Effects of ingesting $[13C]$glucose early or late into cold exposure on substrate utilization

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Blondin DP, Péronnet F, Haman F. Effects of ingesting $[13C]$glucose early or late into cold exposure on substrate utilization. J Appl Physiol 109: 654–662, 2010. First published July 22, 2010; doi:10.1152/japplphysiol.00440.2010.—One of the factors limiting the oxidation of exogenous glucose during cold exposure may be the delay in establishing a shivering steady state (~60 min), reducing glucose uptake into skeletal muscle. Therefore, using indirect calorimetry and isotopic methodologies in non-cold-acclimatized men, the main purpose of this study was to determine whether ingesting glucose at a moment coinciding with the maximal shivering intensity could increase the utilization rate of the ingested glucose. $13C$-enriched glucose was ingested (800 mg/min) from the onset (G0) or after 60 min (G60) of cold exposure when the thermogenic rate was stabilized to low-intensity shivering (~2.5 times resting metabolic rate). For the same quantity of glucose ingested, the oxidation rate of exogenous glucose was 35% higher in G60 (159 ± 17 vs. 118 ± 17 mg/min in G0) between minutes 60 and 90. By the end of cold exposure, exogenous glucose oxidation was significantly greater in G0, reaching 231 ± 14 mg/min, ~15% higher than the only rates previously reported. This considerably reduced the utilization of endogenous reserves over time and compared with the G60 condition. This study also demonstrates a fall in muscle glycogen utilization, when glucose was ingested from the onset of cold exposure (from ~150 to ~75 mg/min). Together, these findings indicate the importance of ingesting glucose immediately on exposure to a cold condition, relying on shivering thermogenesis and sustaining that consumption for as long as possible. This substrate not only provides an auxiliary fuel source for shivering thermogenesis, but, more importantly, preserves the limited endogenous glucose reserves.

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During shivering, carbohydrate (CHO) represent a substantial source of metabolic substrate, with its utilization accounting for up to ~80% of total heat production ($\dot{H}$) (15). Given the limited CHO reserves, several cold-exposure studies have focused on the effects of CHO ingestion on whole body fuel selection. Evidence from this research has shown that the ingestion of food rich in CHO alters substrate utilization to varying degrees (1, 8, 33). During very-low- (8) to low-intensity shivering (33), CHO ingestion has been shown to increase CHO utilization and reduce lipid oxidation. However, more recent findings demonstrated that, when proteins are also considered in the energy balance, ingesting glucose at a rate of 400 or 800 mg/min over 2 h of cold exposure does not alter total CHO, lipid, or protein utilization (1). Using isotopic methodologies, combined with indirect calorimetry, this latter study also showed that exogenous glucose oxidation peaked and reached a plateau at a rate of ~195 mg/min at the lower ingestion rate of 400 mg/min. Interestingly, this oxidation rate was one-third less than what is found during exercise eliciting a similar metabolic rate (28). Glucose ingestion also modified the utilization of endogenous glycogen reserves. Utilization of liver-derived glucose decreased in a dose-dependent manner, suggesting that liver glycogen was being spared, whereas no effect on muscle glycogen utilization was observed.

The lower exogenous glucose oxidation rate found during cold exposure, relative to the rates seen during exercise of a similar metabolic rate, may be due to differences in establishing a metabolic steady state between these two conditions. During low-intensity shivering, there is a substantial delay in establishing a metabolic steady state (~60 min (1, 13)) compared with exercise (~2–3 min). The limited muscle activity early during cold exposure could drastically limit contraction-mediated glucose uptake in skeletal muscles and ultimately the utilization or storage of exogenous glucose. Consequently, this could reduce the potential sparing effect of glucose ingestion on endogenous CHO stores. Therefore, the purpose of this study was to determine whether ingesting glucose at a moment coinciding with the maximal metabolic rate for this cold stress could increase the utilization rate of the ingested glucose and increase the sparing effect compared with when it is ingested immediately upon cold exposure. We hypothesized that ingesting glucose after 60 min of cold exposure, a time that generally coincides with the insulinenic peak and maximal shivering intensity, would optimize the entry and oxidation of glucose in the skeletal muscles. We anticipated that the increased skeletal muscle glucose uptake would be associated with an increase in exogenous glucose oxidation and a reduction in the use of stored CHO sources (i.e., liver and muscle glycogen). In addition, by experimental design, ingesting glucose from the onset of cold exposure provided an additional 40 g of glucose over 2.5 h compared with the ingestion beginning after 60 min or compared with previous work [2-h cold exposure (1)]. Therefore, as a secondary objective, we examined whether ingesting an additional 40 g of glucose over an additional 30 min of shivering could increase the rate of exogenous glucose oxidation over values previously measured during 2-h of cold exposure at the same intensity.

METHODS AND MATERIALS

Subjects. Six healthy, non-cold-acclimatized men volunteered for this study, which was approved by the Health Sciences Ethical Committee of the University of Ottawa, with the written consent of all participants. Age, body mass, height, and percent body fat (estimated by dual-energy X-ray absorptiometry; Lunar Prodigy, General Electric, Madison, WI) of the subjects were 27 ± 2 yr, 84.8 ± 3.8 kg,
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181.3 ± 2.8 cm, and 187.7 ± 1.5%, respectively. Peak oxygen consumption (\(V\text{O}_2\)), estimated 5–7 days before the experiments by incremental treadmill exercise to volitional fatigue, averaged 4.6 ± 0.3 l/min.

Experimental protocol. Each subject participated in two experimental trials, separated by at least 7 days. The order of the trials was randomly assigned following a crossover design. Each trial consisted of a 120-min baseline period, followed by 150 min of shivering at an intensity equivalent to 2.5 times the resting metabolic rate (RMR). Subjects ingested drinks containing glucose at a rate of ~800 mg/min, beginning from the onset of cold exposure (G0) or 60 min into cold exposure (G60), with each traced using [U-13C]glucose.

Experiments were conducted between 0800 and 1300 h, following 36-h without heavy physical activity. The last evening meal was standardized (3.220 kJ or 770 kcal, 42% CHO, 28% fat, and 30% protein), and subjects were asked to report to the laboratory at 0730 h the next morning after a 12–14 h fast. Over the 2 days preceding the experiment, ingestion of CHO from plants naturally rich in \(^{13}\)C (C\(_4\) photosynthetic cycle) was avoided to maintain low \(^{13}\)C background in plasma glucose and expired CO\(_2\) (26). On their arrival in the laboratory, subjects wearing only shorts were weighed and instrumented with thermal probes. Participants were fitted with a liquid-conditioned suit (Three Piece, Allen-Vanguard, Ottawa, ON), and an indwelling catheter (22 G, 25.4 mm, Medex) was then placed in an antecubital vein for blood sampling (left arm). Subjects were then asked to empty their bladder and remain seated for 120 min at ambient temperature (~23–25°C). Following this baseline period, the liquid-conditioned suit was perfused with 4°C water (time = 0) at a rate of 600 ml/min using a temperature and flow-controlled circulation bath (Endocal, NESLAB and Model 200–00, Micropump, Vancouver, WA). Thermal response, metabolic rate, and fuel utilization were measured at baseline before cooling and every 30 min during the subsequent 150 min of cold exposure.

Thermal response. Changes in \(H\) were calculated by indirect respiratory calorimetry corrected for protein oxidation (as described in Ref. 13). Mean skin temperatures (\(T_{\text{s}}\),mm) were monitored continuously before and during cold exposure using heat flux thermocouples [Concept Engineering, Old Saybrook, CT; area-weighted equation from 12 sites: forehead, chest, biceps, forearm, abdomen, lower and upper back, front and back calf, quadriceps, hamstrings, and hand (16)].

Plasma concentrations. Blood samples were used for the measurement of insulin, measured using a commercial human insulin ELISA kit (no. EZHI-14K, Linco), and plasma glucose, measured spectrophotometrically at 340 nm on a Synergy HT Series Multi-Detection Reader (Bio-Tek Instruments, Highland Park, Winooski, VT) using a biochemical hexokinase assay.

Fuel utilization. \(V\text{O}_2\) and carbon dioxide production (\(V\text{CO}_2\)) were measured using a metabolic system (MOXUS, Applied Electrochemistry, Pittsburgh, PA) and expressed in standard temperature and measured using a 3-liter calibration syringe, whereas the gas analyzers were superimposed columns (resins: AG 50W-X8 H\(_2\)O, H\(_3\)PO\(_4\)) and carbon dioxide analyzer (CD-3A) and averaged over 30 min. Gas volume was measured by an electronic volumetric turbine (KTC3 Turbine volumetric system). The volumetric turbine was calibrated using a 3-liter calibration syringe, whereas the gas analyzers were calibrated by using a 4.02% CO\(_2\), 17.0% O\(_2\), balance N\(_2\) mixture. Total protein (\(R\text{P}_{\text{ox}}\), CHO (\(R\text{G}_{\text{ox}}\)), and lipid oxidation rates (\(R\text{F}_{\text{ox}}\)) (in g/min) were calculated as described previously (11, 13):

\[
R\text{P}_{\text{ox}}(g/min) = 2.9 \times U\text{rea}_{\text{urine}}(g/min) \tag{1}
\]

\[
R\text{G}_{\text{ox}}(g/min) = 4.59 V\text{CO}_2(l/min) - 3.23 V\text{O}_2(l/min) \tag{2}
\]

\[
R\text{F}_{\text{ox}}(g/min) = -1.70 V\text{CO}_2(l/min) + 2.17 V\text{O}_2(l/min) \tag{3}
\]

where urinary urea excretion (\(U\text{rea}_{\text{urine}}\)) was measured in urine collected over the 120 min of the baseline period, and 150 min in the cold using a commercial urea assay kit (BioAssay Systems, CA), and \(V\text{CO}_2\) (l/min) and \(V\text{O}_2\) (l/min) were corrected for the volumes of O\(_2\) and CO\(_2\) corresponding to protein oxidation (1.010 and 0.843 g/l, respectively). Energy potentials of 16.3 kJ/g (CHO), 40.8 kJ/g (lipids), and 19.7 kJ/g (proteins) were used to calculate the relative contributions of each fuel to total H (7, 25).

Partitioning of glucose utilization. During the baseline period before cold exposure, rates of plasma and muscle glucose oxidation were quantified by repeated \(^{13}\)C]glucose ingestion (11, 13, 14). For this purpose, 5 g of corn-derived glucose [~11.03‰ difference (\(\delta\) \(^{13}\)C vs. Vienna Pee Dee Belemnitella (V-PDB)] was further enriched with [U-13C]glucose \(^{13}\)C/C > 99%, Isotec, Miamisburg, OH) to obtain a final isotopic composition of +200‰ \(^{13}\)C V-PDB. The [U-13C]-enriched glucose was then diluted in 500 ml of tap water at room temperature and divided into five equal doses. Participants ingested an initial bolus of 200 ml at the beginning of the 120-min baseline period and 100 ml every 30 min thereafter. Resting blood samples of 7 ml were drawn, and 10 ml of expired gases were collected directly from the mixing chamber and filled into vacutainer tubes at the onset and at 15-min intervals during the final 30 min of the baseline period.

During cold exposure, rates of exogenous and plasma glucose, as well as muscle glycogen oxidation, were also quantified by repeated [U-13C]glucose ingestions. To maintain the isotopic enrichment above the background levels during the initial 60 min of cold exposure in the G60 condition, where glucose was ingested late into the exposure period, 5 g of corn-derived glucose further enriched to +200‰ \(^{13}\)C V-PDB was given. The glucose-rich solutions given during the cold exposure contained 120 (G0) and 80 g (G60) of corn-derived glucose, artificially enriched with [U-13C]glucose to +50‰ \(^{13}\)C V-PDB. The [U-13C]-enriched glucose was then diluted in 750 and 500 ml of tap water at room temperature for G0 and G60, respectively. The solutions were subsequently divided into six equal doses for the G0 condition and four equal doses for the G60 condition. In the G0 condition, participants ingested an initial 250-ml bolus of the glucose solution at the onset of cold exposure and 125 ml every 30 min thereafter. Conversely, in the G60 condition, participants ingested the 250-ml bolus of the glucose solution 60 min into the cold-exposure period and 125 ml every 30 min thereafter. This resulted in 80 g of glucose being ingested by 60 min in the G0 condition and 120 min in the G60 condition. Blood samples of 7 ml were drawn, and 10 ml of expired gases filled directly from the mixing chamber were collected in vacutainer tubes just before the ingestion of each [U-13C]glucose dose and every 15 min during the final 30 min of cold exposure. On collection, blood samples were placed on ice and spun in a refrigerated centrifuge. Plasma was separated and stored at ~80°C until analyzed.

For the measurement of \(^{13}\)C/\(^{12}\)C in plasma glucose, 1 ml of plasma was deproteinized, and the glucose was then separated by double-bed ion exchange chromatography by running the supernatants through superimposed columns (resins: AG 50W-X8 H\(_2\)O, 200–400 mesh, and AG 1-X8 chloride, 200–400 mesh), as previously described (27). Following evaporation, glucose was combusted (60 min at 400°C) in the presence of copper oxide, and CO\(_2\) was recovered. Measurements of \(^{13}\)C/\(^{12}\)C in expired CO\(_2\) (\(R_{\text{exp}}\)) and in CO\(_2\) obtained from glucose combustion (\(R_{\text{glu}}\)) were made in a Prism mass spectrometer (VG, Manchester, UK). Isotopic composition of expired CO\(_2\) and in CO\(_2\) obtained from glucose combustion (Fig. 1, A and B) was expressed as \(\delta\) compared with V-PDB standard (6):

\[
\%\delta^{13}\text{C} V\text{-PDB} = \frac{R_{\text{exp}}}{R_{\text{std}}} - 1 \times 1,000 \tag{4}
\]

where \(R_{\text{exp}}\) and \(R_{\text{std}}\) are \(^{13}\text{C}/^{12}\text{C}\) in the sample and standard (1.237%), respectively.

The percentage of plasma glucose (Fig. 1C) derived from exogenous \([^{13}\text{C}]\text{glucose (F}_{\text{exo}})\) was estimated as follows:

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13CO2 recovery values (24) produced in the tissues (24). To take into account this delay between glucose ingestion at the mouth and plasma glucose isotopic composition (Fig. 1, A and B) using the following equation (3, 27, 34).

\[
\text{RG}_{\text{ox}} = \frac{\text{VCO}_2(R_{\text{exp}} - R_{\text{ref-exp}}/R_{\text{glu}} - R_{\text{ref-exp}})}{(1/k1 \cdot k2)}
\]  

Oxidation of glucose released from the liver was calculated by subtracting RG_{\text{ox-exo}} (Eq. 3) from RG_{\text{ox-plasma}} (Eq. 4): 

\[
\text{RG}_{\text{ox-liver}} = \text{RG}_{\text{ox-plasma}} - \text{RG}_{\text{ox-exo}}
\]  

Oxidation of glucose derived from muscle glycogen stores (RG_{\text{ox-mus}}, in mg/min), directly or through the lactate shuttle (2), was calculated by subtracting RG_{\text{ox-plasma}} (Eq. 4) from RG_{\text{ox}} (Eq. 8):

\[
\text{RG}_{\text{ox-mus}} = \text{RG}_{\text{ox}} - \text{RG}_{\text{ox-plasma}}
\]  

Statistical analyses. Data are presented as means ± SE. Statistical significance was set at \( P ≤ 0.05 \). A two-way ANOVA for repeated measures was performed to study the main effects of time and condition, as well as their interaction (SPSS for Windows version 16.0; SPSS, Chicago, IL). Significant interactions were followed up with a 2 (condition) × 2 (time) repeated-measures ANOVA. Significant differences in time and between conditions were followed up with one-way repeated-measures ANOVAs.

Table 1. Absolute oxidation (mg/min) of substrates and their relative contribution to total heat production (%heat production) when given glucose at the onset or after 60 min of cold exposure

<table>
<thead>
<tr>
<th></th>
<th>G0</th>
<th>G60</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Cold 60–90 min</td>
</tr>
<tr>
<td>( H, ) kJ/min</td>
<td>5.9 ± 0.3</td>
<td>12.2 ± 0.6*</td>
</tr>
<tr>
<td>Total glucose mg/min</td>
<td>91 ± 21</td>
<td>333 ± 52*</td>
</tr>
<tr>
<td>( H )</td>
<td>24.1 ± 4.9</td>
<td>43.5 ± 6.4*</td>
</tr>
<tr>
<td>Exogenous glucose mg/min</td>
<td>6 ± 1</td>
<td>118 ± 17*</td>
</tr>
<tr>
<td>( H )</td>
<td>1.3 ± 0.1</td>
<td>15.3 ± 2.1*</td>
</tr>
<tr>
<td>Liver glycogen mg/min</td>
<td>31 ± 3</td>
<td>63 ± 14*</td>
</tr>
<tr>
<td>( H )</td>
<td>8.5 ± 0.7</td>
<td>8.2 ± 1.8</td>
</tr>
<tr>
<td>Muscle glycogen mg/min</td>
<td>55 ± 20</td>
<td>152 ± 36*</td>
</tr>
<tr>
<td>( H )</td>
<td>14.3 ± 4.8</td>
<td>20.0 ± 4.7</td>
</tr>
<tr>
<td>Lipids mg/min</td>
<td>74 ± 7</td>
<td>134 ± 30*</td>
</tr>
<tr>
<td>( H )</td>
<td>52.0 ± 5.0</td>
<td>45.7 ± 6.1</td>
</tr>
<tr>
<td>Proteins mg/min</td>
<td>70 ± 9</td>
<td>66 ± 3</td>
</tr>
<tr>
<td>( H )</td>
<td>23.9 ± 3.0</td>
<td>10.8 ± 0.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 6 \) subjects. G0 and G60, glucose ingested at the onset or after 60 min, respectively; \( H \), heat production. *Significantly different than baseline, \( P < 0.05 \). †Significantly different than G0 (60–90 min), \( P < 0.05 \).
REFERENCES

Stable isotope enrichments. Changes in isotopic composition of expired CO₂, plasma glucose, and Fexo were presented in Fig. 1. The mean resting breath 13CO₂ enrichment (time T = −120 min) was −22.91 ± 0.50 and −22.77 ± 0.40‰ in G0 and G60, respectively, and reached a plateau of −19.40 ± 0.55 and −19.50 ± 0.52‰, respectively, before the start of the cold exposure (Fig. 1A). During cold exposure, breath 13CO₂ enrichment rose progressively following the ingestion of the glucose beverage (T = 0 in G0 and T = 60 in G60), with the increase beginning sooner and being significantly greater in G0 than G60 (Fig. 1A). Mean resting plasma glucose 13C enrichment (T = −120 min) was −23.13 ± 0.97 and −21.17 ± 0.84‰ in G0 and G60, respectively, and reached a plateau of 10.87 ± 2.89 and 11.41 ± 1.85‰, respectively, before the start of the cold exposure (Fig. 1B). During cold exposure, the plasma glucose 13C enrichment rose progressively in both conditions, with a significant transient difference between G0 and G60 observed between 30 and 90 min (Fig. 1B). The percentage of plasma glucose oxidized derived from endogenous glucose rose progressively following the ingestion of the glucose beverage (T = 0 in G0 and T = 60 in G60) with a significant transient difference between G0 and G60 only observed between 30 and 120 min (Fig. 1C).

Thermal response. Changes in H and Tskin are presented in Fig. 2. Total H progressively increased throughout cold exposure in both experimental conditions. However, the rise tended to be transiently greater in G60 at 120 min into cold exposure relative to G0 (P < 0.07), before stabilizing at 2.3 times the RMR by the final 30 min of exposure for both G0 (5.9 ± 0.3 to 14.0 ± 0.2 kJ/min) and G60 (6.1 ± 0.2 to 14.4 ± 0.5 kJ/min; Fig. 2A). Tskin decreased by 17% from the baseline period to the end of the cold-exposure period with no significant difference observed between conditions (from 32.8 ± 0.1 to 27.3 ± 0.5°C in G0 and from 33.0 ± 0.1 to 27.3 ± 0.3°C in G60; Fig. 2B).

Fuel utilization and contribution to H. Changes in absolute Rpox, RGox, and RFox are depicted in Fig. 4, while their relative contribution to total H is represented in Fig. 5. Rpox remained unaffected by CHO ingestion during cold exposure, remaining between 60 ± 6 and 66 ± 3 mg/min by the end of cold exposure vs. 70 ± 9 and 74 ± 8 mg/min during the baseline period (Fig. 4C). However, the relative contribution of RFox to total H decreased similarly, regardless of when glucose ingestion began, from baseline values of 23.7 ± 2.2 to 23.9 ± 3.0‰ H to values between 8.2 ± 0.8 and 9.4 ± 0.5‰ H by the final 30 min of cold exposure (Fig. 5C). CHO oxidation and its relative contribution to H progressively increased over time, reaching a plateau 90 min after ingestion began. Significant differences in both RGox and its relative contribution to energy production, between conditions, were only observed at 90 min (Fig. 4A and 5A). RFox also increased throughout the cold-exposure period; however, the increase was transiently greater at 90 min when glucose was ingested late into cold exposure vs. from the onset (Fig. 4B). Consequently, a trial-by-time interaction was observed for the relative contribution of this substrate to total H at 90 min only (Fig. 5B).

Table 1 summarizes average values measured during the final 30 min of the baseline period and 60 min after glucose ingestion.
ingestion began during cold exposure (60–90 min during the G0 trial and 120–150 min during the G60 trial), for all parameters of total fuel utilization (\( \dot{H} \), \( \dot{R}_{\text{Gox}} \), \( \dot{R}_{\text{Fox}} \), and \( \dot{R}_{\text{Pox}} \)). Total \( \dot{H} \) was 15% higher by the end of cold exposure when glucose was ingested late vs. the period in which the same absolute amount of glucose was ingested from the onset (14.4 ± 0.5 kJ/min at 120–150 min in G60 vs. 12.2 ± 0.6 kJ/min at 60–90 min in G0). The differences in metabolic rate had no effect on total CHO, lipid, or protein utilization, or their relative contribution to \( \dot{H} \) when matching for the quantity of CHO ingested (G0, 60–90 min vs. G60, 120–150 min).

Partitioning of glucose utilization. The oxidation of CHO sources and their relative contribution to total \( \dot{H} \) during the final 30 min of the baseline period and 60 min after glucose ingestion began during cold exposure (60–90 min into the G0 trial and 120–150 min into the G60 trial) are presented in Table 1. During the baseline period, the rate of liver and muscle glycogen utilization was the same between experimental conditions. When glucose was ingested 60 min into cold exposure (G60), the \( \dot{R}_{\text{Gox-exo}} \) was significantly greater than when the same quantity was ingested from the onset of cold exposure (G0) (159 ± 17 vs. 118 ± 17 mg/min). However, the relative contribution of this fuel source to total \( \dot{H} \) did not differ between conditions for the same quantity ingested. \( \dot{R}_{\text{Gox-liver}} \) and \( \dot{R}_{\text{Gox-mus}} \) were not affected by the timing of glucose ingestion during cold exposure, for the same quantity ingested.

Fuel selection over final 30 min of cold exposure. By the end of cold exposure (120–150 min), the \( \dot{R}_{\text{Gox-exo}} \) in the G0 trial increased nearly twofold to a peak of 231 ± 14 mg/min, compared with the period 60 min after glucose ingestion in the same condition (Fig. 6A, left). This oxidation rate was also ~1.5-fold higher than the peak seen at the end of the G60 trial (Fig. 6A, right). This difference by the end of the cold exposure period represented a significant increase in the relative contribution of this fuel source to total energy production from 17.9 ± 1.7% when glucose ingestion was delayed (G60) to 26.8 ± 2.0% when glucose was ingested on the onset of exposure. Although the utilization of the ingested glucose was significantly greater by the end of the exposure period, there was also a significantly greater reliance on \( \dot{R}_{\text{Gox-liver}} \) in the G0 condition by the end of the cold exposure period relative to G60 (73 ± 9 mg/min in G0 vs. 64 ± 12 mg/min in G60; Fig. 6A). This represented a significant increase in the relative contribution of this fuel to total \( \dot{H} \) compared with when participants began ingesting glucose 60 min into cold exposure (8.6 ± 1.1% \( \dot{H} \) in G0 trial vs. 7.3 ± 1.4% \( \dot{H} \) in the G60 trial). Similarly,
utilization of muscle glycogen increased during cold exposure, reaching comparable levels between conditions for the same glucose ingestion; however, ingesting an additional 40 g of glucose in the G0 trial reduced the utilization of muscle glycogen by 48% by the end of cold exposure (Fig. 6B), representing a decrease in the relative contribution of this fuel source from 20.0 ± 4.7% to 8.6 ± 4.3%.

DISCUSSION

This study examined the effects of manipulating the timing of glucose ingestion during 2.5 h of low-intensity shivering on the utilization of exogenous glucose and endogenous CHO sources (i.e., glycogen from liver and muscle). As anticipated, consuming glucose late during cold exposure, at the moment when the metabolic activity had peaked and established a plateau (after 60 min), increased the rate of its utilization by ~35% relative to when the same quantity was ingested from the onset of cold exposure (Table 1). Furthermore, utilization of liver and muscle glucose was diminished by 65 and 45%, respectively, relative to a control condition (1). These changes in the partitioning of endogenous CHO utilization were not significantly affected by the timing of glucose ingestion and occurred without altering the total utilization of CHO, lipids, and proteins. Our secondary objective was to assess the effects of ingesting an additional 40 g of glucose over an additional 30 min of shivering on the oxidation rate of exogenous glucose and endogenous CHO sources. Ingesting glucose from the onset of cold exposure increased its utilization rate to ~230 mg/min and accounted for as much as ~30% of all of the heat produced. This considerably reduced muscle glycogen utilization compared with when glucose was ingested after 60 min of cold exposure (Fig. 6B). Together these findings suggest that glucose should be consumed from the onset of cold exposure and should be sustained as long as possible. Using such a strategy not only supplements the utilization of liver-derived glucose, but also decreases muscle glycogen utilization and may increase glycogen synthesis in the liver and skeletal muscles.

Metabolic fate of exogenous glucose. This study builds on previous estimates of exogenous glucose oxidation rates during cold exposure (1). In this previous work, oxidation rate of ingested glucose was quantified at low (Lo; ~400 mg/min) and high ingestion rates (Hi; ~800 mg/min) during moderate-intensity shivering (~2.5–3 × RMR). Under these conditions, RGox-exo was not different between Lo and Hi, reaching a maximum of ~195 mg/min after 90 min in the cold and
contributed ~20% of all of the heat produced. In the present study, using the same indirect calorimetry and isotopic methodologies, RGox-exo was ~35% greater when the same quantity of glucose was ingested after 60 min of cold exposure relative to ingesting it from the onset (from 159 ± 17 mg/min in G60 vs. 118 ± 17 mg/min in G0; Table 1). This finding is consistent with the hypothesis that shifting the insulinemic peak to a period coinciding with the maximal shivering rate, for a given cold stress, could increase plasma glucose uptake and the oxidation of the ingested glucose. The increase in insulin secretion would result in glucose being indiscriminately taken up by all insulin-sensitive tissues. However, the exact proportion of glucose taken up by individual tissues cannot be determined using the whole body techniques employed in this study. It is likely that the increased insulinemia found here would maximize skeletal muscle glucose uptake via the combined muscle contraction- and insulin-stimulated hemodynamic responses (29) and transmembrane glucose transport (30). Together, these conditions would increase exogenous glucose oxidation in shivering muscles when glucose is ingested late compared with an early ingestion and economize the use of muscle glycogen (see Glucose derived from liver and muscle glycogen below). Interestingly, this effect of glucose ingestion timing on exogenous glucose oxidation appears to be unique to shivering thermogenesis. For instance, in the study by Krzentowski et al. (20), during 240 min of low-intensity exercise, participants ingested 100 g of glucose after 15 or 120 min and found no significant differences in RGox-exo over the 2 subsequent hours. These contrasting outcomes are likely due to the delay in establishing a metabolic steady-state during shivering (~60 min) compared with exercise (~2–3 min).

Glucose derived from liver and muscle glycogen. Compared with a previously reported control condition [177 mg/min; (1)], ingesting glucose at a rate of 800 mg/min suppressed the utilization of liver-derived glucose to a range of 65–75 mg/min (Fig. 6A, left), regardless of the timing of glucose ingestion. Consequently, the utilization of liver-derived glucose contributed <10% to total \( \dot{H} \) (Table 1). This indicates a substantial sparing of liver glycogen reserves, which can be advantageous in providing supplemental energy during prolonged cold exposure, particularly in conditions of low food availability. These findings are consistent with previous estimates found during cold exposure, reporting that ingesting glucose at low (400 mg/min) and high (800 mg/min) rates reduced liver-derived glucose utilization by nearly 50% (1). The reduction in liver glucose utilization is likely attributable to either an insulin-mediated and/or glucoregulatory feedback mechanism suppressing hepatic glucose output, a mechanism previously described when CHO was ingested during exercise (see Ref. 19).

One of the novel findings in this study is that CHO supplementation resulted in an approximately twofold reduction in muscle glycogen utilization (152 ± 36 mg/min in G0 and 129 ± 27 mg/min in G60) compared with ingesting water [279 ± 55 mg/min; (1)]. Exercise studies have shown conflicting evidence on the effects of CHO ingestion on muscle glycogen utilization. While some report that muscle glycogen utilization remains stable over a wide range of CHO ingestion rates (18, 19) and cycling intensities (17), others have shown that this response may be specific to exercise modalities (5, 31) or caused by sex-related differences (34).

Finally, in addition to preserving critical CHO reserves, there is reason to believe that a considerable portion of glucose ingested during low- and moderate-intensity shivering is directed towards nonoxidative disposal (1). However, the exact proportion of glucose destined to glycogen synthesis in the liver and muscle during shivering remains largely unexplored. Given the evidence suggesting that CHO supplementation can increase glycogen synthesis in inactive muscles during exercise (21), along with the current findings demonstrating a reduction in the utilization of liver-derived glucose and muscle glycogen, there may be significant practical importance in further investigating this hypothesis. This glycogen sparing and possible storing may seem inconsequential under thermal conditions eliciting low-intensity shivering, since our laboratory previously demonstrated that, in a glycogen-depleted state, fat and protein oxidation increase, providing the necessary energy to maintain the thermogenic rate (11). However, it is unclear whether this compensatory mechanism can be maintained for a prolonged period or at higher shivering intensities. Combined with the suggestion that a fall in muscle glycogen and/or hypoglycemia can limit the capacity to survive a prolonged cold exposure by effecting a diminished drive to shiver or even inhibiting shivering (see Ref. 9 for review), there is reason to believe that glycogen sparing can have a significant impact on the odds of survival under such conditions. Additionally, we have also demonstrated that interindividual differences in shivering pattern considerably influence the mix of fuels utilized (10). Individuals eliciting a shivering response with a greater burst shivering rate, a reflection of type II muscle fiber recruitment, used relatively more CHO, particularly muscle glycogen, than those with a lower burst shivering rate who used predominantly lipids. Therefore, individuals eliciting a greater burst shivering rate within their unique shivering pattern may be at a greater disadvantage during prolonged cold exposure and would benefit from the glycogen sparing and possible storing observed in the present study.

Overall energy budget: total CHO, lipids, and proteins. Previous investigations have documented the effects of ingesting CHO alone or in combination with other macronutrients before and/or during cold exposure on substrate utilization and thermoregulatory responses (see Ref. 9 for review). Until recently, previous reports suggested that ingesting CHO during cold exposure increased CHO utilization and reduced lipid oxidation, without modifying total \( \dot{H} \) (8, 33). However, in the only study accounting for protein oxidation in the total energy budget, results showed that ingesting glucose at the Lo or Hi rate from the onset of cold exposure had no effect on fuel utilization or thermoregulatory responses and had only a minor influence in the relative contribution of each respective substrate to total energy expenditure compared with control values (1). Results from the present study confirm that, by the end of cold exposure, total CHO, lipid, and protein utilization do not differ compared with ingesting water (1), regardless of the onset of CHO ingestion (Table 1). While total substrate utilization may not necessarily be altered by the nutritional manipulation employed in the present and previous studies, changes in the contribution of intracellular and intravascular substrates utilized during cold exposure can have significant implications. Only examining the use of total energy substrates without considering the specific sources of fuels being affected limits the ability to clearly identify some of the potential effects of the intervention.
Fuel selection over final 30 min of cold exposure. To better understand the factors that limit RG_{exo-exo} in the cold, we also determined the maximal RG_{exo-exo} obtained throughout the full-exposure period for both experimental conditions. While RG_{exo-exo} was clearly higher 60 min after ingestion when glucose ingestion was delayed, by the end of cold exposure the oxidation rate of exogenous glucose increased continuously when glucose was ingested early and even exceeded the values observed in the G60 condition. In the last 30 min of shivering (minutes 120-150), RG_{exo-exo} reached 231 ± 14 mg/min in G0 (Fig. 6A, left), a value 30% higher than the rate measured during the same time period in G60. This exceeded previous estimates by 15% (~195 mg/min), when glucose was ingested on cold exposure (1). In addition, the relative contribution of this fuel to the total thermogenic rate by the end of cold exposure was almost doubled from 17.9 ± 1.7% H when glucose ingestion was delayed to 26.8 ± 2.0% H when glucose was given from the onset. While the rate in which glucose is ingested does not significantly affect exogenous glucose oxidation during cold exposure [400 in Lo vs. 800 mg/min in Hi (1)], the higher rates of exogenous glucose oxidation observed in the G0 condition of the present study suggests that the duration in which glucose is ingested or available may increase the effects of glucose intake on energy partitioning.

A consequence of a continuous rise in exogenous glucose oxidation is a gradual decrease in the dependence of endogenous glucose reserves. In the present experiment, liver-derived glucose remained low and did not fall any further from 60 to 120 min (63 ± 14 and 73 ± 9 mg/min, respectively) when participants began ingesting glucose from the onset of cold exposure. However, muscle glycogen utilization fell by 52% by the end of cold exposure relative to the rate observed after 60–90 min (Fig. 6B, left). This change over time provides further evidence of possible muscle glycogen sparing occurring over time when glucose is ingested early into a cold exposure period. In both experimental conditions, this fuel source contributed <15% to the total thermogenic rate by the final 30 min of cold exposure compared with the ~25% observed in a previous study (1). Glycogen sparing during exercise has been attributed to increased plasma glucose and insulin concentrations, combining with muscle contractions to stimulate insulin- and muscle contraction-induced muscle glucose uptake (5, 32). Consequently, this resulted in increased plasma glucose oxidation and an increase in its relative contribution to total energy yield, thus preserving muscle glycogen utilization. The gradual rise in plasma glucose oxidation over time observed in the present study suggests that a similar mechanism is involved in the muscle glycogen sparing observed in the present study (Fig. 6B).

Conclusion. This study demonstrates that the delay in establishing a metabolic steady state during cold exposure limits skeletal muscle glucose uptake and the subsequent utilization of an ingested glucose load. However, when glucose was ingested immediately on exposure to a cold condition, the rate of exogenous glucose oxidation was higher and the reliance on endogenous CHO reserves was considerably reduced. Together, these observations demonstrate the importance of ingesting glucose immediately on the introduction to a cold stress, relying on shivering thermogenesis and sustaining that consumption for as long as possible. With the limited research available, future studies should be directed at determining the fate of the nonoxidized portion of the ingested CHO and whether higher rates of exogenous glucose oxidation can be achieved by ingesting multiple transportable CHO to further reduce the contribution of endogenous fuels to total thermogenic rate.

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