyruvate dehydrogenase) which may eventually lead to an increased glucose uptake (16).

The accumulation of deuterium oxide in plasma following ingestion of deuterium oxide-labelled beverages has often been used as a qualitative marker of fluid availability (9, 10, 26, 42). An interesting finding of the present study was that ingestion of a GLU drink resulted in lower
plasma deuterium oxide enrichments compared with the ingestion of GLU+FRUC and WAT. In addition, while the plasma deuterium oxide enrichment was lower during the first 30 min (t=60-90 min) following ingestion of GLU+FRUC compared with ingestion of WAT, no difference in deuterium oxide enrichment was observed between GLU+FRUC and WAT thereafter. The present results support the data of a study by Jeukendrup et al. (26) and the work of Davis et al. (9, 10) and suggests that fluid availability during exercise in the heat is lower with a GLU drink compared with a GLU+FRUC drink or WAT. Our data are also in agreement with the findings of Shi et al. (44) who demonstrated in humans at rest using the segmental perfusion technique that ingestion of a glucose+fructose solution resulted in greater water absorption than a glucose solution. Interestingly, the average HR in GLU was lower when compared with WAT and GLU+FRUC. Moreover, PV changes were greater in GLU (~28-38%) although this was not significantly different. Together these findings suggests that a GLU drink may be less effective for fluid replacement during exercise in the heat.

As mentioned earlier, glucose and fructose use different transport mechanisms for absorption and hence combined ingestion of glucose and fructose might increase the intestinal CHO transport capacity (44). A higher intestinal CHO absorption rate may increase the availability of CHO in the bloodstream for oxidation. Furthermore, increased intestinal solute (i.e. CHO) transport will promote greater fluid absorption (15, 32, 44). Therefore, the higher fluid availability and exogenous CHO oxidation rates in GLU+FRUC compared with GLU could be due, at least in part, to a higher intestinal CHO and fluid absorption rate. It is also possible that the differences observed between GLU+FRUC and GLU are (partly) due to differences in the rate of gastric emptying. Some studies have shown that ingestion of fructose empties faster from the stomach than ingestion of glucose (35, 46). Although speculative, it is likely that both GE and
intestinal absorption were lower in GLU compared with GLU+FRUC. A lower rate of gastric emptying and intestinal absorption may result in an increased accumulation of more CHO and fluid in the GI tract which could also explain why some subjects reported severe GI discomfort in the GLU trial. The higher incidence of GI-discomfort in GLU compared with GLU+FRUC supports our previous findings (23).

In conclusion, the present data demonstrate that ingestion of GLU+FRUC results in high oxidation rates (>1.0 g/min) and reduces endogenous CHO oxidation (by ~26%) when compared with WAT. In addition, fluid availability with a GLU+FRUC is higher in comparison with a GLU beverage but appears to be slightly lower compared with WAT. Therefore, ingestion of a GLU+FRUC beverage may be beneficial for exercise performance in the heat as it results in high energy delivery and fluid availability.
ACKNOWLEDGEMENTS

Dr Roy L.P.G. Jentjens is currently appointed to the Nestlé Research Center, Nutrition & Health Department, PO Box 44, CH-1000 Lausanne 26, Switzerland.
GRANTS

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References


Table 1.

Mean oxygen uptake (VO₂), respiratory exchange ratio (RER), total carbohydrate (CHO) oxidation (CHOtot), total fat oxidation (FATtot), endogenous carbohydrate (CHO) oxidation, and exogenous CHO oxidation during the 60- to 90-min and 90- to 120-min period of exercise with ingestion of WAT, GLU and GLU+FRUC.

<table>
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<tr>
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<th>VO₂ (L/min)</th>
<th>RER</th>
<th>CHOtot (g/min)</th>
<th>FATtot (g/min)</th>
<th>Endogenous CHO oxidation (g/min)</th>
<th>Exogenous CHO oxidation (g/min)</th>
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<tr>
<td><strong>WAT</strong></td>
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<td>60-90</td>
<td>3.09±0.08</td>
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<td>1.12±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
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WAT, ingestion of water only; GLU, ingestion of glucose; GLU+FRUC, ingestion of glucose and fructose. Data are presented as means ± SE; n=8. <sup>b</sup> denotes significant difference between GLU+FRUC and GLU (P<0.05); <sup>c</sup> denotes significantly different from WAT (P<0.05).
Table 2
The effects of ingestion of glucose (GLU) or water (WAT) on various aspects of carbohydrate (CHO) metabolism during exercise in the heat (60- to 120-min exercise period), including total CHO oxidation (CHOtot), exogenous CHO oxidation, muscle glycogen and liver-derived glucose oxidation.

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<th>WAT</th>
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<tr>
<td>CHOtot (µmol/kg/min)</td>
<td>110±11</td>
<td>159±9c</td>
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<td>Exogenous CHO (µmol/kg/min)</td>
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<td>53±8</td>
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<tr>
<td>Muscle glycogen oxidation</td>
<td>69±12</td>
<td>80±11</td>
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<tr>
<td>(µmol/kg/min)</td>
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<tr>
<td>Liver-derived glucose oxidation</td>
<td>41±4</td>
<td>27±7</td>
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Data are presented as means ± SE; n=6. c denotes significantly different from WAT (P<0.05).
Figures legends

Figure 1: Breath $^{13}$CO$_2$ enrichment (A) and exogenous carbohydrate oxidation (B) during exercise in the heat without ingestion of carbohydrate (WAT), with ingestion of glucose (GLU) or with ingestion of glucose+fructose (GLU+FRUC). Values are means ± SE; n=8.

a denotes significant difference between WAT and CHO trials ($P<0.05$);
b denotes significant difference between GLU+FRUC and GLU ($P<0.05$).

Figure 2: Relative contributions of substrates to total energy expenditure calculated for the 60-120 min period of exercise in the heat without ingestion of carbohydrate (WAT), with ingestion of glucose (GLU) or with ingestion of glucose+fructose (GLU+FRUC). Values are means ± SE; n=8.

b denotes significant difference between GLU+FRUC and GLU ($P<0.05$);
c denotes significantly different from WAT ($P<0.05$).

Figure 3: The rate of appearance of plasma glucose (R$_a$ glucose; A), the rate of disappearance of plasma glucose (R$_d$ glucose; A) and metabolic clearance rate (MCR; B) during exercise in the heat without ingestion of carbohydrate (WAT) or with ingestion of glucose (GLU). Values are means ± SE; n=6.

c denotes significantly different from WAT ($P<0.05$).

Figure 4: Plasma glucose (A) and lactate (B) during exercise in the heat without ingestion of carbohydrate (WAT), with ingestion of glucose (GLU) or with ingestion of glucose+fructose (GLU+FRUC). Values are means ± SE; n=8.

a denotes significant difference between WAT and CHO trials ($P<0.05$);
d denotes GLU+FRUC significantly different from GLU and WAT ($P<0.05$).

Figure 5: Rectal temperature (A) and weighted mean skin temperature (B) during exercise without ingestion of carbohydrate (WAT), with ingestion of glucose (GLU) or with ingestion of glucose+fructose (GLU+FRUC). Values are means ± SE; n=8.
Figure 6: Plasma $^2$H$_2$O enrichment during exercise in the heat without ingestion of carbohydrate (WAT), with ingestion of glucose (GLU) or with ingestion of glucose+fructose (GLU+FRUC). Values are means ± SE; $n$=8.

e denotes GLU significantly different from GLU+FRUC and WAT ($P<0.05$); 
f denotes WAT significantly different from GLU+FRUC ($P<0.05$).
Figure 1

A

Breath $^{13}$CO$_2$ enrichment ($\delta$ per mil versus PDB)

- GLU+FRUC
- GLU
- WAT

Time (min)

B

Exogenous carbohydrate oxidation (g/min)

- GLU+FRUC
- GLU

Time (min)
Figure 2

Relative contributions of substrates to total energy expenditures (%)

- Fat
- Exogenous carbohydrate
- Endogenous carbohydrate

<table>
<thead>
<tr>
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<th>WAT</th>
<th>GLU</th>
<th>GLU+FRUC</th>
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<td>Fat</td>
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<tr>
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Figure 3

A

- Ra glucose GLU
- Rd glucose GLU
- Ra glucose WAT
- Rd glucose WAT

B

- GLU
- WAT
Figure 5

A

Rectal temperature (°C)

- GLU+FRUC
- GLU
- WAT

Time (min)

B

Weighted mean skin temperature (°C)

- GLU+FRUC
- GLU
- WAT

Time (min)
Figure 6

Plasma $^2$H$_2$O enrichment (ppm) compared to $t=60$ min sample

- WAT
- GLU+FRUC
- GLU

Time (min)