Oxidation of exogenous $[^{13}C]$galactose and $[^{13}C]$glucose during exercise

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Leijsen, Dorian P. C., Wim H. M. Saris, Asker E. Jeukendrup, and Anton J. M. Wagenmakers. Oxidation of exogenous $[^{13}C]$galactose and $[^{13}C]$glucose during exercise. J. Appl. Physiol. 78(3): 720–725, 1995.—The present study examined the oxidation of exogenous galactose or glucose during prolonged submaximal cycling exercise. Eight highly trained volunteers exercised on two occasions on a cycle ergometer at 65% of maximal workload for 120 min, followed by a 60-min rest period and a second exercise bout of 30 min at 60% maximal workload. At random, subjects ingested a 8% galactose solution to which an $[^{13}C]$galactose tracer was added or a 8% glucose solution to which an $[^{13}C]$glucose tracer was added. Drinks were provided at the end of the warm-up period (8 ml/kg) and every 15 min (2 ml/kg) during the first 120 min of the test. Blood and breath samples were collected every 30 and 15 min, respectively, during the test. The exogenous carbohydrate (CHO) oxidation was calculated from the $CO_2$ production of the expired air. Peak exogenous CHO oxidation during exercise for galactose and glucose was 0.41 ± 0.03 and 0.85 ± 0.04 g/min, respectively. Total CHO and fat oxidation were not significantly different between the treatments. Forty-six percent of the ingested glucose was oxidized, whereas only 21% of the ingested galactose was oxidized. As a consequence, more endogenous CHO was utilized with galactose than with glucose (124.4 ± 6.7 and 100.1 ± 3.6 g, respectively). These results indicate that the oxidation rate of orally ingested galactose is maximally ~50% of the oxidation rate of a comparable amount of orally ingested glucose during 120 min of exercise.

galactose; glucose; carbon-13 labeling; carbohydrate oxidation; blood metabolites

CLASSIC STUDIES conducted in the 1920s and 1930s established that the consumption of a high-carbohydrate (CHO) diet before exercise and the ingestion of glucose during exercise delayed the onset of fatigue (5, 7). Over the years, it became clear that it is important for optimal performance to maximize the CHO delivery from exogenous CHO sources to the working muscle.

Whereas, in the earlier studies, estimates of CHO oxidation were made using respiratory gas-exchange measurements, Benadero et al. (2, 3) were the first to show by using radioactive $^{14}C$ isotope techniques that a significant quantity of ingested glucose is oxidized during exercise. Pursnot et al. (26) came to the same conclusion by using stable $^{13}C$ isotope techniques. The search for the optimum CHO type for ingestion during exercise was initiated in the early 1980s (26).

Comparison between glucose and fructose ingested during exercise showed that the total amount of ingested oxidized fructose was less than that of glucose (16). Oxidation rates of ingested maltose, sucrose, and glucose polymer solutions are very similar to those reported for glucose (9, 16, 21, 32). As far as we know, no information is yet available on oxidation rates of galactose during exercise.

Some metabolic characteristics of galactose have been studied. Galactose competes with glucose for intestinal absorption (31). Absorption of galactose has been suggested to be faster than that of glucose (8, 10, 14). It has been shown that galactose can only indentically stimulate insulin secretion (29). Blood galactose levels have been reported to increase with the dose of galactose ingested. This is in contrast with the blood glucose response, which is strongly reduced by the activity of insulin (28, 29, 34).

The effect of galactose on total CHO oxidation has been measured by using the indirect calorimetry technique (18, 20). These studies showed that galactose ingestion resulted in a significantly higher respiratory quotient than glucose and sucrose in the resting state. No distinction was made in these studies between endogenous or exogenous CHO oxidation. Therefore, the aim of this study was to investigate the effect of orally ingested galactose compared with glucose on endogenous and exogenous CHO oxidation during endurance exercise by using stable-isotope techniques.

METHODS

Subjects. Eight healthy, highly trained male cyclists volunteered after having been informed about the procedures. Their mean age, height, weight, and maximum aerobic work output (Wmax) were 26 ± 7 (SD) yr, 1.86 ± 0.05 m, 77.5 ± 5.0 kg, and 419 ± 44 W, respectively. Written informed consent was obtained. All procedures were approved by the Ethics Committee of the University of Maastricht.

Preliminary testing. Subjects' Wmax was determined by an incremental exertion test on an electronically braked bicycle ergometer (Lode, Groningen, The Netherlands) 1 wk before the experimental trials. After a 5-min warm-up at 100 W, the workload was increased 50 W every 2.5 min until the heart rate exceeded 170 beats/min. The workload was then increased 25 W every 2.5 min until exhaustion (13).

Experiment trials. Each subject performed two exercise tests with repeated beverage ingestion. An exercise test consisted of cycling at 65% Wmax for 120 min, followed by a 60-min rest period and a second exercise bout at 60% Wmax for 30 min. Subjects were encouraged to continue their regular training and competition schedules and were instructed not to consume any food products of high natural $^{12}C$ abundance, like cane sugar, corn, or corn-derived products, such as commercial sport drinks and candy bars for at least 1 wk before and during the experimental period (33). In addition, to prevent carryover of the $^{13}C$ label during the trials, tests were separated by at least 1 wk. If any was present, training began 2 days before the next exercise trial.

Protocol. Subjects reported to the laboratory at 8:00 A.M. after an overnight fast. All subjects received a standardized breakfast (identical for all trials) 1 h before exercise. The
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breakfast included two crackers with 15 g of cheese (14.3 g CHO, 5.9 g fat, and 17.9 g protein) and a cup of coffee or tea. All CHO in the breakfast was of C3 plant origin (low natural enrichment).

A Teflon catheter (Baxter Quick Catheter, DuPont, Ireland) was introduced in an antecubital vein. At 8:45 a.m., resting breath and blood samples were collected. For the determination of the 13C/12C ratio in CO2, expired air was collected directly in duplicate in 20-ml vacutainer tubes from the mixing chamber of the open-circuit system. Oxygen consumption (VO2) and CO2 production (VCO2) were analyzed by indirect calorimetry (Oxycon β, Mijnhardt, The Netherlands). At 8:50 a.m., subjects started cycling to warm up, 2 min at 100 W, 10 min at 40% Wmax, and finally 4 min at 55% Wmax. After this warm-up, the subjects consumed an initial bolus of 8 ml/kg of the test solution. Then the subjects started to exercise for 120 min at 60% Wmax (Fig. 1). They ingested 2 ml/kg of the test solution every 15 min (3 feedings of 2 ml/kg). The subjects were instructed to consume the drink within 1 min. During the test, every 15 min a breath sample was collected and the heart rate was recorded. Every 30 min a blood sample was collected into EDTA-containing tubes, and the blood was centrifuged for 4 min. Aliquots of plasma were frozen immediately in liquid N2 and stored at −20°C until analysis.

A 60-min rest period followed the 120-min exercise period, and then a second exercise bout was started at 60% maximal workload for 30 min. In the rest period and the second exercise bout, no drinks were consumed, but breath and blood sampling and recording of the heart rate continued every 15 and 30 min, respectively.

Test drinks. Drinks were given in randomised order and in a double-blind fashion (for the subjects and investigators). Subjects ingested during the exercise test an 8% (wt/vol) galactose solution or an 8% glucose solution. To the 8% galactose solution (DMV International, Veghel, The Netherlands), 0.35 g/kg of 1-13Cigalactose was added. The glucose solution consisted of 8% dextrose solution (Dextro M, glucose prepared from corn; CPC, Benelux BV), which has a high natural 13C content. To this solution, 0.05 g/kg of [U-13C]glucose was added. To both drinks, 20 mM NaCl were added. The number of labeled carbons in the glucose or galactose molecule ([U-13C] vs. [1-13C]) does not affect the results, as only the total enrichment for all carbon atoms is measured and used in the calculation procedure of exogenous CHO oxidation (see calculations below). The 13C enrichment of the drinks was related to the international standard Pee Dee Belemnitella (PDB). The mean enrichment of the galactose and glucose solution was ~0.048 and 0.053 atom percent excess, respectively. The drinks were provided at room temperature.

Sample analysis. On the basis of the 13C/12C in the ingested drink and in the expired CO2, the exogenous CHO oxidation can be calculated. The collected breath samples were analyzed for 13CO2 isotope enrichment by an isotope ratio mass spectrometer (Finnigan MAT 252, Bremen, Germany). The enrichment of the CHO in the drinks was measured after combustion of the CHO in the Carlo Erba elemental analyzer. The isotopic enrichment of CO2 was expressed as the change per mil difference between the δ13C/13C of the sample and a known laboratory reference standard according to

$$\delta^{13}C = \left(\frac{^{13}C/^{12}C \text{ sample}}{^{13}C/^{12}C \text{ standard}} - 1\right) \times 10^3$$

Exogenous CHO oxidation was calculated every 15 min with the computation procedure developed by Mosora et al. (22). Exogenous CHO oxidation was computed from VCO2, 13C-enrichment of the expired air during exercise (δ exp) and at rest (δ rest), and enrichment of the ingested CHO (δ ing). 13C-enrichment of resting samples was used for background correction.

$$VCO2(\delta \text{ exp} - \delta \text{ rest})/(\delta \text{ ing} - \delta \text{ rest}) \times 1/h$$

(see Ref. 22), where, h = CO2 (in liters) produced during oxidation of 1 g glucose or galactose (1/δ = 1.34).

Total fat oxidation and CHO oxidation (in grams) were calculated from VCO2 and VO2 using the nonprotein respiratory quotient (25).

$$\text{fat} = 1.665 \overline{V}O2 - 1.701 \overline{V}CO2$$

$$\text{CHO} = 4.585 \overline{V}CO2 - 3.226 \overline{V}O2$$

The endogenous CHO oxidation was calculated by subtracting the exogenous CHO oxidation from the total CHO oxidation.

Blood samples were analyzed on a semiautomatic analyzer (Cobas Bio) for glucose (hexokinase method; Unimate 5, Roche), galactose (ultraviolet method; Boehringer), lactate (enzymatic LDH method) and glycerol (GPO-trinder; Sigma Chemical). Insulin was determined by radioimmunoassay (RIA 100, Pharmacia).

Statistics. A Wilcoxon ranked-sign test was used to compare differences between the two ingested solutions in substrate oxidation in a given time period and in changes of blood metabolites during the 210-min test. The level of significance was set at 0.05. All results are expressed as means ± SD.

RESULTS

Oxidation. Endogenous and exogenous CHO oxidation with galactose and glucose ingestion are presented in Fig. 1. The mean rate of exogenous CHO oxidation was significantly higher (P < 0.05) during exercise and the rest period with glucose ingestion than during in-
TABLE 1. Nonprotein oxidation rates (in MJ) and percentual contribution to total substrate utilization of CHO and fat during the glucose and galactose exercise trial

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Glucose</th>
<th>Galactose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0–120 min</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy expenditure</td>
<td>12.27±0.34</td>
<td>11.50±0.53</td>
</tr>
<tr>
<td>CHO</td>
<td>4.58±0.13</td>
<td>37%</td>
</tr>
<tr>
<td>Endogenous</td>
<td>3.40±0.12</td>
<td>28%</td>
</tr>
<tr>
<td>Exogenous</td>
<td>1.16±0.52</td>
<td>9%*</td>
</tr>
<tr>
<td>Fat</td>
<td>7.71±0.25</td>
<td>63%</td>
</tr>
<tr>
<td><strong>60–120 min</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy expenditure</td>
<td>6.58±0.16</td>
<td>6.46±0.19</td>
</tr>
<tr>
<td>CHO</td>
<td>2.39±0.07</td>
<td>36%</td>
</tr>
<tr>
<td>Endogenous</td>
<td>1.62±0.06</td>
<td>24%*</td>
</tr>
<tr>
<td>Exogenous</td>
<td>0.75±0.04</td>
<td>12%*</td>
</tr>
<tr>
<td>Fat</td>
<td>4.20±0.12</td>
<td>64%</td>
</tr>
</tbody>
</table>

Values are means ± SD. CHO, carbohydrate. * Significant difference between galactose and glucose (P < 0.05).

gestion of galactose. The peak exogenous oxidation rates for glucose and galactose were 0.85 ± 0.04 and 0.41 ± 0.03 g/min, respectively (P < 0.05). No significant difference was observed in the postrecovery exercise period. The rate of endogenous CHO oxidation decreased during the first 120 min of exercise in both experimental conditions. The endogenous CHO oxidation was higher with galactose ingestion compared to glucose ingestion but differed only significantly (P < 0.05) at 45 and 60 min after the start of exercise. The total oxidation during the 120-min exercise and total oxidation during the last exercise hour (60–120 min) were both calculated, because possible differences can become significant during the last hour when the CHO depots are becoming smaller.

Over the entire exercise period (0–120 min), the exogenous CHO oxidation was significantly lower (P < 0.05) in the galactose trial. It was calculated that 46% of the amount of glucose ingested (155 g) was oxidized during the 120 min of exercise, whereas only 21% of the ingested galactose was oxidized. During the 60–120 min period of exercise, the endogenous CHO oxidation was significantly higher with ingestion of galactose and the exogenous CHO oxidation was significantly lower with ingestion of galactose (P < 0.05). The endogenous CHO oxidation with ingestion of galactose and glucose in the second hour of exercise was 124.4 ± 6.7 and 100.1 ± 3.6 g, respectively. The exogenous CHO oxidation ingested galactose was 47% of the oxidation of ingested glucose.

Table 1 shows the percentual contribution to total nonprotein energy expenditure from the oxidation of fat and the oxidation of endogenous, exogenous, and total CHO (in MJ) during both drinks. Fat oxidation constitutes most of the energy expenditure with both drinks. Endogenous CHO oxidation was higher than exogenous CHO oxidation with both drinks. Total energy expenditure was not significantly different with glucose or galactose ingestion.

FIG. 2. Mean (±SD) plasma galactose (A) and glucose (B) concentrations (mmol/L) with ingestion of glucose and galactose. * Significant difference between galactose and glucose (P < 0.05).

Plasma variables. The galactose concentration in the plasma increased linearly during the test, until the ingestion of galactose stopped. The postrecovery exercise did not accelerate the rate at which the plasma galactose concentration decreased in time, suggesting that exercise does not increase the rate of disappearance of plasma galactose, similarly to its effect on glucose disposal. With the ingestion of glucose, the plasma galactose concentration was negligible, as expected (Fig. 2). The plasma glucose concentration with ingestion of glucose was significantly higher (P < 0.05) compared with the ingestion of galactose (Fig. 2).

Plasma insulin decreased during the 120 min of exercise but did not differ between the two trials (Fig. 3). Plasma glyceral concentrations were not significantly different with ingestion of the two drinks. There was an increase during both exercise periods (Fig. 3). The lactate concentration was significantly higher with ingestion of galactose than of glucose after 1 h of exercise until the end of the rest period (Fig. 3).

DISCUSSION

Methodology. In previous studies from our laboratory (30, 32, 33) it was shown that instructing the subjects not to eat any products of high natural 13C content during the experimental period was effective in reducing the background shift (change in 13CO2) from endogenous substrate stores in European subjects (33). In the present study, any change in background enrichment
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During exercise, the \(^{13}\text{CO}_2\) production increases severalfold so that a physiological steady-state situation occurs and \(^{13}\text{CO}_2\) in expired air is equilibrated with the \(^{13}\text{CO}_2\text{H}_2\text{CO}_3\) pool. After 60 min of exercise, the dilution of \(^{13}\text{CO}_2\) becomes negligible, and recovery of \(^{13}\text{CO}_2\) approaches 100% (24).

*Exogenous CHO oxidation.* The structure of galactose and glucose is almost identical; only one position of a hydroxy group is different. Because of this difference, galactose has different chemical and biochemical characteristics compared with glucose (14).

The exogenous CHO oxidation can be expressed as percentage of the ingested CHO oxidized during the trial. When the percentage of oxidized glucose (42%) and galactose (21%) is compared with the percentage of oxidized maltodextrin (51%) and sucrose (55%) from another study with a similar protocol in our laboratory (32), it appears that the amount of galactose oxidized is very low compared with glucose, maltodextrine, and sucrose. When the rate of intake of glucose and galactose (0.82 g/min) in this study during 60–120 min of exercise is compared with the oxidation rate of glucose (0.79 g/min) and galactose (0.37 g/min), it can be concluded that the mean oxidation rate of glucose was about the same as the rate of intake, whereas the oxidation rate of galactose was about one-half the rate of intake.

The peak oxidation rate of glucose in the first and second exercise bout of this test reached both times a value between 0.9 and 1.0 g/min. This is in accordance with previous values from our laboratory (32). In contrast, the peak oxidation rate of galactose was much lower in the first exercise bout (0.41 g/min), whereas in the postrecovery exercise period the CHO oxidation after ingestion of galactose approached the oxidation rate of glucose. With ingestion of galactose or glucose during exercise, it can be concluded that the peak oxidation rate of galactose was about one-half of the peak oxidation rate of glucose. Compared with other studies, the peak oxidation rate of galactose (0.41 g/min) tends to be even lower than the peak oxidation rate of fructose (0.43–0.44 g/min) (15, 16). However, Massicotte et al. (17) concluded that the oxidation of fructose was only lower compared with the oxidation of glucose when the exercise started in the fed state (a breakfast of 50 g CHO, 1,700 kJ). When the subjects were fasted for the 15 h before the start of the exercise, the oxidation of fructose and glucose was the same because of a faster gastric emptying and absorption from the gut and/or an increase of the conversion of fructose into glucose.

One explanation for the lower oxidation rate of galactose in the first exercise bout compared with glucose might be that galactose has to be converted to glucose in the liver before it can be transported to the muscle for oxidation.

It is also possible that the ingested galactose is converted into glucose, most probably stored in glycogen, and subsequently oxidized during the second exercise bout. During the second exercise bout, part of the \(^{13}\text{CO}_2\) in the expired air may appear from the oxidation of galactose left in the gut during the first exercise bout or may be from glycolyse stores that have been resynthesized during the recovery period. We could not de-

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**Figure 3.** Mean (±SD) plasma insulin (A; U/l), glycerol (B; mmol/l), and lactate (C; mmol/l) with ingestion of glucose and galactose during 120 min of exercise at 65% \(W_{max}\). *Significant difference between galactose and glucose (P < 0.05).*
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terminate what the source of \( ^{14} \text{CO}_2 \) production was during the second exercise bout. The possibility that the absorption of galactose is rate limiting cannot be excluded, but based on the absorption data in the literature (8, 10) this is unlikely. Figure 2 shows that the lower exogenous CHO oxidation with ingestion of galactose is compensated by a higher endogenous CHO oxidation. These data suggest that whole body glycogen breakdown increases with galactose ingestion. No information is available on muscle glycogen. With ingestion of glucose, the (nonsignificant) decrease of total CHO oxidation during the 120-min exercise is compensated by a significant increase in fat oxidation.

Because of the higher endogenous CHO oxidation with ingestion of galactose, the endogenous CHO stores are depleted sooner. This increased rate of glycogen breakdown with the ingestion of galactose can result in an earlier onset of fatigue (4), since muscle glycogen availability can be a limiting factor for endurance performance (1).

Plasma variables. The plasma glucose concentration with ingestion of galactose is significantly lower during the 30- to 150-min exercise compared with the ingestion of glucose. A possible explanation is that the conversion of galactose to glucose in the liver is rate limiting; however, this is reported to be an efficient process (14). As a result, galactose has only an indirect influence on the blood glucose concentration (31).

Figure 1 shows that, in the absence of oral galactose intake, plasma galactose is negligible. With ingestion of galactose, the blood galactose concentration rose gradually until the ingestion of galactose stopped. This has previously been reported by MacRae et al. (14).

The gradual increase in plasma galactose level is an additional argument that it is unlikely that galactose absorption or gastric emptying are major limiting factors in exogenous galactose oxidation. However, during the 120 min of exercise no plateau was reached.

One of the major regulators of blood glucose is insulin. In the literature it is known that galactose (as well as fructose) cannot directly stimulate the insulin secretion by the pancreas (28, 29, 34). Only after conversion of galactose into glucose is insulin secretion stimulated. It is obvious that the downward regulation of insulin secretion during exercise, due to adrenergic activity, overrules the upward regulation with glucose ingestion (6). Probably because of the decreased insulin secretion during exercise, the expected difference in insulin concentration between glucose and galactose was not observed. However, it can be speculated that if galactose and glucose were ingested before exercise, a significantly lower insulin secretion with ingestion of galactose could be expected.

Both the total fat oxidation and glycerol concentrations (23) indicate that galactose intake did not stimulate lipolysis. The blood lactate concentrations with ingestion of galactose were significantly higher compared with the ingestion of glucose. Similar data were found by Koivisto et al. (12) and McDonald et al. (19) after ingestion of fructose. Despite the difference in lactate concentration, the exercise time until exhaustion showed no difference (12). A possible explanation for the higher lactate values of galactose compared with glucose could be a differential catecholamine response, causing an increased rate of glycolysis and lactate formation. Such a response was observed in subjects who became hypoglycemic (1).

In conclusion, this study shows that galactose ingestion during exercise leads to a 50% lower exogenous oxidation rate compared with glucose and other types of CHO. As a consequence, the reliance on glycogen stores is higher with galactose than with glucose.

This study was supported by a grant from DMV International, a division of Campina Melkunie, Veghel, The Netherlands. Address for correspondence: H. M. Sars, Department of Human Biology, P.O. Box 616, 6200 MD Maastricht, The Netherlands.

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