Exogenous carbohydrate oxidation from drinks ingested during prolonged exercise in a cold environment in humans

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Galloway, Stuart D. R., Steve A. Wootton, Jane L. Murphy, and Ronald J. Maughan. Exogenous carbohydrate oxidation from drinks ingested during prolonged exercise in a cold environment in humans. J Appl Physiol 91: 654–660, 2001.—Six healthy male volunteers performed four rides to exhaustion on a cycle ergometer at ~80% of maximal oxygen consumption. Subjects ingested a bolus volume of fluid (7.14 ml/kg) immediately before exercise and additional fluid volumes (1.43 ml/kg) every 10 min during exercise. The fluids ingested were either a flavored water control or glucose-electrolyte beverages with glucose concentrations of 2, 6, or 12%. The beverages were labeled with [U-13C]glucose (99.2%: 0.05 g/l). Exercise capacity was not different (P = 0.13) between trials; median (range) exercise time was 83.52 (79.85–89.68), 103.19 (78.82–108.22), 100.37 (80.60–124.07), and 94.76 (76.78–114.25) min in the 0, 2, 6, and 12% trials, respectively. The oxidation of exogenous glucose in each 15-min period was significantly lower in the 2% trial (P = 0.02) than in the 6 and 12% trials where oxidation rates were between 0.5 and 0.7 g/min. No difference in endogenous glucose oxidation was observed between trials (P = 0.71). These findings indicate that the oxidation of exogenous glucose during exercise of this intensity and duration in a cold environment is similar to that observed in warmer conditions. Thus a low oxidation of exogenous substrate is unlikely to be a factor limiting the effectiveness of carbohydrate-electrolyte drink ingestion on exercise capacity in a cold environment.

MATERIALS AND METHODS

Six healthy male volunteers were studied. The mean ± SD physical characteristics of the subjects were: age = 28 ± 4 yr; body mass = 74.3 ± 8.8 kg; height = 182 ± 4 cm; and maximal oxygen uptake (V̇O2max) = 4.27 ± 0.33 l/min. All subjects were physically active on a recreational basis but not specifically cycle trained. All subjects were given written information concerning the nature and purpose of the study and gave their written, informed consent to participate. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
The study was approved by the Joint Ethical Committee of the University of Aberdeen and Grampian Health Board. Subjects visited the laboratory on seven separate occasions 1 or 2 wk apart and at the same time of day. The first visit to the laboratory was for determination of maximal 

O O

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consumption (V O max). Each subject then completed six rides to exhaustion on an electronically braked cycle ergometer at ~80% V O max in an environment maintained at an ambient dry bulb temperature (T b) of ~10°C with a relative humidity (RH) of around 70%. The first two of these trials were familiarization trials in which subjects were exercised to exhaustion to ensure that a reliable assessment of exercise capacity would be obtained. Data from our laboratory have shown reliable exercise capacity results following two familiarization trials (unpublished observations). The final four trials were the experimental trials. For these trials, subjects ingested either flavored water as a control (0%), a 2% glucose-electrolyte drink, a 6% glucose-electrolyte drink, or a 12% glucose-electrolyte drink. The drinks were made fresh on a daily basis in distilled water, and the glucose-electrolyte drinks were prepared using Analar grade glucose (BDH, Poole, UK, background enrichment of ~10.99 ± uc vs. PDB, triplicate determination) and were enriched by adding 0.05 g/l [U-13C]glucose (99.2%; CK Gases, Wokingham, UK) to make the final CHO content 2, 6, or 12%. Drink formulations and enrichments are presented in Table 1. Final drink enrichment values were obtained from triplicate determination (IRMS, ANCA system, Europa Scientific) of samples from all of the final drink compositions ingested by each subject. The experimental conditions were administered in a cross-over randomization design. The mean ± SD ambient temperature (°C) was 10.0 ± 0.3, 10.0 ± 0.2, 9.9 ± 0.3, and 10.0 ± 0.2 for the 0, 2, 6, and 12% trials, respectively. The relative humidity (%) was 69 ± 3, 70 ± 3, 71 ± 3, and 69 ± 4 for the 0, 2, 6, and 12% trials. Subjects were instructed not to exercise heavily on the day before the trials and to refrain from consuming foods high in naturally enriched [13C]-labelled CHO (e.g., commercial sports drinks, C4 plants such as corn- and corn starch-based products) from 1 wk before the study and throughout the whole experimental period (a total of 5 wk). For each trial, the subjects visited the laboratory in the morning following an overnight fast and emptied their bladder before nude body mass was obtained. Subjects then inserted a rectal thermistor 10 cm beyond the anal sphincter before resting in a sitting position for 30 min in a standardized environment (25.5 ± 0.1°C). The rectal thermistor was held in place by a bulb on the probe, which passed beyond the sphincter to prevent slippage during exercise. During the rest period, subjects were instrumented with skin temperature thermistors and a heart rate (HR) monitor. The thermistors were positioned on the chest, upper arm, thigh, and lower rectum to prevent slippage during exercise. Where VCO 2 is in liters. The delta unit value observed at each timepoint during the expired gas collection period.

An end-tidal breath sample for baseline [13CO 2] enrichment (where brackets indicate concentration) was then collected into a breath sampling bag before the second resting blood sample was drawn. Three 10-ml samples were then analyzed in duplicate using continuous-flow isotope ratio mass spectrometry (ANCA system, Europa Scientific) to determine the ratio of [13C] to [12C] of expired CO 2.

Immediately after the second resting blood sample, subjects transferred to the climatic chamber where they ingested a bolus of 7.14 ml/kg of the assigned drink before beginning exercise. An additional fluid volume of 0.5 liters was ingested every 10 min during all trials. The drinks were maintained at a temperature of 14°C in a water bath. The subjects maintained a pedal cadence of 60–70 rpm throughout the exercise test, and exhaustion was defined as the point at which the subjects could no longer continue or maintain a cadence above 60 rpm.

Blood samples (6.5 ml) were drawn during exercise at 15-min intervals and at exhaustion (immediately after the subject stopped). The venous cannula was kept patent by a slow saline infusion (0.5 ml/min, 0.9% sterile saline) on all trials. Expired gas was collected into Douglas bags over a 1-min period every 15 min and immediately analyzed to enable calculation of VE, V CO 2, V O 2, and RER. Rates of substrate oxidation were calculated from V O 2 and RER measurements (3).

An end-tidal breath sample was collected immediately after each 1-min collection of expired gas into a Douglas bag. Exogenous glucose oxidation was calculated using the method of Mosora et al. (19). Exogenous glucose oxidation was calculated at each time point (excluding the first 30 min of exercise) using the equation indicated below

Exogenous glucose oxidation (g)

\[
\text{Exog.} = \left[ \frac{\text{VCO}_2 \times (\delta^{13}\text{C}_{\text{observed}} - \delta^{13}\text{C}_{\text{control}})}{\delta^{13}\text{C}_{\text{ingested}} - \delta^{13}\text{C}_{\text{control}}} \right] \times 1.34
\]

where VCO 2 is in liters. The δ 13C values were calculated from isotope ratio data using the equation of Craig (5). δ 13C control is the delta unit value observed at each timepoint during the trials where labeled glucose was ingested. δ 13C ingested is the

### Table 1. Formulation and enrichment of the ingested drinks

<table>
<thead>
<tr>
<th>Constituent</th>
<th>0% Drink</th>
<th>2% Drink</th>
<th>6% Drink</th>
<th>12% Drink</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, g/l</td>
<td>20.0</td>
<td>60.0</td>
<td>120.0</td>
<td></td>
</tr>
<tr>
<td>Na+, mmol/l</td>
<td>1 ± 0.2</td>
<td>70 ± 1</td>
<td>69 ± 1</td>
<td>69 ± 1</td>
</tr>
<tr>
<td>K+, mmol/l</td>
<td>0.4 ± 0.0</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Cl-, mmol/l</td>
<td>1 ± 0.2</td>
<td>56 ± 1</td>
<td>55 ± 1</td>
<td>55 ± 1</td>
</tr>
<tr>
<td>Osmolarity, mmol/kg H 2 O</td>
<td>19 ± 1</td>
<td>240 ± 2</td>
<td>467 ± 3</td>
<td>819 ± 2</td>
</tr>
<tr>
<td>Volume ingested, ml</td>
<td>1.010 ± 190</td>
<td>1.481 ± 284</td>
<td>1.493 ± 171</td>
<td>1.387 ± 157</td>
</tr>
<tr>
<td>Drink enrichment, % vs. PDB</td>
<td>-22.94 ± 1.61</td>
<td>173.06 ± 3.79</td>
<td>61.80 ± 8.08</td>
<td>25.24 ± 4.37</td>
</tr>
</tbody>
</table>

Values are means ± SD. All drinks were made up in distilled water and flavoured with a low calorie lemon drink (125 ml/l).

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delta unit value observed at each corresponding timepoint during the control trial (0% trial). \(^{13}\)C_ingested is the delta unit value of the ingested glucose drink; 1.34 (g/l) is a correction factor to account for the amount of CHO oxidized per liter of CO\(_2\) produced. Exogenous glucose oxidation was then expressed as a rate (g/min).

Ratings of perceived exertion (RPE; Ref. 2) for both overall perception of exertion and exertion localized to the legs were recorded every 10 min during exercise. \(T_{sk}, T_{re}\), and \(T_a\), HR, and RH were recorded every 5 min throughout the exercise period and at exhaustion. Following exercise, subjects showered, dried, and then provided a urine sample before a final nude body mass determination. Mass loss, corrected for fluid mass consumed, urine production, respiratory water losses, and losses due to substrate exchange (18) were taken as representing sweat loss.

Blood glucose was measured using the glucose oxidase method (Boehringer Mannheim Biochemica, Lewes, UK). Blood lactate was determined using the method of Boobis and Maughan (1). Serum FFA levels were measured using an enzymatic colorimetric method (Boehringer Mannheim Biochemica). Serum and urine Na\(^+\) and K\(^+\) were determined by flame photometry (Corning clinical flame photometer 410 C, Essex, UK). Serum, urine, and drink osmolality were determined by freezing point depression (Gonotec osmomat 030, YSI Ltd, Hampshire, UK). Serum and urine Cl\(^-\) were determined by potentiometric titration (Jenway chloride meter, Essex, UK). Microhematocrit (by centrifugation) and hemoglobin (cyanmethemoglobin method) were measured on all samples for calculation of percent changes in plasma volume (6). All blood analyses were performed in duplicate except for hematocrit, which was measured in triplicate. All assay coefficients of variation were below 2.0%.

All data are presented as means \(\pm\) SD in text and tables. Where appropriate, following a Shapiro-Wilks test for normality of distribution, values are expressed as median (range) in the text (time-to-exhaustion data). A two-way, two-factor ANOVA for repeated measures was applied to determine any treatment differences and time effects during the exercise protocol. After observation of a main effect, ANOVA or Kruskal-Wallis tests were performed to determine at which time points an effect was observed. Post hoc analysis by Student’s paired \(t\)-test or Wilcoxon test was performed to determine which trials were significantly different. ANOVA or Kruskal-Wallis tests were applied to determine any initial baseline differences in all variables. In all cases, significance was taken at \(P < 0.05\).

RESULTS

The mean power output maintained during the trials was 241 \(\pm\) 38 W eliciting an oxygen cost corresponding to 79 \(\pm\) 7% of \(V_{O2}^{\text{max}}\). Final enrichment of the ingested glucose drinks was +173.1 (2% trial), +61.8 (6% trial), and +25.2 (12% trial) \(^{\delta}\) /oo vs. PDB (Table 1). No subjects complained of any gastrointestinal discomfort with any of the drinks.

Exercise capacity. There was no main effect of drink ingestion on exercise capacity (\(P = 0.13\)). Median (range) exercise time was 83.52 (79.85–89.68), 103.19 (78.82–108.22), 100.37 (80.60–124.07), and 94.76 (76.78–114.25) min in the 0, 2, 6, and 12% trials, respectively.

Fuel oxidation responses. Significant time effects were observed in the estimated fat oxidation data, with significantly greater increases in the estimated rate of fat oxidation in the 2 and 6% trials from the 45-min sample time onward compared with the other trials. There was no main effect of drink on the estimated rates of total CHO \((P = 0.99)\) or fat \((P = 0.99)\) oxidation (Fig. 1). Mean \(\pm\) SD total CHO oxidation rate calculated over the duration of each trial was 186 \(\pm\) 18 g/h in the 0% trial, 181 \(\pm\) 26 g/h in the 2% trial, 185 \(\pm\) 15 g/h in the 6% trial, and 184 \(\pm\) 33 g/h in the 12% trial. There were no significant differences between any trials. Mean \(\pm\) SD total fat oxidation rate calculated over the duration of each trial was 19 \(\pm\) 13 g/h in the 0% trial, 22 \(\pm\) 9 g/h in the 2% trial, 22 \(\pm\) 9 g/h in the 6% trial, and 22 \(\pm\) 14 g/h in the 12% trial. There were no significant differences between any trials.

A background correction was made for the calculation of exogenous glucose oxidation during CHO inges-

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tion trials by using the data collected during the 0% trial. Background breath enrichment values (means ± SD) during the 0% trial and during 2, 6, and 12% drink-ingestion trials are shown in Table 2. Breath-enrichment values were significantly different between trials (P < 0.01). The main difference was between the 0% trial and all other trials, with the 0% trial having lower breath enrichment. However, there were also differences between the 2 and 12% trials at the 45-, 60-, and 75-min sample times, with the 2% trial having higher breath enrichment values.

Exogenous glucose oxidation was only calculated from the breath samples collected from 45 to 75 min of exercise. Rate of exogenous glucose oxidation was significantly different between the 2, 6, and 12% CHO ingestion trials (P = 0.02; Fig. 2). The percentage of ingested exogenous glucose oxidized over the whole exercise duration was 51.5 ± 24.0, 31.8 ± 14.3, and 21.1 ± 10.5% for the 2, 6, and 12% CHO drinks, respectively. The endogenous CHO contribution to total CHO oxidation between 45 and 75 min of exercise was not significantly different between trials (P = 0.71, Fig. 2).

Blood metabolite and substrate responses. Blood glucose concentration (Fig. 3) was significantly affected by drink ingestion (P < 0.01). Blood glucose concentration was significantly higher throughout exercise in the 6 and 12% trials compared with the 0% trial. No effect (P = 0.98) of CHO drink ingestion was observed on the blood lactate response to exercise (Fig. 3). A significant effect (P < 0.05) of CHO drink ingestion on the serum FFA concentration (Fig. 4) was detected, with a significantly higher serum FFA concentration during exercise and at exhaustion in the 2% trial compared with the 12% trial. There was no main effect of CHO drink ingestion on blood glycerol concentration (P = 0.17; Fig. 4). No differences were observed in serum electrolyte concentrations or osmolality between trials.

Cardiorespiratory, thermoregulatory, fluid balance, and subjective responses. There were no significant differences between trials in any of the cardiorespiratory, thermoregulatory, fluid balance, or subjective responses to exercise in the present study.

**DISCUSSION**

The present results confirm our previous data indicating that there is no effect of CHO drink ingestion on exercise capacity in a cold environment (11). However, the findings are in direct contrast to observations made during exercise of similar intensity and duration in warm or hot environmental conditions (4). Although it is well recognized that endogenous CHO depletion may limit exercise capacity and provision of additional substrates may delay the onset of fatigue, the present results suggest that additional substrate availability does not delay the onset of fatigue in this type and intensity of exercise in the cold. Because it is often suggested that drinks ingested during exercise in a cold environment should contain a high concentration of CHO (13, 16, 17), it is surprising that few studies

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**Table 2. Breath enrichment (δ\(^{13}\)C) at rest and during exercise in the trials with 0%, 2%, 6% and 12% glucose-electrolyte drinks**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Rest</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>75 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ(^{13})C</td>
<td>δ(^{13})C vs. PDB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td>-25.47 ± 0.67</td>
<td>-24.98 ± 0.81</td>
<td>-23.72 ± 1.14(^{ab})</td>
<td>-23.14 ± 1.34(^{ab})</td>
<td>-22.69 ± 1.37(^{ab})</td>
<td>-22.25 ± 1.59(^{ab})</td>
</tr>
<tr>
<td>2%</td>
<td>-25.50 ± 0.72</td>
<td>-23.41 ± 1.60</td>
<td>-17.68 ± 4.93(^{b})</td>
<td>-12.58 ± 5.69(^{b})</td>
<td>-10.32 ± 4.64(^{b})</td>
<td>-9.99 ± 3.50(^{b})</td>
</tr>
<tr>
<td>6%</td>
<td>-25.50 ± 0.43</td>
<td>-24.04 ± 1.44</td>
<td>-20.36 ± 3.71</td>
<td>-16.13 ± 4.12(^{b})</td>
<td>-12.70 ± 4.00(^{a})</td>
<td>-10.39 ± 3.88(^{a})</td>
</tr>
<tr>
<td>12%</td>
<td>-25.30 ± 0.61</td>
<td>-24.22 ± 0.87</td>
<td>-21.18 ± 2.22</td>
<td>-17.28 ± 2.73(^{b})</td>
<td>-15.14 ± 2.56(^{b})</td>
<td>-14.18 ± 3.04(^{b})</td>
</tr>
</tbody>
</table>

Values are means ± SD. Trials with like letters (a, b, or c) are significantly different from each other (P < 0.05).

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have examined CHO provision in a cold environment, and none has examined oxidation of exogenous substrate during exercise in the cold.

In the present study, exogenous glucose oxidation was significantly greater with the concentrated glucose drinks (6 and 12%) than with the dilute glucose drink (2%). The rate of exogenous glucose oxidation for the concentrated drinks was consistent with other $^{13}$C tracer studies conducted in warm environments where oxidation rates for exogenous substrates have been in the range of 0.6 to 1.0 g/min (14, 22). It can therefore be seen from the present data that our hypothesis of low exogenous substrate oxidation in a cold environment was not supported.

Of the previous work conducted in this area, that of Rehrer et al. (22) is closest for comparative purposes. These authors investigated exogenous glucose oxidation in moderate-intensity ($70\%$ $\text{VO}_2\text{max}$) endurance exercise of 80-min duration in a warm environment ($20^\circ\text{C}$, $\text{RH} = 50$–55%). These authors observed that mean oxidation rate of exogenous CHO over the exercise duration was not different between trials when a 4.5% glucose drink (0.42 g/min), a 17% glucose drink (0.56 g/min), or a 17% maltodextrin drink (0.52 g/min) was ingested. However, the amount of exogenous CHO oxidized increased with the amount of substrate ingested (0.53 g/min with the 4.5% glucose drink, 0.78 g/min with the 17% glucose drink, and 0.88 g/min with the 17% maltodextrin drink).

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Fig. 3. Mean blood glucose ($A$) and blood lactate ($B$) concentrations at rest, during 75 min of exercise, and at exhaustion (END) with ingestion of 0, 2, 6, and 12% CHO drinks. Group mean errors are shown to indicate the variance at each time point. Letters a, b, c, and d indicate significance ($P < 0.05$) from trials 0, 2, 6, and 12%, respectively. *Significantly different from the concentration at $t = 0$ (all blood lactate exercise values were higher than at rest).

Fig. 4. Mean serum free fatty acid (FFA; $A$) and blood glycerol ($B$) concentrations at rest, during 75 min of exercise, and at exhaustion (END) with ingestion of 0, 2, 6, and 12% CHO drinks. Group mean errors are shown to indicate the variance at each time point. All glycerol concentrations during exercise were significantly higher than at rest. *Significantly different from the concentration at $t = 0$. Trials with like letters are significantly different from each other ($P < 0.05$).
g/min with the 17% glucose drink, and 0.75 g/min with the 17% maltodextrin drink at 75 min of exercise) and the percent oxidation of the total CHO load decreased with increasing CHO content (54% of 4.5% glucose drink, 17% of 17% glucose drink, and 18% of 17% maltodextrin drink). The majority of the present data are in agreement with this previous work. The lack of any difference in exogenous glucose oxidation in the present study compared with studies conducted in warmer environments indicates that in a cold environment there is not a reduced reliance on exogenous glucose as a fuel source.

Rehrer et al. (22) also highlighted that much of the ingested glucose in the concentrated CHO drinks was not oxidized in an 80-min exercise period. Two of their subjects rested for 1 h following exercise and then completed a further 30 min of exercise without CHO ingestion. A significant increase in exogenous CHO oxidation was observed compared with the previous exercise bout. These authors went on to suggest that the exogenous CHO may have been incorporated into skeletal muscle stores during the initial exercise bout for subsequent oxidation with renewed exercise. Wagenmakers et al. (24) subsequently reported that exogenous substrate may be retained in the gut or other pools during exercise, making it unavailable for oxidation. A similarly low percentage of oxidation of total CHO load for the concentrated drinks was observed in the present study, indicating that a large amount of the exogenous substrate was stored.

In an extreme cold environment, the hormonal response to exercise, especially catecholamine response, may be exaggerated (23) with elevated adrenaline and noradrenaline concentrations on exposure to cold air and cold water (7). An increase in circulating adrenaline concentration above that observed in thermoneutral conditions is generally observed in extreme environmental conditions (20, 25). In addition, the catecholamine response to exercise may be augmented during exercise in a fasted state (10). Because an increase in adrenaline concentration has been associated with an increased muscle glycogen degradation during exercise in humans (9) and increased endogenous glycogen use and decreased utilization in exogenous glucose in the horse (12), it is possible that an exaggerated adrenergic response in a cold environment could lead to an earlier onset of fatigue and possibly a reduced reliance on exogenous substrate as a fuel source. However, in moderate cold conditions, it is unclear whether adrenergic activity is augmented compared with thermoneutral conditions since Parkin et al. (20) have observed a blunted adrenaline response to exercise at 5°C compared with at 20°C. It is likely that, in the present study at an ambient temperature of 10°C, there would not have been an exaggerated catecholamine response to exercise.

The present data, therefore, do not provide evidence to explain why ingestion of a concentrated CHO solution does not improve exercise capacity in a cold environment. However, the data do support the use of concentrated CHO beverages in the range of 6–12% CHO content during prolonged exercise in a cold environment. CHO contents higher than 12% may or may not be beneficial in the cold, but it is recognized that CHO contents ≥15% CHO may result in gastrointestinal distress. Further work remains to be done to examine the effectiveness of concentrated CHO drinks on repeated endurance exercise bouts in a cold environment and to examine more closely the effects of adrenergic activation on human exogenous glucose oxidation in more extreme environmental conditions.

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REFERENCES


