Carbohydrate ingestion can completely suppress endogenous glucose production during exercise

ASKER E. J. EUKENDRUP, ANTON J. M. WAGENMAKERS, JOS H. C. H. STEGEN, ANNEMIE P. GIJSSEN, FRED BROUN, AND WIM H. M. SARIS Department of Human Biology, Nutrition Research Centre, Maastricht University, 6200 MD Maastricht, The Netherlands

Carbohydrate ingestion can completely suppress endogenous glucose production during exercise. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E672–E683, 1999.—The purposes of this study were 1) to investigate the effect of carbohydrate (CHO) ingestion on endogenous glucose production (EGP) during prolonged exercise, 2) to study whether glucose appearance in the circulation could be a limiting factor for exogenous CHO oxidation, and 3) to investigate whether large CHO feedings can reduce muscle glycogen oxidation during exercise. Six well-trained subjects exercised three times for 120 min at 50% maximum workload while ingesting water (FAST), a 4% glucose solution (LO-Glc), or a 22% glucose solution (HI-Glc). A primed continuous intravenous infusion of [6,6-2H2]glucose was given, and the ingested glucose was enriched with [U-13C]glucose. Glucose ingestion significantly elevated CHO oxidation as well as the rates of appearance (Ra) and disappearance. Ra glucose equaled Ra of glucose in gut (Ra gut) during HI-Glc, whereas EGP was completely suppressed. During LO-Glc, EGP was partially suppressed, whereas Ra gut provided most of the total glucose Ra. We conclude that 1) high rates of CHO ingestion can completely block EGP, 2) Ra gut may be a limiting factor for exogenous CHO oxidation, and 3) muscle glycogen oxidation was not reduced by large glucose feedings.

stable isotopes; glucose kinetics; glycogen; endogenous glucose output; glucose metabolism; sport nutrition

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CHO INGESTION CAN COMPLETELY SUPPRESS EGP

confirmed by several other researchers. In contrast with these findings, other studies suggested that CHO ingestion may improve endurance performance by slowing the rate of muscle glycogen degradation (2, 19, 52, 53). For example, Tsintzas and colleagues (52, 53) showed that net muscle glycogen breakdown after CHO ingestion during running at 70% \( \text{VO}_{2\text{max}} \) was reduced, and this "glycogen sparing" occurred almost exclusively in type I muscle fibers.

Several explanations have been given for these apparently contradictory findings. There may be fundamental differences in metabolism between running and cycling (51). Second, caution must be exercised when drawing conclusions about muscle glycogen utilization from the analysis of glycogen concentrations in local mixed muscle samples in studies involving CHO ingestion and prolonged exercise (51). A fairly large variation in muscle glycogen content can be found when repeated biopsies are taken from the same muscle. Furthermore, during CHO ingestion, glycogen breakdown may continue at the same high rate in some muscles, while it is reduced in other muscles, and in some muscles glycogen synthesis may even occur especially at low exercise intensities. The third aim of this study therefore was to use the indirect stable-isotope tracer approach to get an estimate of muscle glycogen oxidation at the whole body level and to investigate the effect of CHO ingestion during prolonged exercise on muscle glycogen oxidation.

In summary, the aims of the present study were 1) to examine the effect of small and large CHO feedings during exercise on EGP, 2) to study whether entrance of glucose in the systemic circulation is a rate-limiting step for exogenous CHO oxidation, and 3) to investigate whether high doses of CHO feedings during exercise can reduce the rate of muscle glycogen oxidation.

To study the aforementioned questions, well-trained cyclists ingested \( ^{13} \text{C} \)-labeled CHO during 2 h of exercise, and at the same time, a primed continuous infusion of \( [6,6-\text{H}_2] \)glucose was given. From the \( [6,6-\text{H}_2] \)glucose tracer the total \( R_a \) of glucose could be determined, whereas the \( [13\text{C}] \)glucose enabled us to calculate the \( R_a \) glucose from the gut (\( R_a \) gut). By subtracting \( R_a \) gut from total \( R_a \) of glucose, an estimate of EGP was obtained.

### MATERIALS AND METHODS

**Subjects.** Six highly trained cyclists aged 21.8 ± 0.2 yr and with a weight of 72.8 ± 2.6 kg volunteered for this study. After the nature and the risks of the experimental procedures were explained to the subjects, their written informed consent was obtained. The study was submitted for approval to the local Medical Ethical Committee.

**Pretesting.** The \( \text{VO}_{2\text{max}} \) of the subjects was measured on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) during an incremental exhaustive exercise test (31) 1 wk before the first experimental trial and averaged 77 ± 2 ml·kg\(^{-1}\)·min\(^{-1}\). The maximal workload attained was 434 ± 15 W. The results of this test were used to determine the 50% \( \text{VO}_{2\text{max}} \) workload of the experimental trials.

Experimental trials. Each subject performed three exercise trials, each separated by at least 7 days. The order of the trials was determined by counterbalancing. Each trial consisted of 120 min of cycling at 50% \( \text{VO}_{2\text{max}} \). Subjects ingested drinks containing no glucose (FAST), a 4% glucose solution (LO-Glc), or a 22% glucose solution (HI-Glc). Subjects were instructed not to consume any products with a high natural abundance of \( ^{13}\text{C} \) during the entire experimental period. This is to minimize a shift in background enrichment due to changes in endogenous substrate utilization (54). Furthermore, subjects were instructed to keep their diet as constant as possible the days before the trials. All subjects performed an additional test for correction for exercise-induced changes in background enrichment with standard procedures (27–29).

**Protocol.** Subjects reported to the laboratory at 8:00 AM after an overnight fast. A Teflon catheter (Baxter Quick Cath Du Pont, Uden, The Netherlands) was inserted into an antecubital vein, and at 8:30 AM, a resting blood sample of 10 ml was drawn. Resting breath samples were collected (Oxycon \( \beta \), Mijnhardt, Mannheim, Germany), and vacutainer tubes were filled directly from the mixing chamber to determine the \( ^{13}\text{C} \)-to-\( ^{12}\text{C} \) ratio in expired CO\(_2\). At 8:50 AM, subjects started a warm-up of 5 min at 100 W. At 8:55 AM, a sodium bicarbonate prime was given (5.5 \( \mu \)mol/kg Na\(^{13}\text{CO}_3 \); Cambridge Isotope Laboratories, Andover, MA), followed by a \( [6,6-\text{H}_2] \)glucose (Cambridge Isotope Laboratories) prime. The doses of the prime were equal to the amount of isotope infused during 1 h. After the glucose pool was primed, a continuous infusion of sterile pyrogen-free \( [6,6-\text{H}_2] \)glucose was started via a calibrated IVAC 560 pump (Ivac, San Diego, CA). The concentration of isotopes in the infusate was determined for each experiment to calculate the exact infusion rates. The infusion rates were 0.526 ± 0.009, 0.775 ± 0.014, and 1.310 ± 0.041 \( \mu \)mol·kg\(^{-1}\)·min\(^{-1}\) for FAST, LO-Glc, and HI-Glc, respectively.

At 9:00 AM, the workload was increased to 50% \( \text{VO}_{2\text{max}} \) for 120 min. During the first minutes, subjects drank an initial bolus (8 ml/kg) of one of the glucose solutions. Thereafter, every 15 min, a beverage volume of 2 ml/kg was provided. This feeding schedule has been shown to result in high rates of gastric emptying (42). The average amount of glucose provided during the 120 min of exercise was ~70 g in the LO-Glc trial and 350 g in the HI-Glc trial. Blood samples were drawn at 15-min intervals until the end of exercise. Expiratory gases were collected every 15 min. Results of stable-isotope analyses will be presented for the 45- to 120-min period because those values guaranteed an isotopic steady state (Fig. 1).

**Glucose solutions.** The CHO ingested was corn-derived glucose (Amylum, Belgium) of high natural \( ^{13}\text{C} \) abundance. A small amount of \( [U-^{13}\text{C}] \)glucose (99%, Cambridge Isotope Laboratories) was added to these CHO solutions. The exact enrichment of the ingested drink was measured (after drying) by on-line combustion-isotope ratio mass spectrometry (Carlo-Erba-Finnigan MAT 252, Bremen, Germany) and was 131.69 \( \delta \)‰ vs. Pee Dee Bellemnite (PDB) for LO-Glc and −10.92 \( \delta \)‰ vs. PDB for HI-Glc. Subjects ingested 71 ± 3 g/120 min of glucose during LO-Glc and 354 ± 13 g/120 min of glucose during HI-Glc. The calculated rates of CHO ingestion during the second hour were 33 ± 1 g/60 min during LO-Glc and 164 ± 5 g/60 min during HI-Glc. The amount of water ingested was similar in all trials and averaged 1,602 ± 56 ml/120 min.

**Analysis.** Blood samples (10 ml) were collected into EDTA tubes and were immediately centrifuged for 4 min at 4°C.
Aliquots of plasma were immediately frozen in liquid nitrogen and stored at −240°C until analysis of glucose (Roche, Uni Kit III), free fatty acids (FFA; NEFA-C test kit, Wako Chemicals, Germany), glycerol (Sigma, GPO trinder 337), and triacylglycerols (plus mono- and diacylglycerols) (Sigma, GPO trinder 337), which were performed with the COBAS BIO semiautomatic analyzer (La Roche, Basel, Switzerland). Insulin was analyzed by radioimmunoassay (Nuclilab Ultrasensitive Human Insulin RIA kit).

Breath samples were analyzed for 13C-to-12C ratio by gas chromatography continuous flow isotope ratio mass spectrometry (Finnigan MAT 252, Bremen, Germany). For determination of 13C-to-12C ratios of plasma glucose, glucose was derivatized to its pentaacetate derivative with previously described procedures (57). Thereafter, the derivative was measured by gas chromatography-isotope ratio mass spectrometry (Finnigan MAT 252). By establishing the relationship between the enrichment of a series of glucose standards of variable enrichment and the enrichment of the glucose pentaacetate derivative of these standards, the enrichment of plasma glucose samples was determined. This procedure was described previously (29).

Plasma [2H]glucose enrichment was determined by gas chromatography-mass spectrometry analysis of the glucose pentaacetate derivatives on an INCOXS (Finnigan INCO XL). For [2H]glucose enrichment, ion masses of 200 and 202 were selectively monitored.

From indirect calorimetry [respiratory quotient; O2 uptake VO2] and stable-isotope measurements (13C/12C; 2H enrichment), total energy expenditure and oxidation rates of total fat, total CHO, and exogenous glucose were calculated as well as Ra and rate of disappearance (Rd) of glucose.

Calculations. From CO2 production (VCO2) and VO2 total, CHO and fat oxidation rates were calculated with the nonprotein respiratory quotient (36).

From the [2H]glucose tracer, the total Ra and Rd of glucose were calculated with the single-pool non-steady-state equations of Steele (50) as modified for use with stable isotopes (57). Total Ra glucose represents the splanchnic Ra glucose from ingested CHO and liver and potentially some kidney glycogenolysis and gluconeogenesis.

The [U-13C]glucose tracer in the drinks was used to calculate the Ra of glucose from the gut and oxidation rates. The 13C isotopic enrichment in breath and plasma was expressed as the delta per thousand difference between the 13C-to-12C ratio of the sample and a known laboratory reference standard according to the formula of Craig (12). The $δ^{13}C$ was related to the international standard PDB (PDB-1).

The Ra glucose into the plasma of the ingested [13C]glucose (gut Ra) was determined by transposition of the Steele equation and the known 13C enrichment of the ingested glucose (41) and was adapted for use with stable isotopes

$$F_2 = \frac{R_a}{(E_{p2} - E_{p1})/(t_2 - t_1) \cdot V} \cdot [(E_{p1} + E_{p2})/2 + (C_2 + C_1)/2 \cdot (E_{p2} - E_{p1})(t_2 - t_1)] \cdot (C_2 - C_1)/2$$

where $F_2$ is the Ra of [13C]glucose in the blood; Ra is the previously determined total Ra of glucose (Eq. 3); $E_{p1}$ and $E_{p2}$ are the 13C enrichments of plasma glucose at time points $t_1$ and $t_2$, respectively; and $C_1$ and $C_2$ are glucose concentrations at $t_1$ and $t_2$, respectively, and V is volume of distribution.

Knowing the Ra of [13C]glucose in the blood, one can determine the absorption rate of glucose from the gut from the known enrichment of the ingested glucose

$$R_a \text{ gut} = F_2 / E_{ing}$$

where Ra gut is the Ra of gut-derived glucose and $E_{ing}$ is the 13C enrichment of the ingested glucose.

The rate of EGP was calculated as the difference between total Ra and the Ra from the gut

$$\text{EGP} = \text{Ra total} - \text{Ra gut}$$

The $^{13}CO_2$ production ($\dot{V}^{13}CO_2$) from the tracer ingestion was calculated as

$$\dot{V}^{13}CO_2 (\mu mol \cdot kg^{-1} \cdot min^{-1}) = (E_{CO_2} - E_{eKG}) \cdot \dot{V}CO_2$$
where \( E_{CO_2} \) is the breath \(^{13}\)C-to-\(^{12}\)C ratio at a given time and \( E_{bkg} \) is the background breath \(^{13}\)C-to-\(^{12}\)C ratio at rest before glucose tracer ingestion. The conversion factor is 1 mol CO\(_2\) equals 22.4 liters.

Plasma glucose oxidation was calculated as

\[
\text{plasma glucose oxidation} = \frac{\dot{V}^{13}CO_2 /\left( (E_{pl2} - E_{pl1})/2 - E_{bkg}\right)}{(1/k)}
\]  

where \( k = 0.7467 \) liters CO\(_2\)/g glucose, which is the amount of CO\(_2\) (in liters) produced by the oxidation of 1 g of glucose.

Muscle glycogen oxidation was calculated as the difference between total CHO oxidation and plasma glucose oxidation.

Exogenous CHO oxidation was calculated with the known enrichment of the ingested glucose and the \(^{13}\)CO\(_2\)-to-\(^{12}\)CO\(_2\) ratio in breath

\[
exogenous glucose oxidation = \dot{V}CO_2 \cdot (E_{CO_2} - E_{bkg}) / (E_{ing} - E_{bkg}) \cdot (1/k)
\]  

where \( E_{bkg} \) is the \(^{13}\)C enrichment of expired air at rest, before glucose ingestion, \( E_{CO_2} \) is the \(^{13}\)C enrichment of breath during exercise at different time points, and \( E_{ing} \) is the \(^{13}\)C enrichment of the ingested glucose.

From total CHO oxidation and exogenous glucose oxidation, endogenous CHO oxidation can be calculated.

Because the endogenous glucose oxidation is derived from either muscle glycogen or liver (and kidney) glucose during exercise, endogenous glucose oxidation was calculated as the difference between endogenous CHO oxidation and muscle glycogen oxidation.

The metabolic clearance rate (MCR) was calculated as the \( R_d \) of glucose divided by the average glucose concentration over that time period

\[
\text{MCR} (\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \frac{R_d}{(C_1 + C_2)/2} 
\]

The percentage of glucose disappearing from the plasma that is oxidized was calculated as

\[
\%R_d \text{ oxidized} = \left( \frac{\text{plasma glucose oxidation}}{R_d} \right) \cdot 100\%
\]

Statistics. Throughout this study glucose kinetics were calculated between two different time points (\( t_1 \) and \( t_2 \)); in Figs. 1–8 and Tables 1 and 2, however, these values were presented as \( t_2 \). For instance, \( R_a \) at \( t = 60 \) in reality is \( R_a \) between 45 and 60 min.

A two-way (treatment \times time) analysis of variance for repeated measures was performed to study differences among the three conditions. A Bonferroni-Dunn post hoc test was applied in case of a significant (\( P, 0.05 \)) F ratio to locate the differences.

RESULTS

Plasma glucose, insulin, free fatty acids, glycerol, and triacylglycerol. In the FAST trial, plasma glucose concentrations were in the range of 4.2–4.6 mmol/l at rest and throughout the exercise bout (Fig. 2). With glucose ingestion in the HI-Glc trial, plasma glucose concentrations peaked within the first 15 min of exercise at 6.0 ± 0.2 mmol/l. Plasma glucose concentrations were higher throughout exercise with HI-Glc compared with FAST (and LO-Glc), but this only reached statistical significance at 15, 105, and 120 min (\( P < 0.05 \)).

Plasma insulin was low at rest and during exercise when subjects were fasted (i.e., 5–7 µU/ml) (Fig. 2) but was significantly elevated by glucose ingestion during exercise (Fig. 2). The highest insulin values were observed after 15 min (8 ± 2 µU/ml with LO-Glc and 11 ± 4 µU/ml with HI-Glc). Plasma insulin decreased in all trials but remained elevated in the HI-Glc trials in comparison with FAST. At 60 min, insulin was also significantly higher during HI-Glc compared with LO-Glc. In all conditions, plasma insulin decreased to or below the resting fasting level at the end of the trials. During FAST and LO-Glc, insulin levels were very low (i.e., 2–3 µU/ml) toward the end of exercise.

Resting plasma FFA concentrations were between 337 and 420 µmol/l. During the FAST trial, plasma FFA concentration initially decreased during the first 10 min and thereafter gradually increased during exercise to about three times basal level (854 ± 104 µmol/l at 120 min; Fig. 3). Plasma FFA in both glucose conditions followed a similar pattern and was suppressed during LO-Glc (to 623 ± 70 µmol/l at 120 min) compared with FAST and even more during HI-Glc (to 382 ± 70 µmol/l at 120 min; \( P < 0.05 \)). From 30 to 120 min, plasma FFA was significantly lower during HI-Glc compared with FAST.
and elicited $\sim 49 \pm 2\% \dot{V}O_{2\text{max}}$ (Table 1). The respiratory exchange ratio decreased in the FAST trial ($P < 0.05$) and remained stable with Glc ingestion. The respiratory exchange ratio was slightly higher with the HI-Glc feeding (Table 1). CHO oxidation decreased during FAST, decreased less with LO-Glc, and remained stable with HI-Glc (Fig. 4). After 120 min of exercise, total CHO oxidation rates were 100 $\pm$ 5 (1.31 $\pm$ 0.07 g/min), 118 $\pm$ 5 (1.55 $\pm$ 0.07 g/min), and 153 $\pm$ 9 µmol·kg$^{-1}$·min$^{-1}$ (2.00 $\pm$ 0.12 g/min) for FAST, LO-Glc, and HI-Glc, respectively (Fig. 4; Table 1). Total fat oxidation was markedly suppressed by the Glc feedings (Fig. 4; $P < 0.05$). After 120 min of exercise, total fat oxidation rates were 48 $\pm$ 2 (0.63 $\pm$ 0.01 g/min), 41 $\pm$ 1 (0.54 $\pm$ 0.01 g/min), and 33 $\pm$ 2 µmol·kg$^{-1}$·min$^{-1}$ (0.43 $\pm$ 0.01 g/min) for FAST, LO-Glc, and HI-Glc, respectively ($P < 0.05$).

Stable-isotope measurements. Glucose ingestion raised $^{13}$CO$_2$-$^{12}$CO$_2$ breath ratios from values around $-26.6 \delta$% vs. PDB to values between $-8$ and $-21.8\delta$% vs. PDB during LO-Glc and HI-Glc, respectively. The breath ratios during the experimental trials with [U-$^{13}$C]glucose tracer ingestion are shown in Fig. 1. Glucose $^{13}$C enrichment was elevated by the [U-$^{13}$C]glucose tracer ingestion and was stable between 45 and 120 min. Plasma glucose $^3$H enrichment was between 1.7 and 2.0%, and there was also an isotopic steady state between 45 and 120 min.

$R_a$ and $R_d$ of plasma glucose and MCR. Both $R_a$ and $R_d$ glucose were markedly elevated with glucose ingestion (45% with LO-Glc and 143% with HI-Glc; $P < 0.001$; Table 2; Fig. 5). During the second hour of exercise, no changes in $R_a$ or $R_d$ glucose were observed over time, although during HI-Glc, $R_a$ and $R_d$ glucose had a tendency to increase toward the end of exercise. This however did not reach statistical significance.

MCR was $7 \pm 1$ ml·kg$^{-1}$·min$^{-1}$ during FAST and was significantly higher during LO-Glc ($10 \pm 1$ ml·kg$^{-1}$·min$^{-1}$; $P < 0.001$) and even higher during HI-Glc ($15 \pm 1$ ml·kg$^{-1}$·min$^{-1}$; Table 2; $P < 0.001$).

Glucose $R_a$ from the gut and EGP. The $R_a$ gut was 31–32 µmol·kg$^{-1}$·min$^{-1}$ (0.41–0.42 g/min) during the second hour of the LO-Glc trials and 73–79 µmol·kg$^{-1}$·min$^{-1}$ (0.96–1.04 g/min) during the HI-Glc trial (Fig. 5; Table 2). This difference was highly significant ($P < 0.0001$). EGP was 30–32 µmol·kg$^{-1}$·min$^{-1}$ (0.40–0.42 g/min) during FAST and was suppressed by the LO-Glc feedings to 12–13 µmol·kg$^{-1}$·min$^{-1}$ (0.16–0.17 g/min). Ingestion of large amounts of glucose during exercise (HI-Glc) resulted in increased $R_a$ gut and completely suppressed EGP (Fig. 5; Table 2). The effects of CHO feedings on the $R_a$ of glucose are summarized in Fig. 6. With FAST, all glucose appearing in the circulation is from the liver; during LO-Glc, EGP is suppressed and glucose is derived both from liver and gut. With the ingestion of large amounts of glucose (HI-Glc), the $R_a$ glucose is increased even though EGP is completely blocked.

Oxidation of plasma glucose. Plasma glucose oxidation was between 22 and 26 µmol·kg$^{-1}$·min$^{-1}$ (0.31–0.34 g/min) during LO-Glc and between 58 and 72
µmol·kg⁻¹·min⁻¹ (0.76–0.94 g/min) during HI-Glc (Fig. 6; Table 1). At all times, plasma glucose oxidation was significantly higher during HI-Glc (P < 0.0001). Plasma glucose oxidation was not measured during FAST, but we assumed that it was 96–99% of Rd glucose as observed in a previous study (29). Plasma oxidation was not significantly different from Rd glucose, and the percentage of Rd oxidized was between 92 and 95% during the last 30 min of exercise.

Muscle glycogen oxidation. Muscle glycogen oxidation (total CHO oxidation – plasma glucose oxidation) did not change as a function of CHO ingestion during LO-Glc and HI-Glc and remained between 77 and 89 µmol·kg⁻¹·min⁻¹ (1.01–1.17 g/min) during the last hour of exercise (Fig. 7; Table 1). This represented ~69% of total CHO oxidation during LO-Glc and 56% of total CHO oxidation during HI-Glc.

Exogenous CHO oxidation. Oxidation rates of the ingested CHO were also relatively stable during the second hour of exercise (Fig. 7, Table 1). During LO-Glc, exogenous glucose oxidation rates were between 22 and 26 µmol·kg⁻¹·min⁻¹ (0.31–0.34 g/min), and this was increased to 58–72 µmol·kg⁻¹·min⁻¹ (0.76–0.94 g/min) during HI-Glc. An overview of substrate oxidation is given in Fig. 8.

DISCUSSION

Effect of CHO ingestion on EGP. Ingestion of moderate amounts of glucose (35 g/60 min) markedly suppressed EGP. With the ingestion of large amounts of glucose (175 g/60 min), EGP was completely blocked and all glucose appearing in the plasma originated from the ingested CHO. With the use of a [6-3H]glucose and a [6,6-2H]glucose tracer, McConell et al. (34) found a 51% reduction in EGP when 200 g of CHO were
Table 2. $R_a$ and $R_d$ of glucose, the $R_a$ gut, HGP, MCR, and percentage of $R_d$ glucose that was oxidized

<table>
<thead>
<tr>
<th>Time Interval, min</th>
<th>$R_a$ Glucose, $\mu$mol·kg$^{-1}$·min$^{-1}$</th>
<th>$R_d$ Glucose, $\mu$mol·kg$^{-1}$·min$^{-1}$</th>
<th>$R_a$ Gut, $\mu$mol·kg$^{-1}$·min$^{-1}$</th>
<th>HGP, ml·kg$^{-1}$·min$^{-1}$</th>
<th>MCR, ml·kg$^{-1}$·min$^{-1}$</th>
<th>%$R_d$ Oxidized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FAST</td>
<td>LO-Glc</td>
<td>HI-Glc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75–90</td>
<td>$31 \pm 3$</td>
<td>$45 \pm 2^+$</td>
<td>$74 \pm 3^+$</td>
<td>$0 \pm 0$</td>
<td>$13 \pm 1^+$</td>
<td>$15 \pm 1^+$</td>
</tr>
<tr>
<td>90–105</td>
<td>$31 \pm 3$</td>
<td>$44 \pm 2^+$</td>
<td>$77 \pm 4^+$</td>
<td>$0 \pm 0^+$</td>
<td>$12 \pm 1$</td>
<td>$15 \pm 1^+$</td>
</tr>
<tr>
<td>105–120</td>
<td>$32 \pm 3$</td>
<td>$43 \pm 3^+$</td>
<td>$79 \pm 4^+$</td>
<td>$0 \pm 0^+$</td>
<td>$10 \pm 1$</td>
<td>$16 \pm 1^+$</td>
</tr>
</tbody>
</table>

Values are means ± SE. $R_a$ and $R_d$, rates of appearance and disappearance, respectively; $R_a$ gut, rate of appearance of glucose in gut; HGP, hepatic glucose production; MCR, metabolic clearance rate. *Significant difference between HI-Glc and FAST; †significant difference between LO-Glc and FAST; ‡significant difference between HI-Glc and LO-Glc; P < 0.001.

Ingested during 2 h of exercise at 69% $V_{O2,max}$ (100 g CHO/h). Similarly, Bosch et al. (3) reported a 70% reduction in EGP when 150 g of CHO were ingested during 3 h of exercise at 70% $V_{O2,max}$ (50 g CHO/h).

These results are comparable with the LO-Glc trial in the present study. In their study (3), the exercise intensity, and thus the absolute rates of EGP, were higher than in the present study. To our knowledge, no study has shown previously that ingestion of large amounts of glucose can completely suppress EGP. There may be several reasons for this suppression. Although the present tracer method cannot distinguish between liver glycogenolysis and gluconeogenesis, it is likely that during LO-Glc both processes were inhibited. During HI-Glc, both gluconeogenesis and glycogenolysis were blocked. We suspect that the effect of glucose ingestion is mainly on glycogenolysis because gluconeogenesis may be negligible in the present conditions. Previously, we used two different isotopic tracers to estimate gluconeogenesis (glucose carbon recycling) in an identical experimental setup as the present study (29), and it was concluded that gluconeogenesis was negligible in these conditions. It has been suggested that increases in plasma glucose concentration and plasma insulin levels are major factors reducing EGP during exercise. However, in the present study, the plasma glucose concentration was only slightly higher during HI-Glc compared with LO-Glc and FAST. Although it has been shown that plasma glucose may inhibit EGP directly (26, 46), plasma glucose concentrations were much higher in those studies compared with the present study in which concentrations during the second hour of exercise were always between 4.5 and 5.5 mmol/l. Increased insulin levels may be a more reasonable explanation for the reduced EGP after CHO ingestion. It has been reported that insulin or an increased insulin-to-glucagon ratio can inhibit EGP (55). Insulin levels were significantly elevated during exercise with HI-Glc, and it is possible that even small changes in insulin have a potent effect on EGP. In this study, we did not measure glucagon or epinephrine or cortisol. McConell et al. (34), however, showed that during the second hour of exercise, glucagon and epinephrine levels were lower after CHO ingestion, and they suggest that these factors may also have contributed to the reduction in EGP. Recently, Hевенер et al. (24) demonstrated that glucosensors in the portal vein are largely responsible for the detection of the portal glucose concentration, which then results in a portal-sympathetic glucoregulatory reflex (i.e., reduced or increased hepatic glucose production). Whatever the mechanism, the present study suggests strong feedback regulation of EGP that helps maintain plasma glucose concentration in a narrow range.

Glucose uptake and MCR are markedly increased by glucose ingestion. A second important factor that helps maintain plasma glucose concentration when large amounts of glucose are ingested is the increased glucose uptake. It has been reported that at low exercise intensities, glucose ingestion increases leg glucose uptake (1). Here, the $R_d$ glucose increased along with the total $R_a$ glucose (EGP and $R_a$ gut). The mechanisms behind this increased glucose uptake are largely unknown but may, in part, be due to increased glucose concentration that through mass action may drive glucose into the cells (21).

However, our observation that MCR ($R_d$/plasma glucose concentration) was significantly increased with CHO feedings does not support this notion. The increased MCR with increasing amounts of ingested CHO suggests that plasma glucose availability (i.e., concentration) is not the sole driving force for the increase in plasma glucose uptake and oxidation during exercise with CHO ingestion. Plasma insulin was higher during exercise during HI-Glc, and contractions and insulin have been shown to have strong synergistic effects on muscle glucose uptake during exercise in humans (13, 56). Higher insulin levels and contraction both stimulate GLUT-4 translocation, but they appear to activate different pools of transporters or differentially activate the same pool (39, 40). It has also been shown that higher insulin levels during exercise in
Combination with lower fatty acid levels may activate phosphofructokinase and pyruvate dehydrogenase; this may lead to lower glucose 6-phosphate concentration, which, in turn, will increase glucose uptake (17). Another factor that may have contributed to the increased glucose uptake and MCR may be the lower fatty acid levels. CHO ingestion during low- to moderate-intensity exercise inhibits lipolysis (11, 25) and thereby attenuates the increase in fatty acid concentration. Increases in plasma fatty acid levels may inhibit muscle glucose uptake directly by yet unknown mechanisms (20), although others did not find an effect of plasma fatty acid concentration on glucose uptake during exercise at 60% VO2max (35).

It is unlikely, however, that the lower plasma fatty acid levels during HI-Glc were responsible for the large increases in MCR. On the other hand, it is possible that the lower fatty acid concentrations may have facilitated the insulin and that contraction induced increased muscle glucose uptake.

An additional remark that can be made with regard to the MCR in the present study is that the calculation of MCR overcorrects for the mass action effect glucose has on stimulating its own disappearance. The reason is that the clearance calculation assumes a linear relationship between concentration and uptake, whereas the relationship is in fact curvilinear. Thus, even if a treatment has no effect on the intrinsic ability of tissues to take up glucose, calculated glucose clearance will tend to be lower when concentration is higher and vice versa. Therefore, the fact that we found a higher MCR even though the concentration is \( \sim 1 \text{ mmol/l} \) higher during HI-Glc is all the more impressive.

Limitations of exogenous CHO oxidation. Maximal exogenous CHO oxidation was 72 µmol·kg\(^{-1}\)·min\(^{-1}\) (0.94 g/min) in the present study. This is in agreement with several other studies that employed either radioactive (3, 23) or stable isotopes (27, 28, 37, 54) to quantify exogenous CHO oxidation during exercise. From those studies, it appeared that exogenous CHO oxidation is limited to \( \sim 1 \text{ g/min} \) as reviewed by Hawley et al. (22). Even when large amounts of CHO were ingested, oxidation rates did not exceed 1 g/min (43, 54). Here we calculated CHO ingestion to be 2.15 g/min during the second hour, but exogenous CHO oxidation did not exceed 0.94 g/min.

During LO-Glc, Ra gut equaled the rate of CHO ingestion during the second hour (32 ± 1 vs. 33 µmol·kg\(^{-1}\)·min\(^{-1}\)). Ra gut and the rate of ingestion were \( \sim 0.43 \text{ g/min} \) during LO-Glc. This implies that...
absorption was not limiting and the liver did not store ingested glucose, but instead all ingested glucose appeared in the bloodstream and most of this glucose was oxidized. When a larger dose of CHO was ingested, $R_a$ gut was less than one-half of the rate of CHO ingestion (73–79 vs. 164 µmol·kg$^{-1}$·min$^{-1}$). $R_a$ gut was 0.96–1.04 g/min, whereas CHO were ingested at a rate of 2.15 g/min during the second hour of exercise during HI-Glc. These numbers indicate that only part of the ingested CHO will enter the systemic circulation and that a large part of the $R_a$ gut is oxidized. The factor(s) limiting exogenous CHO oxidation must thus be proximal from the liver. Most likely, $R_a$ gut during HI-Glc was limited by the rate of digestion and/or absorption of glucose, and a fair amount remained in the gastrointestinal tract. Previous studies have suggested that the absorptive capacity of the intestine possesses a modest excess of nutrient capacity over nutrient intake in resting conditions (47). During exercise, however, a reduced mesenteric blood flow may result in a decreased absorption of glucose and water (4). Although this will occur predominantly at higher exercise intensities (4), decreased blood flow in the intestine may have contributed to a reduced absorption of water and glucose and thus to a low $R_a$ gut relative to the rate of ingestion. However, it cannot be excluded that the liver plays an active role and that glucose is taken up by the liver in the first pass.

Muscle glycogen oxidation is not reduced by CHO feedings. The third purpose of this study was to see whether ingestion of 360 g of CHO during 2 h of cycling exercise reduces muscle glycogen oxidation at the whole body level. Muscle glycogen oxidation was similar with ingestion of either 70 or 350 g of CHO during 2 h of exercise. These findings corroborate findings from several studies with direct measurements of muscle glycogen in muscle biopsies (3, 10, 14, 15, 18) but are in contrast with other studies in which CHO ingestion had a glycogen-sparing effect (2, 19, 52, 53). In runners, it was shown that CHO ingestion (45–50 g/h) reduced the net glycogen breakdown in type I fibers during running at 72–76% $V_{O_2max}$, and this might have been responsible for the observed improvements in endurance capacity (52, 53). Tsintzas and Williams (51) recently argued that the observed effects could be specific to running. Continuous cycling would cause

![Fig. 7. Plasma glucose oxidation, muscle glycogen oxidation, and exogenous CHO oxidation during exercise without ingestion of glucose (FAST; open circles), with ingestion of moderate amounts of glucose (LO-Glc; shaded circles), or with ingestion of large amounts of glucose (HI-Glc; filled circles). a Significant ($P < 0.001$) difference between HI-Glc and FAST; b significant ($P < 0.001$) difference between LO-Glc and FAST; c significant ($P < 0.001$) difference between HI-Glc and LO-Glc.](image)

![Fig. 8. Substrate oxidation during exercise without ingestion of glucose (FAST), with ingestion of moderate amounts of glucose (LO-Glc), or with ingestion of large amounts of glucose (HI-Glc).](image)
less marked changes in plasma glucose concentration and insulin concentrations compared with running, and these differences might play an important role in the effect on glycogen utilization to be observed. However, we observed a relatively large insulin response to the CHO feedings while plasma glucose concentration was also slightly increased. Yet this did not result in glycogen sparing as proposed by Tsintzas and Williams. Therefore, the present study suggests that factors other than insulin are responsible for the discrepant findings in running capacity (52, 53) and cycling (3, 10, 14, 15, 18).

In the present study, CHO feedings were provided as a large bolus at the onset of exercise followed by smaller feedings every 15 min. It is possible, however, that different feeding schedules would have produced slightly different results. Glucose feedings in the hour before exercise may lead to increased insulin concentrations, whereas feedings later in exercise may completely abolish an insulin response to the CHO feedings. The present feeding schedule, however, will lead to high rates of gastric emptying (42) and will result in maximal exogenous CHO oxidation rates (22, 54).

Percentage of $R_d$ oxidized. The percentages of $R_d$ glucose oxidized were high at the end of 2 h of exercise (92–95%), indicating that little or no glucose is used for nonoxidative disposal under these conditions. These high rates of oxidation are in agreement with a study by Coggan et al. (8), who found that 93% of $R_d$ glucose was oxidized during exercise at 70% $V_{O_{2max}}$ when CHO were ingested, and confirm our previous findings (96–100% $R_d$ oxidized) (29). Others have found lower percentages of glucose taken up and oxidized during exercise at intensities comparable with the intensity in the present study (50% $V_{O_{2max}}$) (9, 16, 44). The lower percentages of $R_d$ oxidized can most likely be explained by the fact that the bicarbonate pool was not primed in those studies, which may have led to the entrapment of a fair amount of $^{13}$CO$_2$ in the bicarbonate pool. This may have caused a marked underestimation of the true plasma glucose oxidation. Other studies in which a bicarbonate prime was given report values more comparable with those in the present study (88–94%) (7).

It has been reported that with a $^{13}$C tracer for studying fatty acid metabolism, part of the tracer may be (temporarily) trapped in exchange reactions with the tricarboxylic acid (TCA) cycle (48, 49). For example, some $^{13}$C may be incorporated into the glutamate-glutamine pool via $\alpha$-ketoglutarate or into phosphoenolpyruvate via oxaloacetate. This label fixation results in a decreased recovery of label in the expired gases, and to correct for this loss, the acetate correction factor has been proposed (49). The label loss is dependent on the metabolic rate, and at high oxygen uptakes (37–38 ml/kg in the present study), less label is trapped and recovery of the [1-13C]acetate label was 85–90% (49). Besides that, [U-13C]glucose has six labeled carbons of which two will appear directly in $^{13}$CO$_2$ and therefore do not enter the TCA cycle. Only two-thirds (66%) of the glucose carbons are subject to label fixation. Therefore, the recovery of carbons from [U-13C]glucose will be higher than the recovery of [1-13C]palmitate (49) or [U-12C]palmitate (48), explaining why we find such high percentages of $R_d$ oxidized. The small difference from 100% may be explained by the acetate correction factor, implying that all glucose molecules disappearing from the plasma might have been oxidized in both the presence and absence of glucose ingestion.

General overview of substrate utilization with CHO ingestion. The results of the present study indicate that glucose ingestion during exercise leads to decreased fat oxidation, partly because of an inhibition of lipolysis (25) and an increased CHO oxidation (Fig. 8). The increased CHO oxidation was explained by an increased plasma glucose turnover. The appearance of ingested glucose in the bloodstream ($R_g$ gut) increased with the CHO feedings in a dose-dependent way. At the same time, EGP was suppressed and with large CHO feedings was even completely blocked. This effect is possibly insulin mediated. The oxidation of ingested CHO increased to 0.94 g/min when CHO were ingested at a rate of 2.15 g/min. Factors that limit exogenous CHO oxidation must be situated in the splanchnic area, potentially a gastrointestinal limitation or retention of labeled glucose in the splanchnic bed (34). The increased CHO oxidation with CHO ingestion was due to increased glucose uptake and clearance. The increased clearance may also be insulin mediated. The glucose that disappeared from the plasma (and most likely was taken up by active skeletal muscle) was largely oxidized, and very little or no glucose was directed toward glycojen synthesis. Plasma glucose oxidation represented 21% during LO-Glc and 47% of total CHO oxidation during HI-Glc, confirming that plasma glucose can indeed be an important substrate during exercise, as shown by others (3, 10, 45). Muscle glycogen oxidation was not reduced by the glucose feedings.

In summary, we conclude that small CHO feedings suppress EGP and that large CHO feedings completely block EGP, most likely because of increases in plasma insulin and plasma glucose concentration. The entrance of glucose into the systemic circulation seems to be the limiting factor for exogenous CHO oxidation because a large percentage of $R_g$ gut was oxidized, whereas only a small percentage of the ingested glucose appeared in the bloodstream. Muscle glycogen oxidation at the whole body level was not reduced by glucose ingestion during cycling exercise at 50% $V_{O_{2max}}$.

Address for reprint requests and other correspondence: A. E. Jeukendrup, School of Sport and Exercise Sciences, Univ. of Birmingham, Edgbaston, Birmingham B15 2TT, UK (E-mail: A.E. Jeukendrup@bham.ac.uk). Received 14 July 1998; accepted in final form 1 December 1998.

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