Oxidation of Maltose and Trehalose during Prolonged Moderate-Intensity Exercise

MICHELLE C. VENABLES[1], FRED BROUNS[2,3], ASKER E. JEUKENDRUP[1]

[1]Human Performance Laboratory, School of Sport and Exercise Sciences, University of Birmingham, Edgbaston, UNITED KINGDOM; [2]Cargill R&D Centre Europe, Vilvoorde, BELGIUM; and [3]Department of Human Biology, Maastricht University, Maastricht, NETHERLANDS

ABSTRACT

VENABLES, M. C., F. BROUNS, and A. E. JEUKENDRUP. Oxidation of Maltose and Trehalose during Prolonged Moderate-Intensity Exercise. *Med. Sci. Sports Exerc.*, Vol. 40, No. 9, pp. 1653–1659, 2008. **Purpose:** The aim of the present study was to compare the effects of trehalose (TRE) and maltose (MAL) ingestion on exogenous carbohydrate oxidation rates and blood metabolite responses during prolonged moderate-intensity cycling exercise. **Methods:** Nine trained subjects performed three randomly assigned bouts of exercise separated by at least 1 wk. Each trial consisted of 150 min of cycling at 55% of maximal power output (W_{max}) while ingesting a solution providing either 1.1 g·min^{-1} TRE, 1.1 g·min^{-1} MAL, or water (WAT). **Results:** Total carbohydrate oxidation rates were significantly higher ($P < 0.05$) in both the MAL (2.09 ± 0.18 g·min^{-1}) and TRE (1.92 ± 0.32 g·min^{-1}) trials compared with the WAT trial (1.62 ± 0.28 g·min^{-1}). Peak exogenous carbohydrate oxidation was significantly higher in the MAL trial compared with the TRE trial (1.01 ± 0.24 and 0.73 ± 0.22 g·min^{-1}, respectively, $P < 0.05$). The MAL trial resulted in significantly reduced endogenous carbohydrate oxidation rates compared with the WAT trial (1.20 ± 0.25 and 1.62 ± 0.28 g·min^{-1}, respectively, $P < 0.05$). When compared with the WAT trial, total fat oxidation for the same period was significantly reduced in both carbohydrate trials (0.91 ± 0.19, 0.68 ± 0.19, and 0.79 ± 0.19 g·min^{-1} for WAT, MAL, and TRE, respectively, $P < 0.05$) and tended to be lower in MAL compared with TRE ($P < 0.06$). **Discussion:** Both solutions maintained high plasma glucose concentrations. MAL had a "sparing" effect on endogenous carbohydrate stores. The reduced exogenous carbohydrate oxidation rate of TRE compared to MAL is probably due to a reduced enzymatic hydrolysis rate within the small intestine, causing a slower availability. **Key Words:** CARBOHYDRATE INGESTION, STABLE ISOTOPES, SUBSTRATE UTILIZATION, HYDROLYSIS

It is well established that carbohydrate feeding during moderate- to high-intensity exercise can alleviate the development of fatigue and increase endurance capacity (1,6,18). The mechanisms through which this occurs are believed to include maintenance of high blood glucose concentrations, high rates of carbohydrate oxidation (5,6), and endogenous glycogen sparing (3,22), and there is also evidence to suggest that, in addition to its metabolic effects, carbohydrate can act centrally (4,18).

Oxidation rates of ingested carbohydrate during exercise can be influenced by many factors, including timing of ingestion, amount of carbohydrate ingested, and the type of carbohydrate ingested [for review, see Refs. (13,19,20)]. Numerous studies have investigated exogenous carbohydrate oxidation during exercise when the type and amount of carbohydrate are varied. It has been demonstrated that fructose and galactose are oxidized at relatively low rates (0.41–0.50 g·min^{-1}), whereas glucose, maltose (MAL), maltodextrins, and cooked starches are oxidized at higher rates (0.75–1.1 g·min^{-1}). Interestingly, independent of the amount of carbohydrate ingested, exogenous carbohydrate oxidation rates seem to be maximal at 1.0–1.1 g·min^{-1} when a single carbohydrate is ingested (20); however, rates of exogenous carbohydrate oxidation can be increased past this upper boundary by the ingestion of multiple carbohydrates (15–17).

Trehalose (TRE) is a disaccharide similar to MAL in that it is composed of two glucose molecules. Their difference is that the glucose molecules in MAL are coupled by an α-1,4 linkage, whereas in TRE, they are bound by an α-1,1 glycosidic linkage. The different linkage between the glucose molecules gives TRE some exciting properties: TRE is only mildly sweet, approximately 45% of the sweetness of sucrose (14) and may therefore make it a more palatable solution for athletes; more importantly, it has also been reported to have a low cariogenic property when ingested either on its own or when added to other sugars (24) and could therefore have more favorable effects on dental health.

Address for correspondence: Asker E. Jeukendrup, Ph.D., Human Performance Laboratory, School of Sport and Exercise Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom; E-mail: A.E.Jeukendrup@bham.ac.uk.
Submitted for publication July 2007.
Accepted for publication March 2008.

0195-9131/08/4009-1653/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/mss.0b013e318175716c

Two different intestinal brush border enzymes, namely, trehalase and maltase–glycoamylase, hydrolyze TRE and MAL into their glucose subunits thus allowing absorption to take place. It has been reported in animal studies that the activity of trehalase can be 10 times lower than that of maltase–glycoamylase (8,9). However, no studies exist regarding *in vivo* digestion/absorption of these carbohydrates in humans. It has been suggested that exogenous oxidation rates can be used as a surrogate marker of absorption. If TRE is indeed slowly digested and absorbed, this could also affect postabsorptive glucose supply and insulin secretion, which in turn could affect substrate utilization by favoring more lipid oxidation. Accordingly, we hypothesized that 1) TRE ingestion during exercise will induce insulinemic responses that are lower than after consumption of MAL, 2) the oxidation of TRE will be lower compared with MAL, and 3) a reduced rate of glucose appearance along with reduced insulin secretion should increase the ratio of lipid to carbohydrate oxidation.

METHODS

Subjects. Nine trained male cyclists or triathletes (age 28 ± 5 yr, body mass 75.5 ± 7.4 kg, fat mass 9.3 ± 2.9 kg, fat-free mass 67.0 ± 8.1 kg, maximal oxygen uptake [$\dot{V}O_{2max}$] 64.5 ± 4.7 mL·kg^{-1}·min^{-1}, maximal power output [W_{max}] 364 ± 52 W [mean ± SD]) participated in the study that was approved by the ethics subcommittee of the School of Sport and Exercise Sciences at the University of Birmingham, UK. Subjects were healthy on the basis of a general health questionnaire; all were informed of the purpose and nature of the study and the potential risks involved, after which their written informed consent was given.

Preliminary testing. At least 1 wk before the start of the experimental trials, subjects performed an incremental exercise test to volitional exhaustion on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). Subjects started cycling at 95 W for 3 min, followed by incremental steps of 35 W every 3 min until exhaustion. The W_{max} was determined from the following equation (adapted from Kuipers et al. (23)):

$$W_{max} = W_{out}[(t/180) \times 35]$$

where W_{out} is the power output of the last completed stage and t is the time in seconds spent in the final stage. W_{max} values were used to determine the workload (55% W_{max}) used in the later experimental trials. Breath-by-breath measurements were taken throughout the exercise using an Oxycon Pro automated gas-analysis system (Jaeger, Wuerzeburg, Germany). The gas analyzers were calibrated using a 4.95% CO_2–95.05% N_2 gas mixture (BOC Gases, Surrey, UK), and the volume transducer was calibrated with a 3-L calibration syringe. Heart rate (HR) was measured continuously by telemetry using a Polar Vantage HR monitor (Polar Electro Oy, Kempele, Finland). $\dot{V}O_2$ was considered to be maximal if two of the three following conditions were met: 1) a leveling off of $\dot{V}O_2$ with further increasing workloads (an increase of no more than 2 mL·kg^{-1}·min^{-1}), 2) an HR within 10 beats·min^{-1} of the age-predicted maximum (220 bpm − age), and 3) a respiratory exchange ratio (RER) greater than 1.05.

Experimental design. All subjects completed three exercise trials that were randomly assigned and separated by at least 1 wk. Each trial consisted of cycling for 150 min at 55% W_{max} while ingesting either an 8.5% MAL or TRE solution or water (WAT).

Carbohydrate drinks. To quantify exogenous carbohydrate oxidation, MAL and TRE were produced from corn-derived sources that are known to have a natural high abundance of ^{13}C. The MAL (SPI Pharma, Lewes, DE) and TRE (Cargill, Inc., Vilvoorde, Belgium) used for the drinks had ^{13}C enrichments of −10.35 δ‰ and −11.77 δ‰ versus Pee Dee Bellemnitella (PDB), respectively. The ^{13}C enrichments of the carbohydrate drinks were determined by continuous flow–isotope ratio mass spectroscopy (CF–IRMS; Europa Scientific, Crewe, UK). The drinks consisted of 165 g of carbohydrate aiming to deliver 1.1 g carbohydrate·min^{-1}, dissolved in water up to a volume of 1950 mL, resulting in an 8.5% solution (w/v). Sodium chloride 2.28 g was added to create a 20-mmol·L^{-1} solution.

Diet and exercise. Subjects were asked to record their dietary and activity patterns for the 3 d preceding the first trial and replicate this before the other two trials. In addition, subjects were instructed to perform a bout of glycogen-depleting exercise 5–7 d before the first trial in an attempt to remove any endogenous ^{13}C-enriched glycogen stores. Furthermore, in an attempt to reduce the background shift in $^{13}CO_2$ that may result from changes in endogenous substrate utilization, subjects were asked to maintain a diet from C_3 plant carbohydrate sources with natural low ^{13}C abundance, while avoiding C_4 plant carbohydrate sources (maize, cane sugar) that have a natural high abundance of ^{13}C during the entire experimental period. Previous studies have shown that the dietary intervention performed in the present study is effective in reducing the background shift from endogenous substrate stores in European subjects (31).

Protocol. Subjects reported to the Human Performance Laboratory between 7:00 and 9:00 a.m. after an overnight fast (10 h) and having avoided strenuous exercise and alcohol for the preceding 24 h. On arrival, a 21-gauge Teflon catheter (Quickcath; Baxter, Norfolk, UK) was inserted in an antecubital vein and was attached to a three-way stopcock (Sims Portex, Kingsmead, UK) for blood sampling. The catheter was kept patent by flushing with 1.0 to 1.5 mL of isotonic saline (0.9%; Baxter) after each sample collection.

After voiding, the subject was weighed in cycling shorts to the nearest 0.1 kg on platform scales (Seca Alpha, Hamburg, Germany). The subjects then mounted the cycle ergometer, and duplicate resting breath sample were collected directly

TABLE 1. Oxygen uptake, RER, total fat oxidation, total carbohydrate oxidation, endogenous carbohydrate oxidation, and exogenous carbohydrate oxidation during the 60- to 150-min period of cycling exercise.

	Trial		
	WAT	MAL	TRE
$\dot{V}O_2$ (L·min^{-1})	3.13 ± 0.40	3.06 ± 0.43	3.15 ± 0.43
RER	0.83 ± 0.02	0.87 ± 0.02*	0.85 ± 0.02*
Fat_{tot} (g·min^{-1})	0.91 ± 0.19	0.68 ± 0.19*,***	0.79 ± 0.19*
CHO_{tot} (g·min^{-1})	1.62 ± 0.28	2.09 ± 0.18*	1.92 ± 0.32*
Endogenous CHO (g·min^{-1})	1.62 ± 0.28	1.20 ± 0.25*	1.29 ± 0.33
Average exogenous CHO (g·min^{-1})		0.89 ± 0.23**	0.62 ± 0.17
Peak exogenous CHO (g·min^{-1})		1.01 ± 0.24**	0.73 ± 0.22

Values are mean ± SD (n = 9).
$\dot{V}O_2$, oxygen uptake; Fat_{tot}, total fat oxidation; CHO, carbohydrate; CHO_{tot}, total CHO oxidation.
* Significant difference from WAT trial, $P < 0.05$.
**Significant difference between MAL and TRE trials, $P < 0.05$.
***Trend between MAL and TRE trials, $P = 0.06$.

from a mixing chamber into 10-mL Exetainer tubes (Labco Limited, Brow Works, High Wycombe, UK). A resting blood sample was collected into a 10-mL vacutainer (Becton Dickinson, HMS, UK) and stored on ice for later centrifugation. Additional blood and expiratory breath samples were collected at 15-min intervals throughout the exercise period. $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$, and RER were measured every 15 min for periods of 4 min. HR was recorded continuously, and averages were taken of the final 5 min of each 15-min interval.

Approximately 30 min after catheterization, exercise at a workload of 55% W_{max} was started. An initial bolus of 600 mL of one of the three experimental drinks, namely, MAL, TRE, or WAT, was ingested. This was followed every 15 min by a beverage volume of 150 mL. A standing floor fan was placed in front of the subject to circulate air in all trials, and the laboratory temperature was 18 ± 1.7°C (mean ± SD). Immediately after exercise, subjects voided, and after towel drying, subjects were reweighed (Seca Alpha) in cycling shorts.

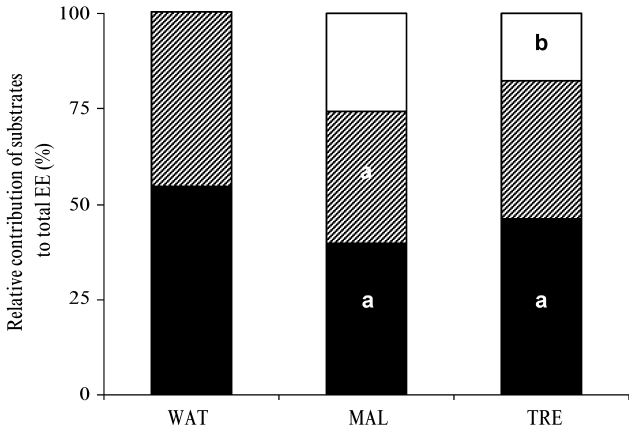

FIGURE 1—Relative contributions of fat (■), endogenous carbohydrate (▨), and exogenous carbohydrate (□) to total energy expenditure calculated for the WAT, MAL, and TRE trials, during the 60- to 150-min exercise period. Values are mean ± SE; n = 9. [a] Significant difference from the WAT trial, $P < 0.05$. [b] Significant difference between MAL and TRE trials, $P < 0.05$.

Questionnaires. Every 30 min, subjects were asked to complete two questionnaires: the Borg category scale (2) and a questionnaire regarding the presence of any gastrointestinal (GI) problems such as GI cramping, bloated feeling, diarrhea, feelings of nausea, dizziness, headaches, belching, vomiting, and urge to urinate and defecate. The possible complaints were scored on a 10-point scale (1 = not at all, 10 = very very much) and were separated into two categories, namely, severe and nonsevere. Severe complaints included nausea, bloated feeling, diarrhea, urge to vomit, and stomach and intestinal cramps because these are symptoms that can impair performance and carry health risks. These symptoms were only registered if a score of 5 or above was recorded. All other symptoms were classified as nonsevere regardless of the rating.

Biochemical analyses. Blood samples (10 mL) were collected in prechilled EDTA containing tubes (Becton Dickinson, Plymouth, Devon, UK) and centrifuged at 3500 rpm at 4°C for 10 min. Aliquots of plasma were stored at −25°C until further analyses. Approximately 1 mL of the EDTA-treated blood was used for measurements of hematocrit and hemoglobin using a Coulter® A^C T diff™ Analyzer (Beckman Coulter, Inc, Miami, FL) so that changes in plasma volume from rest could be calculated as described by Dill and Costill (10). Glucose (glucose HK125; ABX Diagnostics, Montpellier, France), lactate (lactic acid 10; ABX Diagnostics), glycerol (Scil Diagnostics, Martinsried, Germany), and free fatty acid (FFA; NEFA-C; Wako Chemicals, Neuss, Germany) were analyzed on a COBAS MIRA S plus BIO semiautomatic analyzer (la

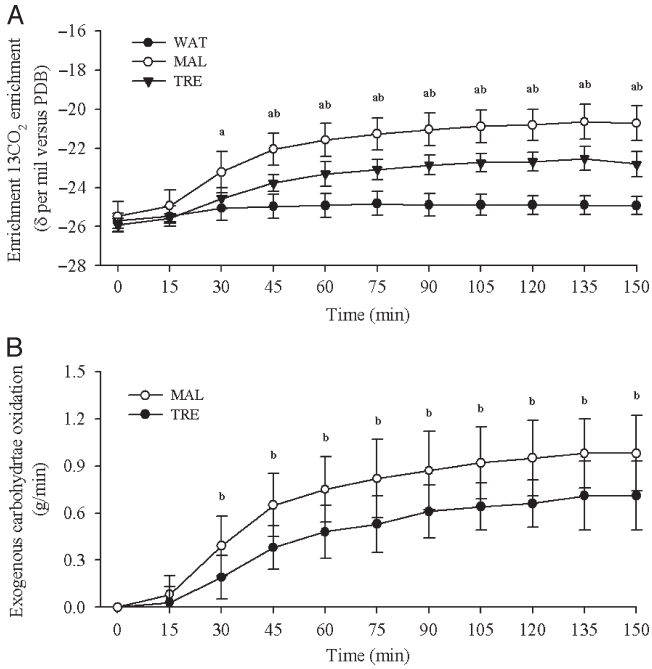

FIGURE 2—Breath $^{13}CO_2$ enrichments (A) and exogenous carbohydrate oxidation (B) during exercise at 55% of maximal power output in the MAL, TRE, and WAT trials. Values are mean ± SE; n = 9. [a] Significant difference between WAT and carbohyrate trials. [b] Significant difference between MAL and TRE trials, $P < 0.05$.

Roche, Basel, Switzerland). Insulin was analyzed by enzyme-linked immunosorbent assay (ELISA DX EIA-2935; IDS Limited, Bolden, UK). Breath samples were analyzed for $^{13}C/^{12}C$ ratios by CF–IRMS (Europa Scientific). From indirect calorimetry ($\dot{V}O_2$ and $\dot{V}CO_2$) and stable isotope measurements (breath $^{13}CO_2/^{12}CO_2$ ratio), oxidation rates of total fat, total carbohydrate, and exogenous MAL or TRE were calculated.

Calculations. Calculations for the determination of endogenous and exogenous substrate utilization during exercise included equations from Jeukendrup and Wallis (21), Craig (7), and Pirnay et al. (27) and have been used previously in our laboratory (17).

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 (29). However, during exercise, the CO_2 production increases several-fold 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. Recovery of $^{13}CO_2$ from ^{13}C carbohydrate oxidation will approach 100% after 60 min of exercise when dilution in the bicarbonate pool becomes negligible (26). Therefore, data from the initial 60 min cannot be used for the calculation of exogenous carbohydrate oxidation. Because of this, all calculations on substrate oxidation were performed during the final 90 min of exercise (60–150 min).

Statistics. A two-way (time × trial) repeated-measures ANOVA was used to compare differences in substrate utilization and in blood-related parameters over time among the three trials. Tukey *post hoc* was applied in the event of a significant *F* ratio. GI questionnaire data among trials were

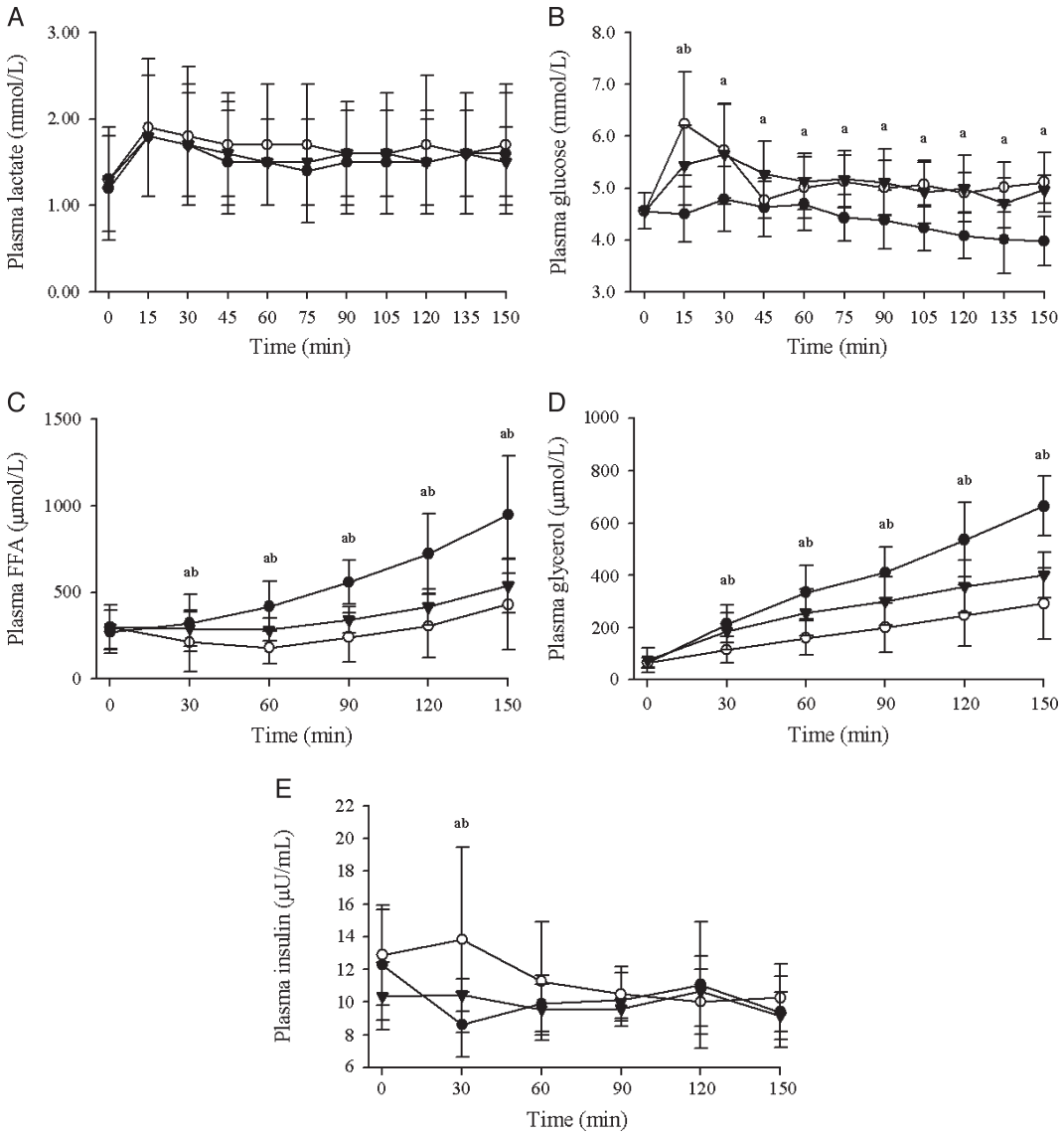

FIGURE 3—Plasma lactate (*A*), glucose (*B*), FFA (*C*), glycerol (*D*), and insulin (*E*) concentrations during 150 min of cycling exercise at 55% of maximal power output in WAT (●), MAL (○), and TRE (▼) trials. Values are mean ± SE; n = 9, except for insulin which is n = 6. [a] Significant difference between WAT and carbohydrate trials. [b] Significant difference between MAL and TRE trials, $P < 0.05$.

TABLE 2. Frequency of reported GI complaints during 150 min of cycling exercise.

Complaint	WAT	MAL	TRE
Nonsevere			
Nausea			1
Dizziness	2	1	1
Headache	1	1	1
Flatulence		2	6
Urge to defecate			3
Urge to urinate	4	5	5
Belching	4	4	6
Severe*,**			
Vomit			2
Stomach burn			1
Bloated feeling		1	3
Stomach cramps			2
Intestine cramps			1
Diarrhea			1

Subjects rated each category every 30 min between 1 and 10, 1 being not at all and 10 being severe. Severe category symptoms were only recorded if rating reported was 5 or above.
Values displayed are number of subjects reporting a symptom.
* Significant difference from WAT trial, $P < 0.05$.
* * Significant difference between MAL and TRE trials, $P < 0.05$.

compared using a Wilcoxon signed-rank test. All statistical tests were carried out using SPSS software for Windows Version 10.0 (SPSS, Inc., Chicago, IL). All data are reported as means ± SD. Statistical significance was set at $P < 0.05$.

RESULTS

$\dot{V}O_2$, RER, total carbohydrate, and fat oxidation. Data for are reported in $\dot{V}O_2$, RER, total carbohydrate, and fat oxidation are reported in Table 1. There was no difference in $\dot{V}O_2$ among the three trials, with the average being 64% $\dot{V}O_{2max}$. RER was significantly lower in the WAT trial than either the MAL or the TRE trials ($P < 0.05$). During the final 90 min of exercise, total carbohydrate oxidation was significantly higher in both the MAL and TRE trials ($P < 0.05$) compared with the WAT trial, with total fat oxidation being suppressed in both carbohydrate trials ($P < 0.05$). Although no significant difference in total carbohydrate oxidation was observed between the MAL and TRE trials, there was a trend for total fat oxidation to be higher in the TRE trial compared with the MAL trial ($P = 0.06$). The relative contribution of substrates to total energy expenditure during this period is depicted in Figure 1.

Stable isotope measurements. Changes in the isotopic composition of expired CO_2 in response to the 150-min cycling exercise with ingestion of WAT, MAL, or TRE are shown in Figure 2A. In both carbohydrate trials, there was a significant increase in the $^{13}CO_2$ enrichment of the expired breath compared with that in the WAT trial ($P < 0.05$). The mean ± SD of $^{13}CO_2$ enrichment at rest was −25.69 ± 0.55 δ‰ versus PDB increasing to −24.94 ± 0.46, −20.71 ± 0.89, and −22.51 ± 0.66 δ‰ versus PDB in the WAT, MAL, and TRE trials, respectively. From the 30-min point onward, there was a significant difference between the MAL and TRE trials. Although the background shift in this study was small, a correction was made for the exogenous carbohydrate oxidation in the MAL and TRE trials by using the data from the WAT trial.

Exogenous and endogenous carbohydrate oxidation. Exogenous carbohydrate oxidation rates rose within the first 135 min of exercise and only leveled off within the final 15 min (Fig. 2B). During the exercise period, 60- to 150-min average exogenous oxidation rates were 0.89 ± 0.23 and 0.62 ± 0.17 $g \cdot min^{-1}$ in the MAL and TRE trials, respectively (Table 1). Peak exogenous carbohydrate oxidation rates were 1.01 ± 0.24 and 0.73 ± 0.22 $g \cdot min^{-1}$ in the MAL and TRE trials, respectively, and were reached within the final 15 min of exercise. Both the average and peak oxidation rates were significantly higher in the MAL trial compared with the TRE trial ($P < 0.05$), with the MAL trial exhibiting 30% higher oxidation rates than the TRE trial. In addition, there was a trend for endogenous carbohydrate oxidation rates to be reduced in the carbohydrate trials when compared to the WAT trial. This, however, only reached statistical significance in the MAL trial ($P < 0.05$; Table 1).

Plasma metabolites. Plasma lactate, glucose, FFA, glycerol, and insulin at rest and during exercise are shown in Figures 3A–E, respectively. No differences were seen in fasted plasma lactate, glucose, FFA, glycerol, or insulin between trials, with the average resting concentrations for each being 1.3 ± 0.6 $mmol \cdot L^{-1}$, 4.5 ± 0.3 $mmol \cdot L^{-1}$, 296 ± 120 $\mu mol \cdot L^{-1}$, 69 ± 30 $\mu mol \cdot L^{-1}$, and 11 ± 4 $\mu U \cdot mL^{-1}$, respectively. After ingestion of the carbohydrate bolus at the beginning of exercise, plasma glucose rose rapidly to peaks of 6.24 ± 0.99 $mmol \cdot L^{-1}$ at $t = 15$ min in the MAL trial and 5.65 ± 0.97 $mmol \cdot L^{-1}$ at $t = 30$ min in the TRE trial; plasma glucose then fell to fasted concentrations and remained constant throughout the exercise (Fig. 3A). In the WAT trial, plasma glucose remained constant for the first 60 min then gradually declined to a value of 3.98 ± 0.47 $mmol \cdot L^{-1}$ after 150 min of exercise. At all time points except $t = 60$ and 90 min, plasma glucose concentrations were significantly higher in both MAL and TRE trials compared with the WAT trial ($P < 0.05$). Interestingly, plasma glucose was also significantly higher in the MAL trial compared with the TRE trial at time point $t = 15$ min ($P < 0.05$). Plasma lactate concentrations were similar in all trials, rising from resting values of 1.3 ± 0.56 to a peak of 1.8 ± 0.7 $mmol \cdot L^{-1}$ at $t = 15$ min. Concentrations then fell and remained constant throughout the exercise (1.7 ± 0.7 $mmol \cdot L^{-1}$; Fig. 3B).

TABLE 3. Mean scores for GI complaints during 150 min of cycling exercise.

Complaint	WAT	MAL	TRE
Nausea	1.0 ± 0.0 (1.0–1.0)	1.0 ± 0.0 (1.0–1.0)	1.0 ± 0.1 (1.0–1.0)
Dizziness/ headache	1.2 ± 0.4 (1.0–7.0)	1.0 ± 0.1 (1.0–2.0)	1.0 ± 0.1 (1.0–3.0)
Flatulence	1.0 ± 0.0 (1.0–1.0)	1.2 ± 0.4 (1.0–4.0)	1.9 ± 1.0*,** (1.0–9.0)
Defecate	1.0 ± 0.0 (1.0–1.0)	1.0 ± 0.0 (1.0–1.0)	1.6 ± 1.5 (1.0–9.0)
Urinate	1.6 ± 0.9 (1.0–8.0)	2.4 ± 1.8 (1.0–9.0)	2.2 ± 1.2 (1.0–8.0)
Belching	1.1 ± 0.2 (1.0–4.0)	1.4 ± 0.5 (1.0–4.0)	1.8 ± 1.0*,** (1.0–6.0)
Bloated feeling	1.1 ± 0.2 (1.0–4.0)	1.4 ± 0.8 (1.0–7.0)	2.0 ± 1.2* (1.0–10.0)

Subjects rated each category every 30 min between 1 and 10, 1 being not at all and 10 being severe.
Values displayed are mean scores ± SD (range).
* Significant difference from WAT trial ($P < 0.05$).
* *Significant difference between MAL and TRE trials ($P < 0.05$).

Plasma FFA and glycerol followed very similar patterns in all three trials (Figs. 3C and D, respectively), rising continuously during the course of the exercise. From t = 30 min, plasma FFA and glycerol were significantly lower ($P < 0.05$) in both the carbohydrate trials compared with the WAT trial, and from t = 60 min, plasma FFA and glycerol were also significantly lower ($P < 0.05$) in the MAL trial compared with the TRE trial. Finally, an effect of trial was found on plasma insulin such that plasma insulin was higher in the MAL group ($P < 0.05$; Fig. 3E).

Plasma volume and body weight changes. Although there was no significant time or drink effect for either change in body mass ($-0.7 \pm 0.2\%$, $-0.6 \pm 0.2\%$, and $-0.9 \pm 0.2\%$ for WAT, MAL, and TRE, respectively) or plasma volume ($-6.7 \pm 2.0\%$, $-6.7 \pm 1.4\%$, and $-11.0 \pm 1.9\%$ for WAT, MAL, and TRE, respectively), there was a trend for TRE to result in a greater loss of plasma volume ($P = 0.08$).

Ratings of perceived exertion and GI distress. Although the reporting of GI complaints was low and mainly nonsevere, the frequency of reported GI complaints was significantly higher (Table 2; $P < 0.05$) in the TRE trial compared with either the WAT or the MAL trials. Mean scores for flatulence, belching, and bloated feeling were also significantly higher (Table 3; $P < 0.05$) during the TRE trial compared with either the WAT or the MAL trials. GI cramps were only reported in one subject who, after exercise, suffered from vomiting and diarrhea after ingesting TRE. Despite the increased number of reported GI complaints associated with the TRE trial, there was no difference among any of the three trials in ratings of perceived exertion, with the average rating being 12 ± 2.

DISCUSSION

The main finding of the present study is that, when ingested at a modest rate (1.1 $g \cdot min^{-1}$) during prolonged cycling exercise, TRE can achieve peak exogenous carbohydrate oxidation rates of 0.73 ± 0.22 $g \cdot min^{-1}$. However, the average and peak exogenous oxidation rates of TRE were approximately 27% lower than those of MAL when ingested at the same rate.

Reviews by Jeukendrup and Jentjens (19,20) have highlighted several factors that could affect exogenous carbohydrate oxidation rates, namely type and amount of the ingested carbohydrate, gastric emptying, hydrolysis, and intestinal absorption. It has been demonstrated by Hawley et al. (12) that during exercise when glucose was either infused or ingested, a greater percentage of the infused glucose was oxidized compared to when ingested, such that oxidation rates higher than 1.1 $g \cdot min^{-1}$ could be achieved. This would imply that the oxidation of exogenous carbohydrate is not limited at the muscular level; moreover, it could be limited by delivery to the systemic circulation, i.e., gastric emptying, hydrolysis, and intestinal absorption. Rehrer et al. (28) have demonstrated that the exogenous oxidation rate of some orally ingested carbohydrates (4.5% glucose, 17% glucose, and 17% maltodextrin) is not dependent on gastric emptying rate, as exogenous carbohydrate oxidation remained quite similar (31.5, 42.0, and 39.1 g) although gastric emptying varied considerably (55, 133, and 147 g). In addition, a study performed in rats has shown that the gastric emptying rate after feeding TRE was more rapid compared to after feeding MAL or glucose (9).

Intestinal digestion rate and subsequent absorption remain as potential factors limiting exogenous carbohydrate oxidation, and it is therefore not unlikely that a reduced rate of digestion–absorption of TRE is responsible for the lower oxidation rates observed in our study. Intestinal glucose transport occurs via a specific sodium-dependent glucose transporter (i.e., SGLT1), and as both MAL and TRE are composed of two subunits of glucose, we would not expect intestinal transport to differ. However, the disaccharides MAL and TRE need to be hydrolyzed into their respective monosaccharides before they can be subsequently absorbed. MAL and TRE are composed of two glucose subunits with an α-1,4 bond and an α-1,1 glycosidic bond and are hydrolyzed by different intestinal brush border enzymes, maltase–glycoamylase and trehalase (11). A reduced hydrolysis rate of either disaccharide could result in a lower rate of carbohydrate availability for absorption. In the studies by Dahlqvist (8) and Dahlqvist and Thomson (9), the enzyme activity of both maltase and trehalase was investigated, and it was found that, in the digestive tract of the pig, the activity of maltase (3.7 $U \cdot mg^{-1}$ protein in the jejunum to 4.2 $U \cdot mg^{-1}$ protein in the ileum) was up to 10 times that of trehalase (0.32 $U \cdot mg^{-1}$ protein in the jejunum down to 0.05 $U \cdot mg^{-1}$ protein in the ileum) and that as much as 20% of the fed TRE reached the large intestine unhydrolyzed. In the present study, we can infer that TRE is absorbed more slowly than MAL because glucose concentration has a much reduced and later peak; consequently, the insulin peak is reduced and fat oxidation is increased. In the present study, we have calculated that during the course of the 150 min of exercise, 111 ± 10 g of MAL and 74 ± 8 g of TRE, that is 67% and 45%, respectively, of the ingested carbohydrate were oxidized. This suggests that either a large proportion of the TRE (55%) remained unabsorbed in the GI tract or that a postabsorptive fraction of TRE was redistributed and stored in endogenous carbohydrate pools.

Ingestion of higher amounts of carbohydrate than apparently are being oxidized during exercise has been shown to lead to GI distress (30). Accordingly, the observed increase in reported symptoms of GI distress during the TRE trial may be primarily related to its relatively high dose of intake compared to its relatively slow digestion–absorption, causing a fraction of unhydrolyzed TRE to induce osmotic effects. The latter may also explain the observation that plasma volume during TRE decreased more than during MAL. Usually, unhydrolyzed TRE can be fully digested by colonic bacteria,

which leads to an increase in flatulence as we have reported in this study; however, Oku and Nakamura (25) have observed that when much TRE are not completely digested, diarrhea can be a side effect. The slow rate of hydrolysis of TRE and its possible impact on osmotic effects due to intestinal accumulation, when ingested in high amounts, could therefore explain the increased number of GI symptoms associated with its ingestion during exercise.

Although it has sometimes been suggested that a low-glycemic index carbohydrate can be beneficial for endurance performance, because of its slow release and oxidation, it could also be speculated that a lower ingestion rate of a higher-glycemic index carbohydrate at regular intervals would have similar effects. Both questions, however, have not been addressed in this study or in the available literature and would warrant future research.

In summary, we have shown that during prolonged cycling exercise, TRE ingestion is accompanied with a much reduced and later glucose peak, is oxidized at approximately 27% lower rates (when ingested at 1.1 $g \cdot min^{-1}$), and has a trend to increase fat oxidation when compared to MAL. This is most likely due to a reduced digestion rate of the disaccharide in the small intestine and may explain some of the GI symptoms.

This study was funded by a grant from Cargill, Inc, Minneapolis, MN, USA. Author Fred Brouns works for Cargill R&D Centre Europe. However, the results presented in this article have been collected and reported in an independent manner.

REFERENCES

1. Below PR, Mora-Rodriguez R, Gonzalez-Alonso J, Coyle EF. Fluid and carbohydrate ingestion independently improve performance during 1 h of intense exercise. *Med Sci Sports Exerc*. 1995; 27(2):200–10.
2. Borg G. Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med*. 1970;2:92–8.
3. Bosch AN, Dennis SC, Noakes TD. Influence of carbohydrate ingestion on fuel substrate turnover and oxidation during prolonged exercise. *J Appl Physiol*. 1994;76:2364–72.
4. Carter JM, Jeukendrup AE, Jones DA. The effect of carbohydrate mouth rinse on 1-h cycle time trial performance. *Med Sci Sports Exerc*. 2004;36(12):2107–11.
5. Coggan AR, Coyle EF. Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion. *J Appl Physiol*. 1987;63:2388–95.
6. Coyle EF, Coggan AR, Hemmert MK, Ivy JL. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol*. 1986;61:165–72.
7. Craig H. Isotopic standards for carbon and oxygen and correction factors. *Geochim Cosmochim Acta*. 1957;12:133–49.
8. Dahlqvist A. The location of carbohydrates in the digestive tract of the pig. *Biochem J*. 1961;78:282–88.
9. Dahlqvist A, Thomson DL. The digestion and absorption of maltose and trehalose by the intact rat. *Acta Physiol Scand*. 1963;59:111–25.
10. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol*. 1974;37:247–8.
11. Gudmand-Hoyer E, Skovbjerg H. Disaccharide digestion and maldigestion. *Scand J Gastroenterol Suppl*. 1996;216:111–21.
12. Hawley JA, Bosch AN, Weltan SM, Dennis SC, Noakes TD. Effects of glucose ingestion or glucose infusion on fuel substrate kinetics during prolonged exercise. *Eur J Appl Physiol Occup Physiol*. 1994;68:381–89.
13. Hawley JA, Dennis SC, Nowitz A, Brouns F, Noakes TD. Exogenous carbohydrate oxidation from maltose and glucose ingested during prolonged exercise. *Eur J Appl Physiol Occup Physiol*. 1992;64:523–7.
14. Higashiyama T. Novel functions and applications of trehalose. *Pure Appl Chem*. 2002;1263–69.
15. Jentjens RL, Achten J, Jeukendrup AE. High oxidation rates from combined carbohydrates ingested during exercise. *Med Sci Sports Exerc*. 2004;36(9):1551–58.
16. Jentjens RL, Moseley L, Waring RH, Harding LK, Jeukendrup AE. Oxidation of combined ingestion of glucose and fructose during exercise. *J Appl Physiol*. 2004;96:1277–84.
17. Jentjens RL, Venables MC, Jeukendrup AE. Oxidation of exogenous glucose, sucrose, and maltose during prolonged cycling exercise. *J Appl Physiol*. 2004;96:1285–91.
18. Jeukendrup A, Brouns F, Wagenmakers AJ, Saris WH. Carbohydrate–electrolyte feedings improve 1 h time trial cycling performance. *Int J Sports Med*. 1997;18:125–9.
19. Jeukendrup AE. Carbohydrate intake during exercise and performance. *Nutrition*. 2004;20:669–77.
20. Jeukendrup AE, Jentjens R. Oxidation of carbohydrate feedings during prolonged exercise: current thoughts, guidelines and directions for future research. *Sports Med*. 2000;29:407–24.
21. Jeukendrup AE, Wallis GA. Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int J Sports Med*. 2005;26(Suppl 1):S28–37.
22. Jeukendrup AE, Wagenmakers AJ, Stegen JH, Gijsen AP, Brouns F, Saris WH. Carbohydrate ingestion can completely suppress endogenous glucose production during exercise. *Am J Physiol*. 1999;276:E672–83.
23. Kuipers H, Keizer HA, Brouns F, Saris WH. Carbohydrate feeding and glycogen synthesis during exercise in man. *Pflugers Arch*. 1987;410:652–56.
24. Neta T, Takada K, Hirasawa M. Low-cariogenicity of trehalose as a substrate. *J Dent*. 2000;28:571–76.
25. Oku T, Nakamura S. Estimation of intestinal trehalase activity from a laxative threshold of trehalose and lactulose on healthy female subjects. *Eur J Clin Nutr*. 2000;54:783–88.
26. Pallikarakis N, Sphiris N, Lefebvre P. Influence of the bicarbonate pool and on the occurrence of $^{13}CO_2$ in exhaled air. *Eur J Appl Physiol Occup Physiol*. 1991;63:179–83.
27. Pirnay F, Lacroix M, Morosa A, Luyckx AS, Lefebvre P. Glucose oxidation during prolonged exercise evaluated with naturally labeled [^{13}C]glucose. *J Appl Physiol*. 1977;43:258–61.
28. Rehrer NJ, Wagenmakers AJ, Beckers EJ, et al. Gastric emptying, absorption, and carbohydrate oxidation during prolonged exercise. *J Appl Physiol*. 1992;72:468–75.
29. Robert JJ, Koziet J, Chauvet D, Darmaun D, Desjeux JF, Young VR. Use of ^{13}C-labeled glucose for estimating glucose oxidation: some design considerations. *J Appl Physiol*. 1987;63:1725–32.
30. Wagenmakers AJ, Brouns F, Saris WH, Halliday D. Oxidation rates of orally ingested carbohydrates during prolonged exercise in men. *J Appl Physiol*. 1993;75:2774–80.
31. Wagenmakers AJ, Rehrer NJ, Brouns F, Saris WH, Halliday D. Breath $^{13}CO_2$ background enrichment during exercise: diet-related differences between Europe and America. *J Appl Physiol*. 1993; 74:2353–57.