Fuel selection and cycling endurance performance with ingestion of $^{13}$C-glucose: evidence for a carbohydrate dose response

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The mechanisms by which carbohydrate (CHO) ingestion increases performance during prolonged exercise (7, 20, 21) remains a matter of debate (25). Carter et al. (3), Potter et al. (38), and Chambers et al. (5) have shown that a 1-h time trial performance was improved by merely rinsing the mouth at regular intervals with a CHO solution. The beneficial effect of CHO ingested during exercise, thus, could be due at least in part to the stimulation of CHO receptors in the oral cavity, which could modulate central motor drive and reduce the rate of perceived exertion. However, improved performance while ingesting CHO during exercise could also be due in part to a better maintenance of plasma glucose level and/or to the associated changes in fuel selection. Indeed, the increased availability of blood glucose results in a higher rate of oxidation, particularly late in the exercise period (6, 20). This is mainly due to exogenous CHO oxidation (20) with few changes, if any, in muscle glycogen oxidation and with a reduction in glucose released from the liver (22, 23).

Based on the idea that the beneficial effect of CHO ingestion on endurance performance is at least in part related to changes in fuel selection due to exogenous CHO oxidation, it can be hypothesized that this effect will increase with the amount of exogenous CHO actually oxidized. In support of this hypothesis, Currell and Jeukendrup (9) have reported that the improvement in performance for an ~1-h time trial [following a 2-h ride at 55% peak $O_2$ uptake ($\dot{V}O_2$peak)] was larger with ingestion of a 2:1 glucose-fructose mixture than with ingestion of glucose only. Although in this study the oxidation rate of ingested CHO was not measured, the authors speculated that this result could be due to the fact that multiple transportable CHO (such as a glucose-fructose mixture) are oxidized at a higher rate than glucose or glucose polymers alone (21).

Indeed, in a previous experiment, these authors (19) showed that compared with glucose ingestion (1.8 g/min), the oxidation rate of a 2:1 mixture of glucose and fructose also ingested at 1.8 g/min was 53% higher and that although this did not reach significance, endogenous CHO oxidation was 24% lower (19). However, the existence of a relationship between the amount of CHO ingested and the effect on endurance performance, as well as the optimal dose of CHO to be administered during exercise, remains a matter of debate (21, 25).

The purpose of the present study was to further investigate the relationship among the rate of glucose ingestion, fuel selection, and performance during a 20-km time trial preceded by a 2-h constant load ride in recreational cyclists. The doses of glucose administered (15, 30, and 60 g/h) were equal to or lower than that generally recommended to avoid gastrointestinal discomfort (~60 g/h) (4, 43). Fat and CHO oxidation as well as the oxidation rate of glucose from various sources (exogenous glucose, plasma glucose, glucose released from the liver and from muscle glycogen) were computed from indirect respiratory calorimetry combined with a tracer technique using $^{13}$C labeling of the glucose ingested (14, 49). Based on observations that glucose ingestion at doses ranging from 10 (28) to >100 g/h (10, 11, 20) have been shown to improve endurance performance, we hypothesized that compared with ingestion of a placebo, the time needed to complete the 20-km time trial would be lower with glucose ingestion and that the differences would be meaningful in terms of performance (17, 18). In addition, based on the suggestion by Currell and Jeukendrup (9) that the beneficial effect of CHO ingestion is related to the...
amount of exogenous CHO actually oxidized, we hypothesized that the improvement in performance would increase with the rate of glucose ingestion and oxidation and with the associated changes in fuel selection.

**METHODS**

Twelve trained, recreational, healthy male cyclists or triathletes volunteered to participate in this study, which was conducted in accordance with the guidelines of the Declaration of Helsinki. All participants read and signed an informed consent approved by a Human Subject Ethics Committee before beginning the study. Mean and standard deviation (SD) age, height, body mass, VO\textsubscript{peak}, and peak power output were 31.7 ± 3.8 yr, 1.82 ± 0.07 m, 77.6 ± 6.9 kg, 55.3 ± 3.6 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} (4.3 ± 0.4 l/min), and 388 ± 41 W, respectively.

Preliminary testing [VO\textsubscript{peak} and onset of blood lactate accumulation (OBLA) (34)] and the experimental exercises (2-h ride at 95% VO\textsubscript{2peak}) were performed in a laboratory maintained at 23 ± 1°C and 35–40% relative humidity with the participants exercising on their own bicycle affixed to a bicycle trainer (Compu Trainer Pro; RacerMate, Seattle, WA) calibrated according to the manufacturer’s recommendations. VO\textsubscript{peak} was determined using an incremental multi-stage cycling protocol (40): after a 10-min warm-up at 100 W, athletes cycled at 150 W for 5 min, and then power output was increased by 50 W every 3 min until 250 W, after which power output was increased by 25 W every minute until volitional exhaustion. VO\textsubscript{2} and carbon dioxide production (V\textsubscript{CO2}) were computed from expiratory gases collected during the last 30 s of each stage in Douglas bags and then analyzed (Vacumed spirometer, Ventura, CA; Ametek S-3A/I oxygen analyzer and Ametek CD-3A carbon dioxide analyzer, Pittsburgh, PA). On a second occasion, separated from the measurement of VO\textsubscript{peak}, by at least 7 days, the subjects exercised for 3.5 min at 55, 60, 65, 70, 75, 80, 85, and 90% VO\textsubscript{2peak}, and 3-ml blood samples were collected during the final 30 s of each stage for measuring blood lactate concentration and determining OBLA (34).

Before the experimental trials, participants performed three familiarization rides, with at least 7 days between trials, i.e., familiarization with the 20-km time trial course, the 2-h ride followed by the 20-km time trial, and, finally, the entire testing procedure (2-h ride with ingestion of 2,000 ml of water, collection of expired gases, and blood sampling following by the 20-km course). Participants then completed four exercise trials with at least 7 days between trials. For each trial, participants reported to the laboratory at 5:00 AM following a 10-h overnight fast, having abstained from exercise over the preceding 24 h. After voiding and verification that urine specific gravity was ≤1.020, body mass was recorded and an intravenous catheter (BD Insyte Autoguard; Becton Dickinson Infusion Therapy Systems, Sandy, UT) was inserted into an antecubital vein to collect blood samples throughout the trial. After a 10-min warm-up at 100 W, participants began the 2-h constant load ride (average workload = 228 ± 26 W; 77 ± 5% VO\textsubscript{2peak}). Gas exchanges and heart rate (Polar Electro, Lake Success, NY) were measured every 15 min. For measurement of the isotopic composition (1\textsuperscript{3}C/1\textsuperscript{2}C) of expired CO\textsubscript{2}, 10-ml samples of the expired gases collected were directly transferred to vacutainers (BD Vacutainer, Franklin Lakes, NJ) through a line from the Douglas bag following thorough mixing. At regular intervals during the 2-h constant load ride, blood samples were collected for the measurement of plasma glucose, lactate, free fatty acid, insulin concentrations and of 1\textsuperscript{3}C/1\textsuperscript{2}C in plasma glucose. Two minutes after completing the 2-h ride, which allowed for removal of the catheter and heart rate monitor, participants began the simulated 20-km time trial on an undulating course (9.04 km of incline at a 2% average slope and 10.96 km of decline at a ~1.95% average slope: ~180 m uphill and ~213 m downhill) and were asked to complete the course as quickly as possible. Participants were aware of their position on the course, but no verbal stimuli or other information was given.

During each 2-h ride, participants ingested 2,000 ml of one of four beverages (250 ml every 15 min, beginning at min 15 and ending at min 120): a placebo (water with electrolytes: 18 mmol/l Na\textsuperscript{+}, 3 mmol/l K\textsuperscript{+}, and 11 mmol/l Cl\textsuperscript{−}) or a 1.5, 3.0, or 6.0% glucose solution in water with the electrolytes (15, 30, or 60 g/h glucose). No fluid was ingested during the 20-km time trial. The four beverages, which were presented in a balanced order and given in opaque plastic containers, were formulated to be similar in flavor and sweetness and were kept at 1°C to minimize differences in taste. Uniformly labeled [\textsuperscript{13}C]glucose (\textsuperscript{13}C/\textsuperscript{12}C >99%; Isotec, Miamisburg, OH) was added to the beverages containing glucose (~1.75 mg/g) to obtain a final 1\textsuperscript{3}C/1\textsuperscript{2}C ratio of glucose close to 145% [\textsuperscript{6-13}C]PDB\textsubscript{4} (actual values measured by mass spectrometry: 136 ± 8‰ [\textsuperscript{6-13}C]PDB\textsubscript{4}).

Total CHO and fat oxidation rates were calculated from VO\textsubscript{2} and V\textsubscript{CO2} (both in l/min), neglecting the small contribution of protein oxidation to the energy yield (24):

\[
CHOCO (g/min) = (4.210 \times V\textsubscript{CO2}) - (2.962 \times V\textsubscript{O2})
\]

\[
Fat (g/min) = (1.695 \times V\textsubscript{O2}) - (1.701 \times V\textsubscript{CO2})
\]

Plasma glucose \textsuperscript{13}C/\textsuperscript{12}C was measured as previously described (2). Briefly, plasma glucose was separated by double-bed ion-exchange chromatography (AG 50W-X8 H\textsuperscript{+} and AG 1-X8 chloride, 200–400 mesh; Bio-Rad, Mississauga, ON, Canada) after deproteinization with barium hydroxide and zinc sulfate (0.3 N). The eluate was evaporated to dryness (Virtis Research Equipment, New York, NY) and then combusted (60 min at 400°C with copper oxide), and the CO\textsubscript{2} was recovered for the isotopic analysis.

Measurement of 1\textsuperscript{3}C/1\textsuperscript{2}C in expired CO\textsubscript{2} (BreathMat Plus; Finnigan MAT, Bremen, Germany) and in CO\textsubscript{2} from combustion of plasma glucose (Prism, Manchester, UK) was performed by mass spectrometry. The isotopic composition of ingested glucose, expired CO\textsubscript{2}, and plasma glucose was expressed as %\textsubscript{d} difference by comparison with the PDB\textsubscript{4} Chicago standard: %d\textsuperscript{[\textsuperscript{13}C]/\textsuperscript{12}C]PDB\textsubscript{4} = [(Rsp1/Rstd) – 1] \times 1,000, where Rspl and Rstd are the \textsuperscript{13}C-to-\textsuperscript{12}C ratio in the sample and standard (1.1237%), respectively (2).

The oxidation rate of exogenous glucose (in g/min) was computed as follows (23, 37):

\[
\text{Exogenous glucose (g/min)} = \frac{VCO2[(Rexp - Rref)/(Rexp - Rref)]}{k}
\]

In this equation, V\textsubscript{CO2} is in liters per minute, Rexp is the observed isotopic composition of expired CO\textsubscript{2}, Rref is the isotopic composition of expired CO\textsubscript{2} observed in response to exercise with ingestion of the placebo, Rglu is the isotopic composition of the exogenous glucose ingested, and k (0.747 l/g) is the volume of CO\textsubscript{2} provided by the complete oxidation of glucose. In addition, based on the isotopic composition of expired CO\textsubscript{2}, the percentage of plasma glucose derived from exogenous glucose (Eq. 4) and the oxidation rate of plasma glucose (in g/min, Eq. 5), were computed (14, 49):

\[
\text{Percent from exogenous} = \frac{(Rglu - Rglu-ref)/(Rexp - Rglu-ref)}{Rglu-ref} \times 100
\]

\[
\text{Plasma glucose (g/min)} = \frac{VCO2[(Rexp - Rref)/(Rglu - Rref)]}{k}
\]

In Eq. 4, Rglu-ref is the baseline isotopic composition of plasma glucose. The oxidation rate of muscle glycogen (expressed in g/min), either directly or through the lactate shuttle (1), was computed as the difference between the rate of total glucose oxidation (Eq. 1) and the oxidation rate of plasma glucose (Eq. 4). Finally, the oxidation rate of glucose released by the liver was estimated as the difference between the oxidation rate of plasma and exogenous glucose. These computations are based on the observation that during exercise, \textsuperscript{13}C provided

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from $[13C]$glucose is not irreversibly lost in pools of the tricarboxylic acid cycle intermediates and/or bicarbonate, that $^{13}CO_2$ recovery in expired gases is, thus, complete or almost complete (42, 44), and that the recycling of $[13C]$glucose is minimal (22). However, the $^{13}CO_2$ in expired $CO_2$ only slowly equilibrates with $^{13}C/^{12}C$ in the $CO_2$ produced in tissues (35). To take into account the delay between $^{13}CO_2$ production in tissues and at the mouth, we only computed exogenous and plasma glucose oxidation, as well as oxidation of glucose released from the liver and muscle glycogen, during the last 60 min of exercise, thus allowing for a 60-min equilibration period.

Energy expenditure, the amount of energy provided by the oxidation of CHO and fat, and the respective contributions of these substrates to the energy yield were computed from the amounts oxidized and their respective energy potential (24). Blood samples were collected through the catheter inserted into an antecubital vein before the beginning of exercise. Between samplings, the catheter was kept patent using a saline lock (Solution-Plus, Mansfield, MA). After centrifugation, plasma samples were stored at $-20^\circ C$ until analysis. Blood lactate concentration was measured using a liquid glucose (hexokinase) reagent set kit (Pointe Scientific, Canton, MI), plasma insulin concentration was measured using a human insulin ELISA kit (Millipore, St. Charles, MO), and plasma free fatty acid concentration was measured using a nonessential fatty acid HR2 series reagent kit (Wako Diagnostics, Richmond, VA). All samples were analyzed on a multisample spectrophotometer (Synergy HT; Bio-tek Instruments, Winooski, MA).

The mean value observed in a given situation is presented with the associated standard deviation (mean $\pm SD$), and the mean difference between two situations is presented with the associated confidence limits at the 90% confidence level with Cohen’s effect size [mean difference, lower limit to upper limit, Cohen’s effect size (ES)] as recommended by Hopkins et al. (18). A probabilistic magnitude-based inferential analysis was used to analyze the effect of glucose ingestion on the average power output sustained over the 20-km time trial in terms of meaningful enhancement of performance (17, 18). Based on the coefficient of variation for a 40-min simulated cycling time trial computed by Hopkins et al. (17) using the data reported by Palmer et al. (36) ($\sim 2.4\%$) and on the smallest worthwhile change in performance in athletes ($\sim 0.3$–$0.7 \times $ coefficient of variation) (17), the smallest meaningful improvement in power output chosen for the analysis of the effect of glucose ingestion was $1.2\%$ (i.e., $2.4\% \times $ the average value between 0.3 and 0.7). Uncertainty in the estimate of the effect of glucose ingestion on the power output was expressed as the 90% confidence or likely limits of the true value of the effect (Table 1). The effect of each of the three doses of glucose administered was expressed as percent change relative to the placebo following back transformation of the mean of the natural logarithm of power outputs (17). The chances that the true value of the effect was larger than the smallest meaningful effect on the 20-km time trial were then computed (16), and qualitative terms to these chances were assigned as suggested: $<1\%$, almost certainly not; $<5\%$, very unlikely; $<25\%$, unlikely or probably not; $<50\%$, possibly not; $>50\%$, possibly; $>75\%$, likely or probable; $>95\%$, very likely; $>99\%$, almost certain (46). For the other variables (heart rate, VO$_2$, substrate oxidation, and plasma glucose, lactate, free fatty acid, and insulin concentrations) for which the smallest worthwhile change was difficult to ascertain, statistical comparisons were also made using Cohen’s ES with threshold values for small, moderate, large, very large, and extremely large effects as 0.2, 0.6, 1.2, 2.0, and 4.0 (18). Although the discussion is based on the probabilistic magnitude-based inferential analysis, statistical comparisons were also made for all the variables using one-way (dose or time) or two-way (dose $\times$ time) analysis of variance for repeated measures and Duncan’s post hoc test when needed (Statistica; Statsoft, Tulsa, OK). For the comparison of performance with ingestion of the placebo and the three doses of glucose, ANOVA was performed after the power outputs were log-transformed. Exact $P$ values are reported if they are $<0.10$ and are expressed to three decimal places. Values of $P$ less than 0.001 are reported as $P < 0.001$.

**RESULTS**

*Time trial performance.* The reduction in body mass over the exercise period was not different among the four experimental situations (average pooled value $\pm SD$: 1.6 $\pm$ 0.8 kg, $n = 48$), indicating dehydration was not a confounding factor in the results. Compared with the placebo, glucose ingestion increased the average power output sustained and improved the 20-km time trial performance (Table 1). When 1.2% was used as the smallest meaningful improvement in performance, the analysis confirmed that the three doses of glucose were very likely to increase the chances of completing the 20-km time trial meaningfully faster and that the true effect of ingesting glucose was not detrimental (<1.6% chance of deterioration in performance). In addition, although increasing the dose from 15 to 30 g/h was very unlikely to result in further improvement in performance, compared with the lower doses (15 and 30 g/h) the chances of obtaining a further beneficial effect were likely

<table>
<thead>
<tr>
<th>Time Trial Performance</th>
<th>Improvement in Power Output, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minutes</td>
<td>Watts</td>
</tr>
<tr>
<td>Placebo</td>
<td>36.4 $\pm$ 2.9</td>
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<td></td>
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<td></td>
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<tr>
<td>15 g/h Glucose</td>
<td>35.2 $\pm$ 2.8</td>
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<td></td>
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<tr>
<td>30 g/h Glucose</td>
<td>35.0 $\pm$ 2.6</td>
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<td></td>
<td></td>
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<tr>
<td>60 g/h Glucose</td>
<td>34.7 $\pm$ 2.1</td>
</tr>
</tbody>
</table>

Table 1. Performance and improvement in power output during 20-km time trials

Data indicate performance in the 20-km time trials with ingestion of placebo and 15, 30, and 60 g/h glucose (mean performance time and power output $\pm$ SD) and % improvement in power output [1st line: % improvement, 90% confidence interval limits, and Cohen’s effect size (ES, in parentheses); 2nd line: chances (% and qualitative) of meaningful improvement (i.e., $>1.2\%$) and sample size needed for a magnitude-based inference about the practical significance of the observed changes in performance for a power of 80% (in parentheses); 3rd line: exact $P$ value from 1-way ANOVA and Duncan’s post hoc test]. The chances of substantial decline in performance relative to placebo were <1.6% (very unlikely) for the 3 doses of glucose.

J Appl Physiol • VOL 108 • JUNE 2010 • www.jap.org
Table 2. Heart rate and oxygen consumption over the first and second hour of exercise

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>15 g/h Glucose</th>
<th>30 g/h Glucose</th>
<th>60 g/h Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First hour</strong></td>
<td></td>
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</tr>
<tr>
<td>HR, beats/min</td>
<td>148 ± 11</td>
<td>147 ± 12</td>
<td>148 ± 12</td>
<td>146 ± 11</td>
</tr>
<tr>
<td>V̇O₂, l/min</td>
<td>3.16 ± 0.28</td>
<td>3.15 ± 0.31</td>
<td>3.13 ± 0.36</td>
<td>3.17 ± 0.32</td>
</tr>
<tr>
<td><strong>Second hour</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>152 ± 12</td>
<td>152 ± 13</td>
<td>151 ± 14</td>
<td>152 ± 11</td>
</tr>
<tr>
<td>V̇O₂, l/min</td>
<td>3.18 ± 0.27</td>
<td>3.25 ± 0.30</td>
<td>3.21 ± 0.35</td>
<td>3.15 ± 0.32</td>
</tr>
</tbody>
</table>

Data are heart rate (HR) and oxygen consumption (V̇O₂) (means ± SD) over the first and second hours of the 2-h ride with ingestion of the placebo and 15, 30, and 60 g/h glucose. See text for Cohen’s ES and statistical comparisons.

Table 3. Comparisons of total CHO and fat oxidation over the second hour of exercise

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>15 g/h Glucose</th>
<th>30 g/h Glucose</th>
<th>60 g/h Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHO</strong></td>
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<tr>
<td>Total Oxidation, g</td>
<td>171.0 ± 34.9</td>
<td>173.3 ± 36.4</td>
<td>169.4 ± 35.3</td>
<td>186.8 ± 37.2</td>
</tr>
<tr>
<td>Difference in Total Oxidation, g</td>
<td>23.3, −9.6 to 14.1</td>
<td>ES = 0.07, P = 0.468</td>
<td>−1.6, −10.7 to 7.5</td>
<td>ES = 0.05, P = 0.608</td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total Oxidation, g</td>
<td>32.8 ± 13.2</td>
<td>34.1 ± 15.3</td>
<td>34.4 ± 14.2</td>
<td>26.7 ± 16.7</td>
</tr>
<tr>
<td>Difference in Total Oxidation, g</td>
<td>13.3, −3.1 to 5.8</td>
<td>ES = 0.09, P = 0.251</td>
<td>1.6, −2.1 to 5.4</td>
<td>ES = 0.12, P = 0.182</td>
</tr>
</tbody>
</table>

Data are comparisons of total carbohydrate (CHO) and fat oxidation over the second hour of exercise at 77% peak V̇O₂ in the 4 experimental trials (placebo and ingestion of 15, 30, and 60 g/h glucose) [1st line: means ± SD and differences among the trials with the associated 90% confidence limits; 2nd line: Cohen’s ES and P values (ANOVA and Duncan’s post hoc test)].
with the ingestion rate (in % [δ-13C] PDB): 9.6, 8.6 to 10.6 for 15 g/h glucose; 16.3, 15.0 to 17.7 for 30 g/h glucose; and 23.8, 21.9 to 25.7 for 60 g/h glucose; ES = 6.2, 8.2, and 9.1, respectively; P < 0.001 with the 3 doses). During the second hour of exercise, based on the average value of 13C/12C observed in plasma glucose (13.2 ± 7.8, 37.0 ± 9.4, and 57.2 ± 10.9% [δ-13C] PDB; with 15, 30, and 60 g/h glucose ingested, respectively), the percentage of plasma glucose deriving from exogenous glucose (22.2 ± 5.9% with ingestion of 15 g/h glucose) increased with the glucose ingestion rate (from 15 to 30 g/h ingested: 14.5, 11.6 to 17.4%, ES = 2.7, P < 0.001; from 30 to 60 g/h ingested: 13.4, 10.8 to 15.9%, ES = 2.1, P < 0.001) (Fig. 2).

Sources of glucose oxidized. During the second hour of exercise, compared with the value observed with ingestion of 15 g/h glucose, the rate of exogenous glucose oxidation markedly increased with the ingestion rate (ES > 3.0, P < 0.001, Fig. 3 and Table 4). For 15, 30, and 60 g/h ingestion rates, the average oxidation rates were 68, 68, and 53% of the ingestion rates, respectively, and the peak oxidation rates of exogenous glucose observed at min 120 were 0.22 ± 0.05, 0.39 ± 0.07, and 0.65 ± 0.09 g/min (Fig. 3). The energy yield from exogenous glucose oxidation also increased with the amount ingested (Fig. 1): average values for 15, 30, and 60 g/h ingestion rates: 3.9, 7.8 and 12.4%, respectively (ES > 2.5, P < 0.001).

Compared with the placebo trial (endogenous glucose oxidation = total CHO oxidation = 171.0 ± 34.9 g; see Table 3), the reduction of endogenous glucose oxidation over the second hour of the constant load ride (Table 4) was, respectively, slight, moderate, and small with ingestion of 15, 30, and 60 g/h glucose (−7.7, −19.6 to 4.0 g, ES = 0.24, P = 0.283; −21.3, −30.4 to −12.3 g, ES = 0.63, P = 0.009; and −15.4, −28.5 to −2.4 g, ES = 0.46, P = 0.047, respectively). A small reduction of endogenous CHO oxidation was observed when the dose of glucose ingested increased from 15 to 30 g/h, but no consistent changes were observed when the dose of glucose was increased from 15 to 60 g/h and from 30 to 60 g/h (Table 4).

In the three trials the oxidation rate of plasma glucose and glucose released from the liver increased with time, whereas the oxidation rate of glucose released from muscle glycogen decreased (Fig. 3). Compared with the ingestion of 15 g/h glucose, moderate and large reductions in the oxidation of glucose released from the liver were observed, respectively, with ingestion of 30 and 60 g/h glucose (Table 4). In contrast, oxidation of glucose from muscle glycogen and of plasma glucose did not decrease with the dose of glucose ingested (Table 4). As shown in Fig. 1, compared with the value observed with ingestion of 15 g/h glucose, the energy yields from the oxidation of plasma glucose and of glucose released from muscle glycogen (24.7 ± 6.5 and 42.3 ± 11.5%, respec-
tively) were only slightly modified when the dose of glucose increased (e.g., 2.8, 0.4 to 5.2%, ES = 0.47, P = 0.120; and 4.1, −2.8 to 10.9%, ES = 0.30, P = 0.115, respectively, from 15 to 60 g/h glucose ingested). In contrast, when the rate of glucose ingestion and oxidation increased, the energy yields from the oxidation of glucose released from the liver markedly decreased (Fig. 1; e.g., 20.7 ± 6.3 to 15.1 ± 5.5% when the ingestion rate increased from 15 to 60 g/h: −5.7, −7.7 to −3.7%, ES = 1.0, P < 0.001).

**Plasma metabolite and insulin concentrations.** Baseline plasma glucose, insulin, lactate, and free fatty acid concentrations were similar in the four experimental situations (Fig. 4). Plasma lactate concentration increased in response to exercise and remained stable, slightly below 3 mmol/l, over the second hour of exercise with no differences among the four experimental situations. Compared with placebo (3.0 ± 1.7 mmol/l), the differences were only 0.0, −0.6 to 0.6 mmol/l for 15 g/h glucose ingested; −0.2, −0.9 to 0.4 mmol/l for 30 g/h glucose; and 0.1 −0.3 to 0.5 mmol/l for 60 g/h glucose (ES = 0.02, 0.13, and 0.06; P = 0.686, 0.097, and 0.560, respectively). Plasma glucose concentration, which decreased during exercise when the placebo was ingested (rest: 5.2 ± 0.9 mmol/l, average decrease over the second hour of exercise: −0.4, −0.8 to 0.0 mmol/l, ES = 0.58, P = 0.019) slightly increased when 15 g/h glucose were ingested (0.2, −0.2 to 0.7 mmol/l; ES = 0.32, P = 0.209) and markedly increased with 30 and 60 g/h intakes (0.4, −0.2 to 1.0 and 0.6, 0.2 to 1.0 mmol/l, ES = 0.51 and 0.73, P = 0.017 and 0.002, respectively). Plasma insulin concentration decreased in the four trials and, compared with the placebo trial (average value ± SD over the second hour of exercise: 1.3 ± 1.7 mU/l), was similar to that with ingestion of 15 g/h glucose (−0.1, −0.9 to 0.6 mU/l, ES = 0.08, P = 0.921) but moderately and markedly higher with ingestion of 30 and 60 g/h glucose (0.8, 0.0 to 1.6 and 1.8, 0.4 to 3.2 mU/l, ES = 0.44 and 0.96, P = 0.434 and 0.099, respectively). As for free fatty acid concentration, compared with the marked increase observed when the placebo was ingested (rest: 0.24 ± 0.25 mmol/l; average increase over the second hour of exercise: 0.16, 0.08 to 0.23 mmol/l, ES = 0.77, P < 0.001), the increase was similar when glucose was ingested at a rate of 15 and 30 g/h (0.18, 0.08 to 0.29 and 0.12, 0.05 to 0.19 mmol/l, ES = 0.71 and 0.96, P < 0.001 and P = 0.005, respectively) but was slightly blunted at the highest ingestion rate (0.05, 0.01 to 0.09 mmol/l, ES = 0.43, P = 0.268).

**DISCUSSION**

Results from the present experiment show that glucose ingested at low doses over a 2-h exercise period at ~77% V\(\text{O}_{2\text{peak}}\) substantially improved performance during a subse-
Fig. 4. Plasma glucose, insulin, lactate, and free fatty acid (FFA) concentrations in response to exercise with ingestion of the placebo and 15, 30, and 60 g/h glucose. Values are means ± SD (see text for statistical comparisons).

quent 20-km simulated cycling time-trial completed in ~35 min. In terms of meaningful effect on performance for the recreational cyclists studied, the chances of improvement increased with the dose ingested, with ingestion of 60 g/h glucose being higher than with the lower doses. The progressive reduction in endogenous CHO oxidation with increasing glucose ingestion rates was due to a reduction in the oxidation of glucose released from the liver without any significant change in muscle glycogen oxidation. Compared with the placebo trial, the largest change in fuel selection, i.e., higher CHO oxidation, was observed with the highest dose of glucose ingested, which was associated with the highest chances of improvement in performance.

It has been suggested that CHO ingestion during exercise increases endurance performance by maintaining plasma glucose level and CHO oxidation (6, 20, 21, 45). For this reason, several studies have been conducted for the explicit purpose of identifying the types and amounts of CHO to be ingested to maximize exogenous CHO oxidation. Results from these studies show that when large amounts of mixtures of multiple transportable CHO (glucose and fructose as monomers or in the form of glucose polymers and disaccharides) are ingested (up to 2.4 g/min or 144 g/h), the oxidation rate of exogenous CHO could reach 1.75 g/min (see Jeukendrup (21) for review). Currell and Jeukendrup (9) have reported a larger improvement in performance with the ingestion of a mixture of glucose and fructose (1.2 and 0.6 g/min) than of glucose only (1.8 g/min) (8% quicker time to complete ~925 kJ of work when glucose and fructose are ingested compared with glucose only and a 19% quicker time when glucose and fructose are ingested compared with water). This could suggest that the improvement in performance increases with the amount of CHO ingested and oxidized, although in this study the oxidation rate of exogenous CHO was not measured. In fact, a relationship between the dose of CHO ingested and improvement in performance has not been clearly established (21). In some studies that compared various doses of CHO (0.41 to 1.3 g/min), performance was improved with increasing ingestion rates (32, 41). However, this has not been a uniform observation, since higher ingestion rates tested in some studies did not result in peak performance (29, 31). In the present experiment, compared with the placebo, the average 20-km time was lower with the lower ingestion rate (15 g/h). Increasing the ingestion rate to 30 g/h further reduced the 20-km time, but compared with the 15-g/h ingestion rate, the improvement was small and did not result in a substantial increase in the chances of improving performance. In contrast, compared with the lower doses of glucose, the chances of improving performance increased when 60 g/h glucose were ingested.

Our data indicate that compared with the ingestion of a placebo, a substantial improvement in endurance performance can be obtained with an ingestion rate of CHO as small as 15 or 30 g/h, but chances of improving performance are higher with an ingestion rate of 60 g/h. Although improvements in performance have also been reported with higher ingestion rates (32), direct comparative studies over a large range of ingestion rates (up to 90 and 120 g/h) such as in the study by Currell and Jeukendrup (9) are currently lacking to conclude that the beneficial effects are larger at ingestion rates greater than 60 g/h. It should be recognized that post hoc power analyses using magnitude-based inferences showed that the number of subjects needed to firmly conclude that the time trial performance did not improve when the dose of glucose ingested increased from 15 to 30 g/h was much higher than the actual sample size (~200 vs. 12) and is very unlikely to be achieved in research on this topic. In contrast, the number of subjects needed to firmly conclude the comparisons between the lower and the higher dose ingested (15 and 30 vs. 60 g/h) was much lower (\(n = 22\) and \(15\)) and for the comparison between 30 and 60 g/h glucose ingested was in fact close to the actual sample size used in the present experiment. These observations add support to the hypothesis of a relationship between the dose of glucose ingested in the range from 15 to 60 g/h and improvement in endurance performance. This hypothesis could well be tested with sample sizes in the range computed in the present experiment (\(n = 15–22\)). In addition, the sample size needed can be reduced by using tests with lower coefficients of variation and including elite athletes familiar with the tests chosen and able, in a given situation, to consistently reproduce their performance with only small difference from one trial to the other.

In the present experiment, due to the low ingestion rates of exogenous glucose used, only modest changes in fuel selection were observed. As expected, exogenous glucose oxidation increased with the ingestion rates. The average values computed over the second hour of exercise (0.17, 0.34, and 0.53 g/min) are in good accordance with data compiled from the literature (20). As discussed by several authors (e.g., Refs. 47, 49), with increasing ingestion rates the percentage of exogenous glucose that escapes oxidation increases (~30% for ingestion rates of 15 and 30 g/h, up to ~45% for 60 g/h), but the fate of the glucose ingested and not oxidized remains to be determined. Jeukendrup et al. (22) showed that over a wide ingestion rate (~36 to ~180 g/h), the rate of plasma glucose disappearance was not significantly different from its rate of oxidation. This obser-
vation strongly suggests that intestinal absorption could be the limiting step for the oxidation of exogenous CHO that may accumulate in the stomach and gut leading to intestinal discomforts particularly for ingestion rates >1 g/min (39). However, for lower ingestion rates such as those used in the present experiment (15 and 30 g/h), a portion of the glucose absorbed can also be removed by the liver on first pass (27).

In the present experiment, the energy yield from exogenous glucose oxidation increased with the ingestion rate from 3.9 ± 1.6% for 15 g/h ingested to 12.4 ± 4.3% for 60 g/h ingested. These values are in good agreement with those computed from the data reported, for example, by Galloway et al. (12) at similar %V̇O₂peak (80%) values: ~5.5, ~11, and ~14.5% for 17, 53, and 105 g/h glucose ingested. These contributions of exogenous glucose oxidation to the energy yield resulted in only modest changes in overall fuel selection that were not closely related to the progressive improvement in performance with increasing ingestion rate. The reduction in endogenous CHO oxidation, which was modest (ES = 0.23 to 0.63), did not increase with the dose administered. As for the increase in total CHO oxidation, it was higher with the highest ingestion rate, at which the chances for a meaningful improvement in performance were also the highest. This could support the hypothesis that the improvement in endurance performance with CHO ingestion is due to the maintenance of a high rate of CHO oxidation (8). However, an improvement in performance, albeit smaller, was also observed when 15 and 30 g/h glucose were ingested, although total CHO oxidation was not different from that observed with ingestion of the placebo.

Consistent data show that glucose release from the liver is reduced when CHO is ingested during exercise (22, 23, 30, 49) and can even be totally suppressed with a very high ingestion rate of glucose (164 g/h) (22, 23). In contrast, except for some observations made during constant prolonged running (33) and variable load cycling (13), all the studies using muscle biopsy to track muscle glycogen utilization (including all experiments during prolonged constant load cycling) failed to observe muscle glycogen sparing when CHO was ingested during exercise (31). The same observation was made when muscle glycogen oxidation was computed as the difference between total CHO oxidation measured from gas exchange at the mouth and plasma glucose oxidation or turnover (22, 48). Data from the present experiment are well in line with these observations. As shown in Fig. 1, when the ingestion rate increased, the percentage of plasma glucose derived from the ingested [13C]glucose (average value over the last hour: 22.2 ± 6.1, 36.7 ± 7.5, and 50.1 ± 8.7% with ingestion of 15, 30, and 60 g/h glucose, respectively) increased almost in parallel with the oxidation rate (average values over the last hour: 0.17 ± 0.07, 0.33 ± 0.11, and 0.52 ± 0.17 g/min), whereas the oxidation rate of glucose released from the liver decreased. As a consequence, the oxidation rate of plasma glucose remained unaffected and no reduction in muscle glycogen oxidation was observed (Fig. 1). It should be recognized that since [13C]glucose is a recycling tracer, the oxidation rate of plasma glucose and, thus, of glucose released from the liver could be underestimated. However, Jeukendrup et al. (22) have shown that this phenomenon, which is large at rest, is small in response to exercise, where the rate of plasma glucose disappearance from [13C]glucose only underestimates by ~6–8% the value computed using 6,6-[2H]glucose, which does not recycle. Moreover, this underestimation decreases with the amount of glucose ingested, probably because of a reduction in the flux through gluconeogenesis (22). In the present experiment, the underestimation of plasma glucose oxidation was, thus, probably larger when 15 rather than 60 g/h glucose were ingested, which reinforces the conclusion that when glucose ingestion increases, glucose release from the liver decreases without any changes in muscle glycogen utilization.

Changes in fuel selection with ingestion of CHO were at least partly associated with those in plasma glucose, insulin, and free fatty acids, all of which play a role in plasma glucose utilization during exercise (30). Compared with placebo, no changes in these variables were observed with ingestion of the lower dose of glucose. The contribution of exogenous glucose oxidation to the energy yield was small (~4%), and fat vs. CHO (total and endogenous) oxidation was not modified. In contrast, ingestion of 60 g/h glucose compared with ingestion of 15 g/h glucose resulted in higher plasma insulin concentrations, which could have contributed to the reduction in glucose release from the liver, in plasma free fatty acid concentration, and in fat oxidation over the second hour of exercise. In fact, the progressive reduction in glucose release from the liver with increasing doses of glucose ingested closely followed the increase in plasma insulin concentration. In contrast, the rate of plasma glucose oxidation was not related to the amount of glucose ingested despite the marked difference in plasma insulin concentration. This is in line with the observation that the regulation of plasma glucose uptake in working skeletal muscle through the translocation of GLUT4 transporters is more under the control of muscle contraction than the endocrine effect of insulin (26).

In conclusion, glucose ingested at low rates (15 to 60 g/h) during prolonged exercise has a beneficial effect on endurance performance, and this effect appears to increase with the dose ingested, suggesting that a dose-performance relationship may exist, at least within the range of the doses studied and for exercise lasting ~150 min. Exogenous glucose oxidation, which, as expected, increased with the ingestion rate, increased total CHO oxidation and reduced fat and endogenous CHO oxidation in a dose-dependent manner. The reduction in endogenous CHO oxidation with increasing ingestion rate of exogenous glucose was entirely due to a progressive inhibition of glucose released from the liver (probably related to the higher plasma insulin concentration) without evidence for any muscle glycogen sparing. Although the effect was small, these observations suggest that the improvement in endurance performance due to CHO ingestion during exercise may be associated with the increased oxidation rate of CHO but not with a reduction in muscle glycogen utilization.

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