Effect of carbohydrate ingestion on metabolism during running and cycling

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Arkinstall, Melissa J., Clinton R. Bruce, Vasilis Nikolopoulos, Andrew P. Garnham, and John A. Hawley. Effect of carbohydrate ingestion on metabolism during running and cycling. J Appl Physiol 91: 2125–2134, 2001.—The effects of carbohydrate or water ingestion on metabolism were investigated in seven male subjects during two running and two cycling trials lasting 60 min at individual lactate threshold using indirect calorimetry, U-14C-labeled tracer-derived measures of the rates of oxidation of plasma glucose, and direct determination of mixed muscle glycogen content from the vastus lateralis before and after exercise. Subjects ingested 8 ml/kg body mass of either a 6.4% carbohydrate-electrolyte solution (CHO) or water 10 min before exercise and an additional 2 ml/kg body mass of the same fluid after 20 and 40 min of exercise. Plasma glucose oxidation was greater with CHO than with water during both running (65 ± 20 vs. 42 ± 16 g/h; P < 0.01) and cycling (57 ± 16 vs. 35 ± 12 g/h; P < 0.01). Accordingly, the contribution from plasma glucose oxidation to total carbohydrate oxidation was greater during both running (33 ± 4 vs. 23 ± 3%; P < 0.01) and cycling (36 ± 5 vs. 22 ± 3%; P < 0.01) with CHO ingestion. However, muscle glycogen utilization was not reduced by the ingestion of CHO compared with water during either running (112 ± 32 vs. 141 ± 34 mmol/kg dry mass) or cycling (227 ± 36 vs. 216 ± 39 mmol/kg dry mass). We conclude that, compared with water, 1) the ingestion of carbohydrate during running and cycling enhanced the contribution of plasma glucose oxidation to total carbohydrate oxidation but 2) did not attenuate mixed muscle glycogen utilization during 1 h of continuous submaximal exercise at individual lactate threshold.

muscle metabolism; plasma glucose oxidation; U-14C; whole body glycogen oxidation

IT IS WELL ESTABLISHED that the ingestion of carbohydrate-electrolyte solutions before (24), during (20), or in combination (16) can delay the onset of fatigue during prolonged, submaximal exercise. However, the precise mechanism(s) underlying this ergogenic effect of carbohydrate is not completely understood. During constant-speed treadmill running, ingestion of carbohydrate-electrolyte solutions before and during exercise has been reported to reduce mixed muscle glycogen utilization of the vastus lateralis by ~25% (39, 40). In contrast, consuming carbohydrate during constant-load cycling suppresses hepatic glucose production (6, 16, 28), maintains euglycemia (i.e., 4–5 mM) and high rates of blood glucose oxidation late in exercise (11), but does not reduce muscle glycogenolysis (6, 11, 17, 18, 21). The reasons for these discrepant findings are not clear but could relate to differences in the mode and relative intensity of exercise, the amount and timing of carbohydrate ingested, preexercise muscle glycogen levels, and/or the training status of the subjects under investigation.

With regard to differences in the mode of exercise, it has been proposed that carbohydrate ingestion results in more marked elevations in blood glucose and insulin concentrations during running compared with cycling at the same relative exercise intensity (38). Presumably, the combined effect of this greater blood glucose availability and elevated insulin concentration would be to increase muscle glucose uptake (25, 31, 32) and reduce glycogenolysis; such a mechanism might explain the observed differences in muscle metabolism between exercise modalities.

On the other hand, a comparison of the rates of muscle glycogen utilization between running and cycling studies is complicated because previous investigations have used exercise protocols based on a relative measure of exercise intensity [i.e., a predetermined percentage of maximal O2 uptake (Vo2 max)]. Rates of muscle glycogen utilization have been demonstrated to differ significantly between individuals with a similar aerobic capacity when exercising at the same percentage of Vo2 max (12). Accordingly, when attempting to normalize exercise intensity, it is more appropriate to select a work rate or power output relative to the individuals’ lactate threshold (LT) for that mode of exercise (i.e., with the same acid-base status) rather than a given percentage of peak O2 uptake (Vo2 peak) (2, 12, 13).

Therefore, the aim of the present investigation was to determine the effect of carbohydrate vs. water ingestion on muscle metabolism in the same subjects during continuous, submaximal running and cycling at indi-
vidual LT. We used a combination of indirect calorimetry to estimate whole body rates of carbohydrate and fat oxidation, U-14C-labeled tracer-derived measures of the rates of oxidation of plasma glucose, and direct determination of muscle glycogen concentration before and after each exercise bout. In view of the results of previous investigations, we hypothesized that, when carbohydrate was ingested before and during exercise, mixed muscle glycogen utilization from the vastus lateralis would be attenuated during running (39, 40) but not during constant-load cycling (6, 11, 18, 21).

METHODS

Subjects

Seven male subjects who were moderately trained in both running and cycling exercise participated as subjects in this study, which was approved by the Human Research Ethics Committee of RMIT University. The subjects’ age, body mass (BM), and VO2 peak for running and cycling, respectively, were 31 ± 2 yr, 83.2 ± 4 kg, 4.50 ± 0.22, and 4.28 ± 0.24 l/min (means ± SE). As U-14C-tracer was infused and multiple muscle biopsies and blood samples were taken, all procedures and risks were carefully explained to each subject before written consent was obtained.

Preliminary Testing

All subjects performed a random order of two progressive, incremental tests to volitional fatigue, 1 wk apart, for the determination of individual LT (described subsequently) and VO2 peak for both running and cycling exercise modalities. The cycle test was undertaken on a Lode ergometer ( Groningen, The Netherlands), and the running test was performed on a custom-built motorized treadmill. All tests were conducted under standard laboratory conditions (21–22°C, 40–50% relative humidity). During the 24-h period before each maximal test, subjects refrained from heavy exercise and consumed a preprepared food pack consisting of three meals with a total energy content of ~45 kcal/kg body mass (BM) (carbohydrate: ~24 kcal/kg BM, fat: ~15 kcal/kg BM, protein: ~6 kcal/kg BM).

On the day of a maximal test, subjects reported to the laboratory between 0700 and 0800 h, after a 12- to 14-h overnight fast. After quiet rest for 10 min, an indwelling sterile cannula was inserted into an antecubital vein of one arm and a stopcock was attached to the cannula to allow for repeated blood sampling during exercise. Patency of the cannula and stopcock was maintained by flushing with 2–3 ml of 0.9% saline solution after each blood draw. Blood samples were collected in EDTA Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and immediately analyzed in duplicate for blood glucose and blood lactate concentration by use of a YSI 2300 STAT PLUS blood glucose and lactate analyzer (YSI, Yellow Springs, OH). The LT test commenced at a speed of 10 km/h (running) or a workload of 100 W (cycling). This speed or workload was maintained for 6 min, after which subjects stopped exercise for 1 min while a blood sample (5 ml) was taken. The speed or workload was then increased by 0.5 km/h or 25 W, respectively, for an additional 6 min, with blood samples being drawn at the end of each work bout. Once a workload of 200 W was attained on the cycle ergometer, subsequent workload increments were reduced to 15 W while the same work-rest ratio was maintained to allow for regular blood sampling. Blood lactate concentration was measured on-line, and the LT test was terminated once subjects had reached a concentration of ~3–4 mmol/l. Individual LT was determined according to the methods of Coyle et al. (13). In brief, the LT of each subject for each exercise mode was taken as the speed or workload at which blood lactate concentration rose 1 mmol/l above baseline (taken as the average blood lactate concentration of the first 2–3 exercise bouts).

On completion of the LT test, subjects rested for 2–3 min and then commenced an incremental test to exhaustion for the determination of VO2 peak (i.e., maximal test). During each stage of the LT test and throughout the maximal test, subjects breathed through a mouthpiece attached to a Quark b2 metabolic cart (COSMED, Rome, Italy). Expired gas was passed through a flowmeter, an O2 analyzer, and a CO2 analyzer that were calibrated before testing using a 3-liter Hans-Rudolph syringe and gases of known concentration (4.00% CO2 and 16.00% O2). The flowmeter and gas analyzers were connected to a computer that calculated minute ventilation, oxygen uptake (V˙O2), CO2 production (V˙CO2), and respiratory exchange ratio (RER) from conventional equations (29).

The initial running speed (km/h) or workload (W) for each maximal test was the same as in the final stage completed by a subject during the LT test. Running speed was increased by 0.5 km/h every 60 s until a speed of 16 km/h was reached. Thereafter, the treadmill gradient was increased by 1% (0.9°) every 60 s. During the cycle test, the workload was increased by 25 W/150 s. Tests were terminated at the point of volitional fatigue, which coincided with the inability of a subject to keep pace with the treadmill or maintain a cadence >70 rev/min on the cycle ergometer and/or an RER > 1.15. The highest VO2 for any 60 s was taken as the subject’s VO2 peak. Maximal running speed was determined as the final speed at which subjects exercised. Peak sustained power output was calculated by adding the work completed on the final (uncompleted) workload to the last successfully completed workload (W). Heart rate (HR) was monitored throughout all exercise testing and the subsequently described experimental trials by use of a POLAR Vantage XL heart rate monitor (Polar Electro Oy, Kempele, Finland). The results of these preliminary tests were used to estimate the exercise intensity that corresponded to each individual’s LT for both running and cycling to be performed in the experimental trials.

Experimental Trials

All subjects performed a random order of four experimental trials consisting of two running and two cycling trials with either water or CHO ingestion, separated by 1 wk. In an attempt to standardize pretrial muscle and liver glycogen stores, 48 h before an experiment subjects completed a 30-min exercise bout at ~70% of VO2 peak in the exercise mode to be subsequently tested. Subjects then consumed a standard diet containing an energy content of 45 kcal/kg BM (of which 6 g/kg BM comprised carbohydrate) during the 24-h period before each trial and were also instructed to refrain from formal exercise during this time.

On the morning of an experiment, subjects reported to the laboratory between 0700–0800 h after a 12- to 14-h overnight fast. After 10 min of rest, an indwelling sterile cannula was inserted into a forearm vein, a stopcock was attached, and a resting venous blood sample (10 ml) was taken. After blood collection, local anesthesia [2–3 ml of 1% Xylocaine (lignocaine)] was administered to the skin, subcutaneous tissue, and fascia of the vastus lateralis in preparation for a muscle biopsy. A resting biopsy was taken by using a 6-mm Bergström needle with suction applied (15). Approximately 100
mg of muscle were removed and immediately frozen in liquid nitrogen. Samples were stored at −80°C until subsequent analysis. At this time, a separate site on the same leg (~5 cm distal) was prepared for a second biopsy to be taken immediately after exercise.

After resting for 5 min, subjects were given a single bolus infusion of trace amounts of [U-14C]glucose (10 μCi/370 MBq; Amersham Pharmacia Biotech) in 10 ml of 0.9% saline for subsequent determination of the rates of oxidation of plasma glucose during exercise. Five minutes later, subjects commenced a 5-min warmup that consisted of either running at 8.0 km/h for 150 s and then 9.0 km/h for a further 150 s or cycling at 100 W for 150 s followed by 125 W. On completion of a warm-up, subjects remained standing on the treadmill or sitting on the cycle ergometer for 10 min while ingesting 8 ml/kg BM of either a 6.4% carbohydrate-electrolyte solution (CHO; Lucozade Sport, Glaxo SmithKline PLC) or water. An additional 2 ml/kg BM of the same fluid was ingested 8 ml/kg BM of either a 6.4% carbohydrate-electrolyte solution (CHO; Lucozade Sport, Glaxo SmithKline PLC) or water. An additional 2 ml/kg BM of the same fluid was ingested by subjects after 20 and 40 min of exercise for a total fluid intake of 12 ml/kg BM. On average, subjects ingested 99 ± 37 ml of fluid which resulted in a total carbohydrate intake of 64 ± 3 g during the CHO trials.

Immediately before the start of an exercise bout, a blood sample (10 ml) was taken, and subjects then commenced running or cycling at the speed or workload corresponding to their individual LT. After 5, 15, 25, 35, 45, and 55 min, subjects breathed into a mouthpiece attached to the previously described automated gas analyzer for a 5-min period. In addition, expired air was trapped in an ambulatory bag and bubbled for 2–3 min through a solution that contained 1 ml of 1 N hyamine hydroxide in methanol, 1 ml of 96% ethanol, and two to three drops of phenolphthalein until the solution became clear. At that point, exactly 1 mmol of 14CO2 was trapped (36). Liquid scintillation cocktail (Ultima Gold XR, Packard BioScience BV, Groningen, The Netherlands) was then added to the solution, and 14CO2 radioactivity in disintegrations/min (dpm, later converted to dpm/mmol) was subsequently counted in a liquid scintillation counter (Tri-Carb 1500, Packard Instruments). Expired 14CO2 gas and blood samples were collected after 10, 20, 30, 40, 50, and 59 min of the exercise bout. Ratings of perceived exertion (RPE) (5) and instantaneous HR measures were also taken at these time points.

**Analytical Procedures**

**Rates of carbohydrate and fat oxidation.** Instantaneous rates of whole body carbohydrate and fat oxidation (g/min) were calculated for each 10-min exercise interval from VCO2 and VO2 values (33). Rates of carbohydrate oxidation (μmol·kg⁻¹·min⁻¹) were determined by converting the rate of carbohydrate oxidation (g/min) to its molar equivalent, assuming that 6 mol of O2 is consumed and 6 mol of CO2 is produced for each mole (180 g) oxidized. Rates of fatty acid oxidation (μmol·kg⁻¹·min⁻¹) were determined by converting the rate of triglyceride oxidation (g/min) to its molar equivalent, assuming the average molecular weight of human triglyceride to be 855.3 g per mol oxidized, and multiplying the molar rate of triglyceride oxidation by three because each molecule contains 3 mmol of fatty acid.

Whole body muscle glycogen oxidation was calculated as the difference between total carbohydrate oxidation (estimated from RER data) and the tracer-derived measures of plasma glucose oxidation. Such a calculation is based on the assumption that the original sources of carbohydrate oxidized during exercise are muscle glycogen and blood glucose (10).

**Plasma glucose oxidation.** The rates of oxidation of plasma glucose (Rox) in mmol/min were calculated from the following equation

\[
R_{ox} = (SA_{CO2}/SA_{glu}) \times VCO2
\]

In this equation Rox is the rate of plasma glucose oxidation in mmol/min; SA CO2 is the specific (radio) activity of expired 14CO2 (in dpm/mmol); SA glu is the corresponding specific 14C activity of the plasma glucose (in dpm/mmol); and VCO2 is the volume of expired CO2 in (mmol/min), calculated from the l/min VO2 and the 22.4 mmol gas volume. Because the complete conversion of one molecule of [U-14C]glucose to six molecules of 14CO2 decreases the dpm/mmol specific radioactivity by a factor of six, the VCO2 values did not need to be divided by six to allow for six CO2 molecules arising from oxidation of one glucose molecule.

This formula does not take into account the time required to equilibrate 14CO2 with the plasma CO2/HCO3 that has been reported to vary between 5 and 30 min during steady-state exercise at 65–80% of VO2 max (7). However, Barstow et al. (3) have shown that a near complete equilibrium of the CO2/HCO3 pool is attained after ~15 min of moderate-intensity exercise. Others (6, 14) have previously shown that 14CO2 values closely track specific activities of [14C]glucose in blood and therefore the possible small underestimation of the contribution of the oxidation of plasma glucose to total CHO oxidation would be very similar between trials. Separation of glucose from lactate in the plasma samples was not undertaken because several previous investigations have shown that plasma lactate counts are not significantly different to background counts at exercise intensities up to 80% of VO2 max (e.g., Ref. 14) and in subjects exercising at the individual LT (23). Accordingly, plasma glucose oxidation represents the total labeled plasma products (i.e., lactate, glutamate, glutamine).

**Blood substrates.** Five milliliters of whole blood were placed into a tube containing sodium fluoride EDTA, mixed, and spun in a centrifuge at 4,000 revolutions/min for 8 min at 0°C. The plasma was later analyzed for glucose and lactate concentration using an automated glucose/lactate analyzer (YSI 2300 STAT PLUS). Four milliliters of whole blood were placed into a tube containing lithium heparin, mixed, and spun in a centrifuge (as above). A 500-μl aliquot of this plasma was placed in a tube containing 500 μl of ice-cold 3 M perchloric acid, mixed vigorously, and spun at 15,000 revolutions/min for 3 min. The supernatant was subsequently analyzed for plasma glycerol concentration by using an enzymatic spectrophotometric analysis (34). The remaining plasma was stored at −80°C for later analysis of plasma insulin concentration by radioimmunoassay (Phadeiph, insulin RIA, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Blood (3 ml) for the determination of plasma free fatty acid (FFA) concentration was placed in tubes containing EGTA and reduced glutathione and spun in a centrifuge at 0°C for 15 min at 4,000 revolutions/min. The supernatant was then stored at −80°C until analysis. Plasma FFA concentration was measured by an enzymatic colorimetric method (Wako, NEFA C code 279-75409, Tokyo, Japan). Muscle glycogen concentration was determined in duplicate as glucose residues after hydrolysis in 2 M HCl at 100°C for 2 h (30).

**Statistical Analyses**

Differences in plasma glucose oxidation and muscle glycogen utilization between trials were determined by using a
two-factor (treatment and exercise mode) ANOVA with repeated measures. Total carbohydrate and fat oxidation between trials were compared using a one-way ANOVA. When a significant difference was found, Tukey’s post hoc test was used to identify where the difference occurred. Statistical significance was established at $P < 0.05$. All data are reported as means ± SE.

RESULTS

\textbf{\textit{\textit{V}}\textsubscript{\textit{O}}\textsubscript{2}, Energy Expenditure, Blood Lactate Concentration, HR, and RPE}

Table 1 displays the instantaneous $\text{V}_{\text{O}_2}$ values together with the prevailing blood lactate concentration, HR, and RPE values for each time point. The average percentage of $\text{V}_{\text{O}_2}\text{peak}$ at individual LT was significantly greater running vs. cycling ($78 ± 3\%$ vs. $69 ± 2\%$; $P < 0.05$). However, the rate of energy expenditure was similar between treatment conditions for both running (water: $893 ± 28$ J·kg$^{-1}$·min$^{-1}$, CHO: $922 ± 25$ J·kg$^{-1}$·min$^{-1}$) and cycling (water: $755 ± 17$, CHO: $713 ± 28$ J·kg$^{-1}$·min$^{-1}$). As intended, there were no differences in blood lactate concentration between water and CHO conditions within exercise modalities. Likewise, HR and RPE values were similar between treatments within each exercise mode.

\textit{RER and Instantaneous Rates of Substrate Oxidation}

RER values together with the corresponding rates of carbohydrate and fat oxidation during running and cycling when ingesting water and CHO are displayed in Table 2. RER values were significantly lower at the end of exercise (60 min) compared with 10-min values for both running and cycling with water and CHO ingestion ($P < 0.05$). The average rate of carbohydrate oxidation was not significantly different for either running ($203 ± 11$ vs. $221 ± 9$ μmol·kg$^{-1}$·min$^{-1}$) or cycling ($175 ± 6$ vs. $182 ± 6$ μmol·kg$^{-1}$·min$^{-1}$) between water and CHO trials, respectively. Rates of fat oxidation were significantly greater with water ingestion during cycling compared with CHO ingestion after 10 ($15 ± 6$ vs. $9 ± 3$ μmol·kg$^{-1}$·min$^{-1}$; $P < 0.05$) and 60 min ($27 ± 6$ vs. $18 ± 6$ μmol·kg$^{-1}$·min$^{-1}$; $P < 0.05$) of exercise. There were no significant differences in the rates of fat oxidation during running when subjects ingested water or CHO.

Table 1. $\text{V}_{\text{O}_2}$, blood lactate concentration, HR, and RPE during running and cycling when subjects ingested water and CHO

<table>
<thead>
<tr>
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<th>10 min</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
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<tbody>
<tr>
<td></td>
<td>Water</td>
<td>CHO</td>
<td>Water</td>
<td>CHO</td>
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<tr>
<td>$\text{V}_{\text{O}_2}$, l/min</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Running</td>
<td>3.51 ± 0.59</td>
<td>3.61 ± 0.51</td>
<td>3.48 ± 0.57</td>
<td>3.60 ± 0.49</td>
</tr>
<tr>
<td>Cycling</td>
<td>2.85 ± 0.32</td>
<td>2.68 ± 0.27</td>
<td>2.89 ± 0.35</td>
<td>2.67 ± 0.25</td>
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<tr>
<td>Blood lactate concentration, mmol/l</td>
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<tr>
<td>Running</td>
<td>2.11 ± 0.06</td>
<td>1.94 ± 0.16</td>
<td>2.16 ± 0.21</td>
<td>1.98 ± 0.20</td>
</tr>
<tr>
<td>Cycling</td>
<td>2.22 ± 0.26</td>
<td>2.33 ± 0.20</td>
<td>2.39 ± 0.35</td>
<td>2.29 ± 0.18</td>
</tr>
<tr>
<td>HR, beats/min</td>
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<td></td>
<td></td>
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<tr>
<td>Running</td>
<td>149 ± 16</td>
<td>152 ± 13</td>
<td>153 ± 16</td>
<td>155 ± 14</td>
</tr>
<tr>
<td>Cycling</td>
<td>135 ± 16</td>
<td>133 ± 16</td>
<td>132 ± 18</td>
<td>135 ± 15</td>
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<tr>
<td>RPE</td>
<td></td>
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<tr>
<td>Running</td>
<td>11 ± 2</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
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<tr>
<td>Cycling</td>
<td>10 ± 2</td>
<td>11 ± 2</td>
<td>10 ± 2</td>
<td>11 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 7$. $\text{V}_{\text{O}_2}$, O$_2$ uptake; HR, heart rate; RPE, rating of perceived exertion; CHO, carbohydrate-electrolyte solution.

Table 2. RER, instantaneous rates of carbohydrate and fat oxidation during running and cycling when subjects ingested water and CHO

<table>
<thead>
<tr>
<th></th>
<th>10 min</th>
<th>20 min</th>
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<th>60 min</th>
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<tbody>
<tr>
<td></td>
<td>Water</td>
<td>CHO</td>
<td>Water</td>
<td>CHO</td>
</tr>
<tr>
<td>RER</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Running</td>
<td>0.92 ± 0.03</td>
<td>0.92 ± 0.03</td>
<td>0.90 ± 0.02</td>
<td>0.90 ± 0.03</td>
</tr>
<tr>
<td>Cycling</td>
<td>0.92 ± 0.02</td>
<td>0.95 ± 0.02</td>
<td>0.91 ± 0.02</td>
<td>0.93 ± 0.04</td>
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<tr>
<td>Carbohydrate oxidation, μmol·min$^{-1}·kg^{-1}$</td>
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<td></td>
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<tr>
<td>Running</td>
<td>235 ± 36</td>
<td>239 ± 38</td>
<td>214 ± 27</td>
<td>217 ± 30</td>
</tr>
<tr>
<td>Cycling</td>
<td>192 ± 16</td>
<td>204 ± 23</td>
<td>186 ± 20</td>
<td>185 ± 32</td>
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<tr>
<td>Fat oxidation, μmol·min$^{-1}·kg^{-1}$</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Running</td>
<td>19 ± 6</td>
<td>20 ± 8</td>
<td>23 ± 5</td>
<td>25 ± 8</td>
</tr>
<tr>
<td>Cycling</td>
<td>15 ± 6†</td>
<td>9 ± 3</td>
<td>17 ± 5</td>
<td>13 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 7$. RER, respiratory exchange ratio. *Water significantly different from CHO trial, $P < 0.05$; †10-min value significantly different from 60-min value, $P < 0.05$. 

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Blood Metabolites

Plasma glucose and plasma insulin concentrations are displayed in Fig. 1, and plasma FFA and plasma glycerol concentrations are displayed in Fig. 2. Plasma glucose concentrations were similar at rest (4.6 mmol/l) for all treatment conditions but were elevated by the ingestion of CHO to ~5.4 mmol/l at the start of exercise (Fig. 1A). Plasma glucose concentration peaked after 10 min of exercise during both the running (6.5 ± 0.4 mmol/l) and cycling (6.6 ± 0.3 mmol/l) trials when CHO was ingested. During running, plasma glucose concentration rose significantly above resting values after 10 and 30 min of exercise with CHO and water ingestion and remained elevated until after 50 min of exercise for both treatment conditions. When CHO was ingested during cycling, plasma glucose concentration was only greater than resting values after 10 and 20 min. Although plasma glucose concentration remained above 5 mmol/l throughout the running trial with CHO ingestion, it gradually declined during cycling such that after 60 min of exercise it was similar to resting values. During running with water ingestion, plasma glucose concentration was greater than at rest after 30 min and remained elevated above resting values for the remainder of the exercise bout. In contrast, plasma glucose concentration remained at ~4.8 mmol/l for the duration of the cycle trial when water was ingested. The area under the glucose × time curve (AUC) was significantly greater for both running and cycling when CHO was ingested compared with water (running: 405 ± 13 vs. 363 ± 13 units; *P < 0.05 and cycling: 380 ± 10 vs. 334 ± 7 units; **P < 0.01). However, there were no differences in the AUC between exercise modes when either water or CHO was ingested.

Plasma insulin concentration (Fig. 1B) peaked during both running and cycling trials after 20 min when CHO was ingested, with differences between treatment conditions for both exercise modes being significant after 40 min of exercise (*P < 0.05). During running trials, plasma insulin concentration after 20 and 40 min of exercise was significantly greater than values at rest when CHO was ingested. Differences between plasma insulin concentration at rest and during exercise were observed only after 20 min during cycling. In contrast, there were no significant differences between resting and exercise plasma insulin concentrations for either running or cycling with water ingestion. A comparison of AUC (insulin × time) for running and cycling revealed that the AUC was significantly greater for running and cycling when CHO was ingested compared with water (running: 454 ± 52 vs.

Fig. 1. Plasma glucose (A) and plasma insulin (B) concentrations (brackets denote concentration) during running and cycling when subjects ingested water (W) and carbohydrate (CHO). All values are means ± SE; n = 7. W significantly different from CHO trial: *P < 0.05, **P < 0.01, †P < 0.001. Exercise values significantly different from rest: ×P < 0.05, ‡P < 0.01, ⊥P < 0.001.

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Fig. 2. Plasma free fatty acid (FFA) (A) and plasma glycerol (B) concentrations during running and cycling when subjects ingested W and CHO. All values are means ± SE; n = 7; W significantly different from CHO trial: *P < 0.05, **P < 0.01, †P < 0.001. Exercise values significantly different from rest: ×P < 0.05, ‡P < 0.01, ⊥P < 0.001.
322 ± 14 units; \(P < 0.05\) and cycling: 571 ± 121 vs. 332 ± 46 units; \(P < 0.05\)). However, there were no differences in the AUC between exercise modalities when either water or CHO was ingested.

Plasma FFA concentration (Fig. 2A) increased with water ingestion during both running and cycling trials until 40 min. At this time and at 60 min, plasma FFA concentration was significantly greater with water than CHO ingestion during running \((P < 0.01)\) but not cycling. CHO ingestion suppressed FFA concentration during both running and cycling trials such that, after 20, 40, and 60 min of cycling, values were significantly lower than at rest. Plasma FFA concentration during running was significantly greater than at rest after 40 and 60 min when water was ingested. However, during cycling, such a difference was only observed after 40 min of exercise.

Plasma glycerol concentration (Fig. 2B) increased with water and CHO ingestion throughout running and cycling trials. During running, plasma glycerol concentrations were significantly greater with water ingestion than CHO after 40 and 60 min \((P < 0.01)\), whereas during cycling no differences between treatment conditions occurred. Plasma glycerol concentration during running and cycling was significantly greater than rest after 20, 40, and 60 min when water was ingested. CHO ingestion elevated plasma glycerol concentration above rest after 40 and 60 min during running and after 60 min during cycling.

**Total Fuel Utilization**

RER (Fig. 3A) and plasma glucose oxidation (Fig. 3B) averaged for the 60-min exercise bouts, together with pre- and postexercise mixed muscle glycogen concentration (Fig. 3C) during running and cycling with water or CHO ingestion, are shown in Fig. 3. Although RER was similar between water and CHO treatments for running, the ingestion of CHO during cycling resulted in a significantly higher RER compared with water \((0.92 ± 0.01 \text{ vs. } 0.90 ± 0.01; P < 0.05)\). However, because the average VO\(_2\) was slightly higher during cycling when subjects ingested water \((P < 0.05)\), there were no differences in total carbohydrate oxidation between treatment conditions \((157 ± 25 \text{ vs. } 162 ± 15 \text{ g/h for water and CHO trials, respectively})\). Similarly, there were no differences in total carbohydrate oxidation between water and CHO trials during running \((181 ± 26 \text{ vs. } 197 ± 19 \text{ g/h})\). There was a significant main treatment effect for plasma glucose oxidation, with the ingestion of CHO elevating the rates of oxidation of plasma glucose during both running \((65 ± 20 \text{ vs. } 42 ± 16 \text{ g/h}; P < 0.01)\) and cycling \((57 ± 16 \text{ vs. } 35 ± 12 \text{ g/h}; P < 0.01)\). Accordingly, the contribution from plasma glucose oxidation to total carbohydrate oxidation was significantly greater with CHO than water ingestion during both running \((33 ± 4 \text{ vs. } 23 ± 3%; P < 0.01)\) and cycling trials \((36 ± 5 \text{ vs. } 22 ± 3%; P < 0.01)\). Mixed muscle glycogen concentrations at rest were not significantly different between treatment conditions or exercise modalities (Fig. 3C). Furthermore, muscle glycogen utilization was not reduced by the ingestion of CHO compared with water during either running \([112 ± 32 \text{ vs. } 141 ± 34 \text{ mmol/kg dry mass (dm)}]\) or cycling \([227 ± 36 \text{ vs. } 216 ± 39 \text{ mmol/kg dm}; P = 0.595]\). However, there was a significant main treatment effect for exercise mode, with muscle glycogen utilization being less during running than cycling (Fig. 3C). There was no exercise mode × treatment interaction \((P = 0.41)\).
Figure 4 displays the relative contribution of fat, plasma glucose, and whole body muscle glycogen oxidation to total energy expenditure during running and cycling when water and CHO are ingested. There were no differences in the contribution of whole body muscle glycogen oxidation to energy expenditure either between treatment conditions or between exercise modalities: the oxidation of whole body muscle glycogen contributed ~52% to total energy expenditure (Fig. 4). On the other hand, the contribution of plasma glucose oxidation to energy was increased by the ingestion of CHO during both running (water: 16 ± 2%; CHO: 23 ± 2%; P < 0.05) and cycling (water: 16 ± 2%; CHO: 28 ± 3%; P < 0.001). Similarly, the contribution of fat oxidation to total energy expenditure was lower (P < 0.01) during cycling with CHO (22 ± 2%) compared with both running with CHO (27 ± 3%) and cycling with water ingestion (30 ± 3%; P < 0.05).

DISCUSSION

The major finding of the present study was that the ingestion of ~60 g of carbohydrate before and during 60 min of continuous, submaximal running and cycling at LT did not result in an attenuation of mixed muscle glycogen utilization compared with water. To the best of our knowledge, this is the first investigation to utilize a combination of indirect and direct techniques (i.e., conventional gas measures of whole body rates of substrate oxidation, tracer-derived estimates of plasma glucose oxidation, and muscle biopsies to determine glycogen disappearance) to study the effects of carbohydrate ingestion and mode of exercise on fuel metabolism in the same subjects.

The cycling data from the present investigation are in agreement with the results of several previous studies that have directly measured muscle glycogen before and after continuous submaximal cycling and failed to demonstrate a glycogen-sparing effect as a result of either liquid (6, 11, 18, 21) or solid (17) carbohydrate feedings. In addition, others have estimated whole body muscle glycogen disappearance from the difference between total carbohydrate oxidation and tracer-derived measures of the oxidation of plasma glucose and have also reported that muscle glycogen oxidation was not reduced by carbohydrate ingestion during submaximal cycling (16, 27, 28). We also failed to detect a statistically significant attenuation of muscle glycogen utilization with carbohydrate ingestion during treadmill running despite the fact that the relative exercise intensity (expressed as a percentage of $\dot{V}O_2$ peak) was higher during running (78%) than cycling (70%), conditions that would favor a greater oxidation of intramuscular glycogen during running (35).

In contrast to our findings, one laboratory has reported that in moderately trained subjects carbohydrate ingestion before and during constant-speed running resulted in a reduction in mixed muscle glycogen utilization compared with water (39, 40). The magnitude of this glycogen “sparing” effect was 28% after 1 h (39) and 24% after 104 min of constant-pace treadmill running at 70% of $\dot{V}O_2$ max (40). The reduction in muscle glycogen utilization was confined mainly to type I fibers (39). Although we did not determine the differential depletion of glycogen from type I and type II fibers, the values for mixed muscle glycogen utilization during 1 h of running with and without carbohydrate feedings in the present investigation (112 ± 32 and 141 ± 34 mmol/kg dm) are almost identical to those previously reported (109 vs. 151 mmol/kg dm) by Tsintzas et al. (39).

It should be noted that, although we were unable to detect a statistical difference in muscle glycogen utilization during running with and without carbohydrate ingestion, there was a ~20% attenuation in glycogenolysis with carbohydrate feedings. The inability to detect significant differences in muscle glycogen utilization between treatments may be due to the lack of statistical power given the small number of subjects. Even with the stringent dietary and exercise controls imposed in the current investigation, there remains variability in muscle glycogen content from human biopsy samples. Furthermore, there is likely to be variability both between and within subjects in the amount of muscle mass they can activate when performing the different exercise tasks (i.e., running vs. cycling).
The calculation of whole body muscle glycogen oxidation overcomes limitations of the traditional method of quantifying muscle glycogen utilization from simple changes in concentration within one muscle (10) because there is likely to be less error associated with the estimate of total carbohydrate oxidation from indirect calorimetry and the tracer-derived measures of plasma glucose oxidation than muscle biopsy samples (35). In the present study, the contribution from whole body glycogen oxidation to total energy expenditure was remarkably similar for both water and CHO treatments during running (53 vs. 49%). To the best of our knowledge, such a finding has not been previously reported.

A major difference between the present study and that of Tsintzas et al. (39) is the subjects’ preexercise muscle glycogen levels. Exercise and diet were strictly controlled in the 24-h period before an experimental trial in our moderately trained subjects, with the result that preexercise muscle glycogen concentrations were ~480 mmol/kg dm. In contrast, despite refraining from heavy exercise for 48 h and consuming their normal diet, the “recreational runners” in the study of Tsintzas et al. (39) presented with muscle glycogen levels some 30% lower than in the present investigation (~340 mmol/kg dm). Such values are considerably less than might be expected after such a diet-rest regimen and substantially (~50%) lower than those reported for subjects in cycling studies that have examined the effect of carbohydrate ingestion on muscle metabolism and found no glycogen sparing (6, 11, 21).

Reduced levels of muscle glycogen have been shown to result in lower rates of glycogenolysis during sustained submaximal exercise (19, 22, 41). Conversely, elevated preexercise muscle glycogen concentrations have been reported to increase muscle glycogenolysis during subsequent exercise (19, 22). However, the effect of preexercise muscle glycogen concentration on glucose uptake is less clear. Gollnick et al. (19) observed a greater glucose extraction in the leg that commenced exercise with low glycogen levels compared with the leg that started exercise with normal glycogen concentration. On the other hand, Hargreaves et al. (22) reported no effect of starting muscle glycogen levels on glucose uptake during 40 min of two-legged cycling at 65–70% of VO2 max. Similarly, Weltan et al. (42) also failed to observe an increase in blood glucose oxidation during cycling when euglycemia was maintained by glucose infusion. Finally, Jeukendrup et al. (26) found that tracer-determined rates of ingested glucose oxidation during 2 h of cycling were actually reduced by 28% when subjects commenced exercise with low compared with high starting muscle glycogen content. Taken collectively, these findings strongly suggest that low preexercise muscle glycogen content has little effect on subsequent rates of glucose uptake.

It has been proposed that, at the same relative exercise intensity, carbohydrate ingestion during submaximal running results in more marked elevations in plasma glucose and insulin concentrations compared with cycling (38). An elevation in plasma glucose and insulin concentrations after carbohydrate ingestion has been shown to increase muscle glucose uptake during both low- (1) and moderate- to high-intensity exercise (32). Thus the possibility exists that the mode of exercise may play a role in determining fuel metabolism when carbohydrate is consumed before and during exercise.

Although the prevailing plasma insulin concentrations during running after carbohydrate ingestion were similar in the present study to those reported by Tsintzas et al. (39), our plasma glucose concentrations were substantially higher (average 5.8 mmol/l). Indeed, it might be argued that the ingestion of carbohydrate in the study of Tsintzas et al. merely restored euglycemia (i.e., 4.3 mmol/l) compared with the modest hypoglycemia (i.e., 3.5 mmol/l) that resulted when subjects consumed only water. Of interest in the current study was the observation that carbohydrate ingestion before and during running better maintained plasma glucose concentration than during cycling. Previous studies have reported that plasma glucose concentration during running without carbohydrate ingestion does not decline to the same extent as during cycling before the point of fatigue (11, 37, 43). However, this is the first investigation to measure this effect in the same subjects exercising in the two disciplines.

Others have also observed marked elevations in blood glucose concentration in response to carbohydrate ingestion during moderate-intensity cycling without concomitant glycogen sparing (16, 27, 28). In the current investigation, the areas under both the plasma glucose and plasma insulin vs. time curves were not different between exercise modes when subjects ingested carbohydrate. This is an important finding in view of previous suggestions that differences in muscle metabolism between exercise modalities may be due to differences in plasma glucose and insulin concentrations (38).

A decreased muscle glycogen utilization during 1 h of running with the intake of carbohydrate in the investigation of Tsintzas et al. (39) must be due to an increased oxidation of plasma glucose, because rates of total carbohydrate oxidation in that study were similar with and without carbohydrate ingestion. In agreement with Tsintzas et al., we also found that carbohydrate ingestion failed to increase rates of total carbohydrate oxidation during running. However, our tracer-derived estimates of the rates of oxidation of plasma glucose showed that, compared with water, carbohydrate ingestion increased the contribution from plasma glucose oxidation to total carbohydrate oxidation from ~23 to ~35%, an increase that was remarkably consistent for both exercise modes.

Several other “mode-specific” differences exist between running and cycling. First, during laboratory cycling, there is marked glycogen depletion from the vastus lateralis, whereas during level running the gastrocnemius and soleus muscles have the greatest rate of glycogen utilization (8, 9). We deliberately chose to sample the vastus lateralis muscle in the present study during both running and cycling because previous in-
vestigations have reported glycogen sparing in that muscle during treadmill running (39, 40). Differences in muscle recruitment patterns during the two modes of exercise would also be reflected in the actual muscle mass involved in the contraction process. In this regard, running is likely to involve a greater total (working and nonworking) muscle mass than cycling. Therefore, one would expect a higher absolute oxygen cost and greater rates of whole body carbohydrate oxidation during running compared with cycling exercise at the same LT. This was indeed the case in the present investigation. Yet despite the higher relative exercise intensity and overall rate of carbohydrate oxidation when running than when cycling, the disappearance of muscle glycogen was greater in cycling than running. Our rates of glycogen disappearance are in close agreement with several previous reports (6, 11, 22) and corroborate the results of other studies that report that the vastus lateralis is heavily recruited during cycling (8, 9).

In conclusion, the results of this investigation demonstrate that, in moderately trained subjects exercising at LT, the ingestion of ~60 g of carbohydrate before and during 1 h of continuous submaximal running and cycling elevated plasma glucose and insulin concentrations and increased the oxidation of plasma glucose compared with water. However, carbohydrate ingestion did not change the relative contribution from whole body glycogen oxidation to total energy expenditure nor reduce mixed muscle glycogen utilization compared with water.

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