Oral $^{13}$C-glucose oxidation during prolonged exercise after high- and low-carbohydrate diets

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Péronnet, F., N. Rhéaume, C. Lavoie, C. Hillaire-Marcel, and D. Massicotte. Oral $^{13}$C-glucose oxidation during prolonged exercise after high- and low-carbohydrate diets. J. Appl. Physiol. 85(2): 723–730, 1998.—The effect of a diet either high or low in carbohydrates (CHO) on exogenous $^{13}$C-labeled glucose oxidation (200 g) during exercise (ergocycle: 120 min at 64.0 ± 0.5% maximal oxygen uptake) was studied in six subjects. Between 40 and 80 min, exogenous glucose oxidation was significantly higher after the diet low in CHO (0.63 ± 0.05 vs. 0.52 ± 0.04 g/min), but this difference disappeared between 80 and 120 min (0.71 ± 0.03 vs. 0.69 ± 0.04 g/min). The oxidation rate of plasma glucose, computed from the volume of $^{13}$CO$_2$ produced at the mouth. In addition, the oxidation of plasma glucose was computed (13C/12C) of plasma glucose. Glucose liver output was estimated by the difference between plasma glucose concentration (4.6 ± 0.03 vs. 0.69 ± 0.04 g/min). After a diet low in CHO, the oxidation of exogenous glucose was higher after the diet low in CHO (1.68 ± 0.26 vs. 1.41 ± 0.17 and 1.02 ± 0.20 vs. 0.81 ± 0.14 g/min, respectively). In contrast the oxidation rate of glucose plus lactate from muscle glycogen (computed from the difference between total CHO oxidation and plasma glucose oxidation) was lower (0.31 ± 0.35 vs. 1.59 ± 0.20 g/min). After a diet low in CHO, the oxidation of exogenous glucose and of glucose released from the liver, computed from the difference between plasma glucose and exogenous glucose oxidation, was higher than the difference observed in prolonged exercises: 40 (31) and 57% maximal oxygen uptake (VO$_{2\text{max}}$) (16). The amount of glucose that was ingested was also low [100–125 g for a 2-h exercise period or 0.83–1.04 g/min in both studies (16, 31)]. The oxidation rate of exogenous carbohydrates increases with both workload (22) and with the amount ingested (36). In addition, the subjects studied by Jeukendrup et al. (16) were highly trained endurance athletes, and the amount of carbohydrate ingested before exercise was comparatively low (250–300 g). As a consequence, the subjects relied much less on the oxidation of glucose (43 and 30% of the energy yield during the last 60 min of the 2-h exercise period for the diet low and high in carbohydrates, respectively) than on that of fatty acids.

The purpose of the present study was to describe the effect of endogenous carbohydrate availability on the oxidation of exogenous glucose during prolonged exercise in a situation where the contribution of glucose oxidation to the energy yield is high, i.e., at a moderately high percent VO$_{2\text{max}}$, in active subjects, and when a large amount of glucose is ingested immediately before and during exercise. Ingested glucose was artificially labeled with $^{13}$C to compute its oxidation rate from the volume of $^{13}$CO$_2$ produced at the mouth. In addition, the oxidation of plasma glucose was computed from volume of $^{13}$CO$_2$ produced and the $^{13}$C-to-$^{12}$C ratio ($^{13}$C/$^{12}$C) of plasma glucose. Glucose liver output was estimated by the difference between plasma glucose and exogenous glucose oxidation, and the oxidation of muscle glycogen was calculated by the difference between total glucose and plasma glucose oxidation.

METHODS

Subjects. The experiment was conducted in six active and healthy male subjects who gave their informed written consent to participate in the study, which was approved by the Institutional Board on the use of human subjects in research. Their age, height, weight, VO$_{2\text{max}}$, and power output on the cycle ergometer were 21.6 ± 1.1 (SE) yr; 174.6 ± 3.8 cm; 65.3 ± 2.8 kg; and 62.4 ± 1.1 ml·kg$^{-1}$·min$^{-1}$ (295 ± 19 W), respectively. All the subjects had a normal fasting plasma glucose concentration (4.6 ± 0.3 mmol/l).

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Experimental protocol. VO_2max and experimental workloads on the cycle ergometer (Ergomeca, La Bayette, France) were determined for each subject during a preliminary test session by using open-circuit spirometry (1100 Medical Gas Analyzer, Marquette Electronics, Milwaukee, WI). Subsequently, the subjects performed, 1 wk apart, two exercises of 120 min duration at a workload corresponding to 64.0 ± 0.5% VO_2max (170 ± 10 W). The exercises were performed in a laboratory with controlled temperature and humidity (23°C; 36%) between 8:00 and 11:00 AM. Three days before each of the two trials, between 3:00 and 5:00 PM, the subjects performed a 90-min exercise at 70% VO_2max on a cycle ergometer to reduce glycogen stores (10). For the following 2 days the subjects rested, and a diet low in carbohydrates (3,000 kcal/day with 200 g of carbohydrates or 27% of the energy intake) or high in carbohydrates (3,500 kcal/day with 700 g of carbohydrates or 80% of the energy intake) was ingested. Ingestion of carbohydrates from plants with the C_4 photosynthetic cycle, which are naturally enriched in ^13C (26), was avoided so as to keep a low background ^13C enrichment of plasma glucose and expired CO_2. The subjects were provided with prepackaged meals and were closely monitored by one of the investigators to ensure maximal compliance with the requested diet. They also refrained from drinking coffee and alcohol. Finally, in the morning before the exercise (6:30 AM), the subjects ingested a standardized breakfast (600 kcal) either low (32% carbohydrates or 50 g) or high in carbohydrates (65% carbohydrates or 95 g). The order of presentation of the two diets was randomly balanced among the subjects.

Twenty minutes before the beginning of exercise, the subjects ingested 50 g of glucose in 400 ml of water at room temperature. They subsequently ingested four doses of 37.5 g of glucose in 300 ml of water at 20, 40, 60, and 80 min during the exercise period (total amount of glucose ingested = 200 g in 1,600 ml of water). The glucose, which was purchased from Biopharm (Laval, Quebec), was derived from corn ^13C/12C = −11.03% (8-13C)Pee Dee Bemimtenlla1 (PDB-1)) and was artificially enriched with [U-^13C]glucose (13C/C > 99%, lots etc., Miaminds, OH) to achieve a final isotopic composition close to +50% ^13C/PDB-1 (actual value measured by mass spectrometry: +51.1% [8-13C]PDB-1). This high ^13C enrichment of exogenous glucose provides a very strong signal in plasma glucose as well as in expired CO_2 (Table 1) and allows neglect of the comparatively small changes in background enrichment of expired CO_2 observed from rest to exercise (26).

Measurements and computations. Observations were made at rest immediately before ingestion of the first dose of [^13C]glucose and every 20 min during the exercise period. Total glucose and fatty acid oxidation were computed from indirect respiratory calorimetry corrected for protein oxidation. For this purpose, CO_2 production (VCO_2) and oxygen uptake (VO_2) were measured by using open-circuit spirometry (5 min collection). Urea excretion over the exercise period was estimated from its concentration in urine (260 ± 98 and 167 ± 39 mmol/l after the diet low and high in carbohydrates, respectively) and sweat (3) (15.0 ± 3.9 vs. 12.2 ± 2.1 mmol/l) and from urine (0.20 ± 0.02 and 0.29 ± 0.02 liter) and sweat loss (1.70 ± 0.22 and 1.76 ± 0.33 liters) over the exercise period. Sweat loss was estimated from change in body weight, accounting for fluid intake, weight loss through VCO_2, and water loss in the lungs (20). For the measurement of ^13C/12C in expired CO_2, 80-ml samples of expired gas were collected in Vacutainers (Becton-Dickinson, Franklin Lakes, NJ). Finally, 15-ml blood samples were withdrawn through a catheter (Baxter Health Care, Valencia, CA), which was inserted into an antecubital vein, at 20-min intervals for the measurement of plasma glucose, insulin, lactate, free fatty acid, and urea concentrations and for the measurement of ^13C/12C in plasma glucose (this latter measurement was only performed at rest before ingestion of the first dose of glucose and at 40 and 80 min during exercise). Plasma, urine, and sweat samples were stored at −80°C until analysis.

Protein oxidation and the associated amount of energy provided were computed from the estimated amount of urea excreted during the exercise period (neglecting the small changes in plasma urea concentration: 8.3 ± 1.1 and 6.8 ± 0.8 mmol/l at rest; 8.7 ± 0.6 and 6.6 ± 0.5 mmol/l at the end of exercise, after the diet low and high in carbohydrates, respectively), accounting for 1 g of urea excreted corresponding to 2.9 g of proteins oxidized and the energy potential of proteins being 4.704 kcal/g (21). Glucose (Glu_total) and free fatty acid (FFA_total) oxidation were then computed from VO_2 and VCO_2 (25) corrected for the volume of O_2 and CO_2 corresponding to protein oxidation (1.010 vs. 0.843 l/g, respectively (21).

\[ \text{Glu}_{\text{total}} = 4.5850 \times \text{VCO}_2 - 3.2255 \times \text{VO}_2 \] (1)

\[ \text{FFA}_{\text{total}} = -1.7012 \times \text{VCO}_2 + 1.6946 \times \text{VO}_2 \] (2)

The amount of energy provided by the oxidation of glucose and fat was computed from their respective energy potential (3.8683 and 9.7462 kcal/g) (25).

Plasma glucose, lactate (Sigma Diagnostics, Sigma Chemical, Mississauga, Ontario), and free fatty acid concentrations (Boehringer Mannheim) were measured by using spectrophotometric automated assays, whereas plasma insulin concentration was measured by using an automated radioimmunoassay (KTSP-11001, Immunocorp Sciences, Montreal, Quebec). Plasma, urine, and sweat urea concentrations were measured by using a Synchon Clinical System (CX7, Beckman, Anaheim, CA).

For the measurement of ^13C/12C in plasma glucose, 1 ml of plasma was first deproteinized with barium hydroxide (1.5 ml, 0.3 N) and zinc sulfate (1.5 ml, 0.3 N). The soluble phase was separated from the protein precipitate by centrifugation (20 min, 3,000 g, 4°C), and the remaining protein precipitate was washed with 3 ml of distilled water. The glucose was then separated by double-bed ion-exchange chromatography by running the combined supernatants (7 ml) through superposed columns (0.5 × 2 cm) of AG 50W-X8 H^+ (200–400 mesh) and (0.5 × 2 cm) of AG 1-X8 chloride (200–400 mesh) resins (Bio-Rad, Mississauga, Ontario) equilibrated and eluted with distilled water. The solution obtained (10 ml) was evaporated to dryness ( Virtis Research Equipment, New York, NY). The average percent recovery of glucose was 86 ± 3%. The eluent was then used for 60 min at 400°C in the presence of copper oxide (20 mg), and the CO_2 recovered was measured.

### Table 1. VO_2, VCO_2, and ^13C/12C in expired CO_2 at rest and during exercise after diets low and high in carbohydrates

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Low-CHO</th>
<th>High-CHO</th>
</tr>
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<tbody>
<tr>
<td>VO_2, l/min</td>
<td></td>
<td></td>
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<tr>
<td>40–80</td>
<td>2.70 ± 0.13</td>
<td>2.56 ± 0.09</td>
</tr>
<tr>
<td>80–120</td>
<td>2.70 ± 0.35</td>
<td>2.62 ± 0.12</td>
</tr>
<tr>
<td>VCO_2, l/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–80</td>
<td>2.15 ± 0.09</td>
<td>2.37 ± 0.08</td>
</tr>
<tr>
<td>80–120</td>
<td>2.12 ± 0.11</td>
<td>2.35 ± 0.09</td>
</tr>
<tr>
<td>^13C/12C, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–80</td>
<td>-23.26 ± 0.32</td>
<td>-23.76 ± 0.29</td>
</tr>
<tr>
<td>80–120</td>
<td>-3.84 ± 1.70</td>
<td>-10.39 ± 2.10</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. VO_2, O_2 uptake; VCO_2, CO_2 production; Low-CHO and High-CHO, low- and high-carbohydrate diet, respectively; ^13C/12C, ^13C-to-12C ratio; PDB-1, Pee-Dee Bemimtenlla1.
analyzed by mass spectrometry (Prism, Manchester, UK). This procedure for purification of plasma glucose has been demonstrated to yield values for $^{13}$C/glucose enrichment similar to those obtained by using the more specific isolation procedure of plasma glucose by crystallization as potassium gluconate (38). The material obtained after evaporation was resuspended in 0.5 ml of distilled water for screening for possible contamination by nonglucose carbons. Compared with the amount of glucose present (8.2 mmol/l), the amounts of glycerol (0.07–0.09 mmol/l) and lactate (0–0.04 mmol/l) present were negligible, and no proteins were detectable.

Measurements of $^{13}$C/$^{12}$C in expired CO$_2$ and in CO$_2$ from combustion of plasma glucose were performed by mass spectrometry, after cryodistillation as previously described (22). The isotopic composition of ingested glucose, expired CO$_2$, and plasma glucose was expressed in % difference by comparison with the PDB-1 Chicago Standard: $\%_{\text{rel}}$ = $^{13}$C/12C$_{\text{sample}}$/$^{13}$C/12C$_{\text{standard}}$ (39). This computation is made assuming that, in response to exercise, $^{13}$CO$_2$ recovery is complete or almost complete (6, 19).

The amount of exogenous glucose oxidized (GIUexo, g) was computed as follows

$$\text{GIU}_{\text{exo}} = \frac{\dot{V}_{\text{CO}_2} (R_{\text{exo}} - R_{\text{ref}})/(R_{\text{ref}} - R_{\text{std}})}{k} \quad (3)$$

where $\dot{V}_{\text{CO}_2}$ is in liters per minute, $R_{\text{exo}}$ is the observed isotopic composition of expired CO$_2$, $R_{\text{ref}}$ is the isotopic composition of expired CO$_2$ at rest before ingestion of $^{13}$C/glucose, $R_{\text{std}}$ is the isotopic composition of the exogenous glucose ingested, and $k$ (0.7426 l/g) is the volume of CO$_2$ provided by the complete oxidation of glucose (25). This computation is made assuming that, in response to exercise, $^{13}$CO$_2$ recovery in expired gases is complete or almost complete (6, 19).

However, because of the presence of a large bicarbonate pool in the body, $^{13}$C/$^{12}$C in expired CO$_2$ only slowly equilibrates with $^{13}$C/$^{12}$C in the CO$_2$ produced in the tissues (23). To take into account this delay between $^{13}$CO$_2$ production in the tissues and at the mouth, the above computations were only made during the last 80 min of the observation period. The amount of endogenous glucose oxidized was computed from the difference between the total amount of glucose oxidized computed from indirect respiratory calorimetry (Eq. 1) and the amount of exogenous glucose oxidized (Eq. 3).

On the basis of the isotopic composition of plasma glucose ($R_{\text{GLU}}$), the percentage of plasma glucose derived from exogenous $^{13}$C/glucose ($F_{\text{exo}}$) and the oxidation rate of blood-borne glucose ($\text{GIU}_{\text{blood}}$) were computed as follows, at 40 and 80 min during exercise

$$F_{\text{exo}} = \frac{[R_{\text{GLU}} - R_{\text{GLU}-\text{ref}}]/(R_{\text{ref}} - R_{\text{GLU}-\text{ref}})] \times 100 \quad (4)$$

and

$$\text{GIU}_{\text{blood}} = \frac{\dot{V}_{\text{CO}_2} (R_{\text{exo}} - R_{\text{ref}})/(R_{\text{ref}} - R_{\text{std}})}{k} \quad (5)$$

where $R_{\text{GLU}-\text{ref}}$ is the isotopic composition of plasma glucose observed at rest before ingestion of labeled glucose: $-23.1 \pm 0.13$ and $-22.9 \pm 0.16$% in the PDB-1 after the diet high and low in carbohydrates, respectively. These values were not significantly different from each other and not significantly different from $R_{\text{ref}}$ (see Table 2). The amount of glucose and C$_3$ products oxidized that was derived from muscle glycogen, either directly or through the lactate-pyruvate shuttle (4), was computed as the difference between the total amount of glucose oxidized ($\text{GIU}_{\text{exo}}$ in Eq. 1) and the amount of plasma glucose oxidized ($\text{GIU}_{\text{blood}}$ in Eq. 5). Finally, the amount of glucose released from the liver was estimated as the difference between $\text{GIU}_{\text{blood}}$ and $\text{GIU}_{\text{exo}}$ because under these conditions oxidation appears to be the major metabolic fate of plasma glucose (17).

**RESULTS**

Results from indirect respiratory calorimetry and substrate oxidation during the last 80 min of the exercise period are shown in Tables 1 and 2. Compared with the observations made after the diet low in carbohydrates, total carbohydrate oxidation significantly increased by 57% and fatty acid oxidation significantly decreased by 69% after ingestion of the diet high in carbohydrates. No significant difference in the amount of protein oxidized was observed between the two conditions. On the other hand, the amount of exogenous glucose oxidized was slightly but significantly lower (8%) after the diet high in carbohydrates. The contribution of exogenous glucose oxidation to the energy yield averaged 18.1 ± 2.2 and 19.4 ± 1.5% after the diets high and low in carbohydrates, respectively. In contrast, the amount of endogenous glucose oxidized was significantly higher (93%) after the diet high in carbohydrates.

The average oxidation rates of total, exogenous, and endogenous glucose and of fatty acids computed in the 40- to 80- and 80- to 120-min intervals (assuming that the oxidation rate of proteins was stable over the exercise period) are shown in Fig. 1. Throughout the exercise period, total glucose oxidation was significantly higher after the diet high in carbohydrates than that after the diet low in carbohydrates. After the diet high in carbohydrates, the oxidation rates of endogenous glucose and fatty acids progressively decreased and increased, respectively, over the exercise period and

**Table 2. Average substrate oxidation computed from indirect respiratory calorimetry, corrected for protein oxidation, and volume of $^{13}$CO$_2$ produced at the mouth over the last 80 min of exercise period after diets low and high in carbohydrates**

<table>
<thead>
<tr>
<th></th>
<th>Low-CHO</th>
<th>High-CHO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g/min</td>
<td>0.16 ± 0.05</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>% energy</td>
<td>3.3 ± 1.5</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>0.58 ± 0.06</td>
<td>0.18 ± 0.06†</td>
</tr>
<tr>
<td>g/min</td>
<td>42.7 ± 4.9</td>
<td>12.5 ± 4.1†</td>
</tr>
<tr>
<td>% energy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endogenous</td>
<td>1.20 ± 0.17</td>
<td>2.31 ± 0.18‡</td>
</tr>
<tr>
<td>g/min</td>
<td>34.6 ± 4.7</td>
<td>65.6 ± 3.9†</td>
</tr>
<tr>
<td>% energy</td>
<td>57.0 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>Exogenous</td>
<td>0.66 ± 0.04</td>
<td>0.61 ± 0.05†</td>
</tr>
<tr>
<td>g/min</td>
<td>19.4 ± 1.5</td>
<td>18.1 ± 2.2</td>
</tr>
<tr>
<td>% energy</td>
<td>30.5 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.86 ± 0.17</td>
<td>2.92 ± 0.15†</td>
</tr>
<tr>
<td>g/min</td>
<td>54.0 ± 4.4</td>
<td>83.7 ± 4.01†</td>
</tr>
<tr>
<td>% energy</td>
<td>83.7 ± 4.01†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 6$ subjects. *Values corrected for protein oxidation. †Significantly different from Low-CHO, $P < 0.05$. Statistics. Data are presented as means ± SE. The main effects of time and diet (low- and high-carbohydrate diets) as well as time-diet interactions were tested by repeated-measures analysis of variance (Statistica package). A Newman-Keuls post hoc test was used to identify the location of significant differences ($P < 0.05$) when analysis of variance yielded a significant F-ratio.
were significantly higher and lower, respectively, than after the diet low in carbohydrates. The oxidation rate of exogenous glucose significantly increased over the exercise period in the two conditions. Between minutes 40 and 80, the oxidation rate of exogenous glucose was significantly higher (21%) after the diet low in carbohydrates, whereas during the last 40 min of exercise the oxidation rate of exogenous glucose was similar in both conditions (0.71 ± 0.04 vs. 0.69 ± 0.04 g/min for the diets high and low in carbohydrates, respectively). At the end of the exercise period (80–120 min), the oxidation rate of endogenous glucose was significantly lower after the diet low in carbohydrates (1.03 ± 0.19 vs. 1.97 ± 0.14 g/min), contributing 32 and 60% to the energy yield for the low- and high-carbohydrate diets, respectively. After the diet high in carbohydrates, between 80 and 120 min, 26% of the glucose oxidized was derived from exogenous glucose. This value increased to 40% after the diet low in carbohydrates.

The oxidation rates of plasma glucose, exogenous glucose, liver glucose, and muscle glycogen (plus C₃ products) computed at 40 and 80 min during the exercise period are shown in Fig. 2. Compared with the observations made after the diet high in carbohydrates, the oxidation rate of plasma glucose, which increased from 40 to 80 min in both conditions, was significantly higher after the diet low in carbohydrates. This was due to both an increased oxidation rate of exogenous glucose, as already mentioned, and to an increased liver glucose output (statistically significant at 40 min, only). In contrast, the amount of muscle glycogen and C₃ products oxidized was significantly reduced after the diet low in carbohydrates.

Figure 3 shows changes in plasma glucose, insulin, lactate, and free fatty acid concentrations throughout the exercise period. At rest before ingestion of [13C]glucose (−20 min), plasma glucose concentration was significantly higher and plasma free fatty acid concentration was significantly lower after the diet and the breakfast high in carbohydrates. After the transient peak observed between −20 and 20 min, due to the ingestion of 50 g of [13C]glucose at 20 min before the onset of exercise, plasma glucose concentration remained stable at slightly above 5.5 mmol/l throughout the exercise period and was not significantly different after the diets low and high in carbohydrates, respectively. Plasma free fatty acid concentration significantly increased in response to exercise in both situations but remained significantly lower after the diet high in carbohydrates throughout the exercise period. Plasma insulin concentration, which was high at the onset of exercise, quickly declined over the exercise period and was also similar after the two diets, except at 20 min during the exercise. The transient rise in plasma lactate concentration at the beginning of exercise was significantly larger, and plasma lactate concen-
tration remained slightly higher throughout the exercise period after the diet high in carbohydrates.

**DISCUSSION**

Results from the present experiment indicate that, in response to prolonged moderate exercise, the subjects relied more on exogenous glucose oxidation after the diet poor in carbohydrates, when glycogen availability was presumably low, than after the diet rich in carbohydrates, when glycogen availability was presumably high. This was mainly due to a larger oxidation rate of exogenous glucose between 40 and 80 min of the exercise period (0.63 ± 0.05 vs. 0.52 ± 0.04 g/min, or 17.6 vs. 14.5% of the energy yield, respectively). In contrast, during the last 40 min of exercise the oxidation rate of exogenous glucose was similar after the two diets (0.69 ± 0.03 and 0.71 ± 0.04 g/min, or 19.2 and 20.5% of the energy yield, respectively). However, after the diet low in carbohydrates, during the last 40 min of exercise, a much higher percentage of the carbohydrates oxidized was provided by the glucose ingested (40 vs. 26% after the diet high in carbohydrates).

Ravussin et al. (31) and Jeukendrup et al. (16) have previously studied the effect of changes in glycogen availability, induced by various combination of diet and exercise the day preceding the experiment, on the metabolic response to exercise and on exogenous glucose oxidation. In these studies, as in the present experiment, the actual changes in muscle glycogen content were not measured. However, it has been shown that the types of diet and/or exercise regimen used in the present experiment, as well as by Ravussin et al. and Jeukendrup et al., are associated with marked changes in muscle glycogen contents (1, 2, 33, 37). In addition, changes in the respective contributions of endogenous glucose vs. fatty acid oxidation to the energy yield observed by Ravussin et al. and by Jeukendrup et al. were consistent with the expected changes in glycogen availability. In the present study the respective contributions of endogenous glucose and fatty acid oxidation to the energy yield also confirmed that marked changes in glycogen availability were obtained (endogenous glucose, 70.8 ± 2.7 vs. 37.1 ± 6.7% fatty acid oxidation, 10.1 ± 3.2 vs. 41.8 ± 17.5% of the energy yield after the diet high and low in carbohydrates, respectively). Furthermore, the reduction in the contribution of endogenous glucose oxidation to the energy yield was mainly due to a large decrease in muscle glycogen utilization (Fig. 2). This observation is in line with results from several studies showing that the rate of muscle glycogen breakdown under electrical stimulation of the rat hindlimb (14, 15, 32) and during prolonged submaximal exercise in humans (2, 9, 11, 22, 33) decreases as the initial level of muscle glycogen declines.

In the study by Ravussin et al. (31) two groups of subjects were observed for 2 h at 40% VO2max on a cycle ergometer, 1 h after ingestion of 100 g of glucose. The
amounts of exogenous glucose actually oxidized were not significantly different in the two groups: 41 g in subjects with normal glycogen availability vs. 38 g in subjects with reduced glycogen availability. However, due to the 20% higher energy expenditure observed in the group of subjects with reduced glycogen stores (because of a 15% higher VO2max), exogenous glucose oxidation provided only 16% of the energy yield vs. 20% in the group of subjects with normal glycogen stores. These data suggest that the decrease in glycogen availability was associated with a decrease in the reliance on exogenous glucose oxidation during exercise. In the more recent study by Jeukendrup et al. (16), the same subjects exercised for 2 h at 57% of VO2max, 13 h after either a prolonged exercise period associated with food deprivation, to reduce glycogen availability, or rest associated with a meal high in carbohydrates, to increase glycogen availability. Over the second hour of exercise, the contribution of exogenous glucose oxidation to the energy yield was reduced from 18% after the diet high in carbohydrates (49.3 g oxidized) to 13% after carbohydrate deprivation (35.7 g oxidized). In both studies, from Ravussin et al. and from Jeukendrup et al., the response of plasma free fatty acid concentration to exercise was 2–3 times higher when glycogen availability was reduced, whereas plasma insulin concentration tended to be lower (31) or was significantly lower (16). These authors suggested that this could explain the observed reduction in exogenous glucose oxidation because both high plasma free fatty acid concentration (7, 28) and low plasma insulin level reduce plasma glucose uptake and oxidation.

In the present study, in response to exercise, plasma free fatty concentration was also about twofold higher after the diet low in carbohydrates, whereas plasma insulin concentration was similar in the two situations, except for the values observed early during exercise. However, exogenous glucose oxidation was 14% higher after the diet low in carbohydrates. This difference from results in the studies by Ravussin et al. (31) and by Jeukendrup et al. (16) could be due to differences in the power output sustained and in the amount of glucose ingested. In the present study the subjects exercised at 64% VO2max, whereas the fractional utilization of VO2max was only 40% in the study by Ravussin et al. and 57% in the study by Jeukendrup et al. At these comparatively low relative workloads the reduction in carbohydrate availability was compensated for by a large increase in the contribution of fatty acid oxidation to the energy yield (70% in both studies when glycogen availability was reduced). In contrast, in the present study, because of the higher relative workload sustained, the subjects relied much more on the oxidation of glucose after the diet high in carbohydrates as well as after the diet low
in carbohydrates (85.5 ± 2.8 and 54.4 ± 1.8% for carbohydrates vs. 10.1 ± 3.2 and 41.8 ± 7.5% for fatty acid oxidation, respectively). This increased reliance on carbohydrate oxidation, and the much larger amount of exogenous glucose ingested [200 vs. 100 and 127 g in the studies by Ravussin et al. and Jeukendrup et al., respectively] could explain the compensatory increase in exogenous glucose oxidation when glycogen availability was reduced, despite higher plasma free fatty acid and similar plasma insulin concentrations for most of the exercise period.

The increased oxidation of exogenous glucose observed in the present study after the diet low in carbohydrates is in line with results from studies conducted both in rats (14, 15, 32) and humans (9, 12). In electrically stimulated perfused rat hindquarters, glucose uptake is inversely related to the initial muscle glycogen content; compared with the control level of muscle glycogen, glucose uptake was 50–60% higher when the initial muscle glycogen content was reduced (13) and 30% lower when muscle glycogen content was increased (14, 15, 32). In humans, Golnicken et al. (9) studied plasma glucose uptake across each leg during two-leg exercise with the initial glycogen content in one leg being reduced by 50% from the control level in the other leg. Plasma glucose uptake was approximately five times higher in the leg with a low initial glycogen content. Finally, Hargreaves et al. (12) have shown an inverse relationship between muscle glycogen content and plasma glucose uptake during exercise in humans.

In contrast, Bosch et al. (2) and Hargreaves et al. (11) did not observe any increase in the rate of plasma glucose appearance or disappearance during exercise in subjects with low vs. high muscle glycogen stores. However, in the cross-sectional study by Bosch et al., although the differences did not reach statistical significance, the rate of appearance of plasma glucose was consistently ~45–50% higher, whereas the rate of plasma glucose oxidation was ~20% higher in subjects with reduced muscle glycogen stores. In addition, as discussed by Hargreaves et al., in the study by Bosch et al., as well as in their own study, plasma glucose uptake was not reduced when muscle glycogen availability was low despite lower plasma glucose (2, 10) and insulin concentrations (11). This suggests that the reduction in the availability and utilization of muscle glycogen does not favor plasma glucose uptake and compensates for the low plasma glucose and insulin level. In the present study, despite the reduction in glycogen availability, plasma glucose concentration was maintained at a high level throughout the exercise period (slightly above 5.5 mmol/l) because large amounts of glucose were ingested both before and during exercise, and plasma insulin concentration was significantly higher in the first 20 min of exercise after the diet high in carbohydrates. This could explain that exogenous glucose oxidation was increased when glycogen availability was low, particularly in the first part of exercise.

Results from Bosch et al. (2) suggest that glucose release from the liver could be larger when muscle glycogen availability is low. As already mentioned above, although the difference did not reach statistical significance in this cross-sectional study, the rate of glucose appearance was consistently ~45–50% higher in subjects with low carbohydrate stores (2). In the present study the amounts of glucose released from the liver were estimated from the rate of exogenous and plasma glucose oxidation because, during exercise, most of the glucose released by the liver is oxidized (2). As shown by Bosch et al., compared with the results observed after the diet high in carbohydrates, the amounts of glucose released from the liver also appeared higher after the diet low in carbohydrates, at least in the first part of exercise. This finding of a larger liver glucose output during exercise when carbohydrate stores are low suggests that, in this situation, the liver could at least partly compensate for the reduction in muscle glycogen availability and oxidation. This could be due to the marked increase in the response of counterregulatory hormones, which has been described during exercise when carbohydrate stores are low (8) and can promote liver glycogenolysis as well as liver gluconeogenesis.

An additional finding from the present study is that protein oxidation was not significantly modified by changes in glycogen availability and contributed 4.4 ± 0.7 and 3.8 ± 1.5% of the energy yield after the diet high and low in carbohydrates, respectively. A similar observation was made by Ravussin et al. (31) with protein oxidation contributing to ~5% to the energy yield in subjects with normal and low glycogen stores. In contrast, Lemon and Mullin (20) have shown that a decrease in glycogen availability was associated with a 2.3-fold increase in protein oxidation during a 60-min exercise period at 61% \( V_{\text{O}2\text{max}} \) (5.8–13.7 g vs. 4.4–10.4% of the energy yield). The major difference with the study by Ravussin et al. and the present study is that, in the study by Lemon and Mullin, the subjects did not ingest carbohydrates before and/or during exercise. This suggests that carbohydrate administration could reverse the compensatory increase in amino acid oxidation during exercise that is associated with a reduction in glycogen availability and thus could have a protein-sparing effect in this situation.

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