Effects of carbohydrate availability on sustained shivering
I. Oxidation of plasma glucose, muscle glycogen, and proteins

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2Département de Kinésiologie, Université de Montréal, Montréal, Québec H3C 3P8; and 4Département des Sciences de l’Activité Physique, Université du Québec à Montréal, Montréal, Québec H3C 3P8; and 5Département des Sciences de l’Activité Physique, Université du Québec à Trois-Rivières, Trois-Rivières, Québec, Canada G9A 5H7

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Haman, François, François Péronnet, Glen P. Kenny, Éric Doucet, Denis Massicotte, Carole Lavoie, and Jean-Michel Weber. Effects of carbohydrate availability on sustained shivering. I. Oxidation of plasma glucose, muscle glycogen, and proteins. J Appl Physiol 96: 32–40, 2004. First published August 29, 2003; 10.1152/japplphysiol.00427.2003.—Carbohydrates (CHO) can play an important thermogenic role during shivering, but the effect of their availability on the use of other oxidative fuels is unclear. Using indirect calorimetry and tracer methods ([1U-13C]glucose ingestion), we have determined the specific contributions of plasma glucose, muscle glycogen, proteins, and lipids to total heat production (H˙ prod) in men exposed to cold for 2 h (liquid-conditioned suit perfused with 10°C water). Measurements were made after low-CHO diet and exercise (Lo) and high-CHO diet without exercise (Hi). The size of CHO reserves had no effect on H˙ prod but a major impact on fuel selection before and during shivering. In the cold, a complete shift from lipid oxidation for Lo (53, 28, and 19% H˙ prod for lipids, CHO, and proteins, respectively) to CHO-based metabolism for Hi (23, 65, and 12% H˙ prod for lipids, CHO, and proteins, respectively) was observed. Plasma glucose oxidation remained a minor fuel under all conditions (<13% H˙ prod), failing to 7% H˙ prod for Lo. Therefore, adjusting plasma glucose oxidation to compensate for changes in muscle glycogen oxidation is not a strategy used for maintaining heat production. Instead, proteins and lipids share responsibility for this compensation. We conclude that humans can show remarkable flexibility in oxidative fuel selection to ensure that heat production is not compromised during sustained cold exposure.

Although carbohydrates (CHO) represent only ~1% of energy stores in normal subjects, CHO oxidation can account for up to 60% of total heat production (H˙ prod) during cold exposure (18, 19). Modifying glycogen availability has a profound effect on fuel selection during shivering, to the extent that a complete shift from CHO dominance to lipid dominance can be elicited (22, 43). Several studies show that shivering humans (prolonged immersion in 18°C water) produce ~80% of total heat from CHO oxidation when glycogen reserves are artificially elevated, and the same percentage, but from lipid oxidation, when glycogen reserves are depleted (22, 43). Usually, such drastic changes in fuel selection have no effect on cold tolerance because H˙ prod appears to be independent of glycogen availability (22, 40, 43). However, when glycogen reserves are low, conflicting results have been reported for body cooling rate that show a significant increase in one study (22), but no change in another (43).

Two separate sources of CHO are available for heat production: hepatic glucose provided to shivering muscles by the circulation, and muscle glycogen. The relative importance of these two CHO sources in humans with normal glycogen stores was recently determined during sustained, low-intensity shivering (18). In these subjects, most of the CHO oxidized came from muscle glycogen (~75%), whereas the contribution of circulating glucose was minor (~25% of total CHO oxidized or ~10% of total heat produced). Several earlier studies investigated the effect of changes in the size of glycogen reserves on fuel metabolism during shivering. Using biopsies from the vastus lateralis, Martineau and Jacobs (22) showed higher glycogen use in glycogen-loaded than in glycogen-depleted subjects, whereas Young et al. (43) did not find a significant difference between the two conditions.

The roles played by the oxidation of blood glucose and body proteins have never been characterized in relation to the size of glycogen stores. Consequently, the purpose of this paper was to measure the effects of changes in CHO availability on oxidative fuel selection during sustained, low-intensity shivering. More specifically, the oxidation rates of circulating glucose, proteins, and lipids were quantified to determine their role in compensating for low vs. high glycogen availability. Using a combination of indirect calorimetry and stable isotope tracer methods, we monitored fuel oxidation in adult human male subjects with low and high glycogen levels while they were exposed to cold for 2 h. In view of the minor role played by circulating glucose in normal subjects (18), we hypothesized that changes in plasma glucose oxidation would not be used to offset changes in glycogen oxidation. Therefore, we predicted that lipids and proteins would maintain heat production by compensating for the variable contribution from glycogen.

In the second part of this study, reported in the companion article (17), we investigated a potential mechanism for changing fuel selection. It has been suggested that switching oxidative fuels could simply be achieved by recruiting different populations of muscle fibers specialized for lipid or CHO oxidation (30). However, this concept of “fuel-specific fibers” has never been tested directly, probably because it is very difficult to make simultaneous measurements of substrate me-

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32
tabolism and muscle fiber recruitment, particularly during exercise. It occurred to us that shivering could be an ideal model to test this hypothesis because electrical noise caused by limb movements is considerably lower than in exercise. In the companion paper (17), we report electromyography (EMG) data recorded simultaneously with the substrate metabolism results presented here and correlate fuel selection with muscle fiber recruitment.

**METHODS**

_Subjects._ Six healthy male subjects volunteered to participate in this study approved by the Health Sciences Ethical Committee of the University of Ottawa. Physical characteristics of the male subjects are presented in Table 1. Five to 7 days before the experimental trials, maximal oxygen consumption ($VO_{2\text{max}}$) was determined separately for upper and lower body using progressive cycloergometer protocols (90 and 180 W, 2-min ramps for upper and lower body, respectively). Body composition was estimated by underwater weighing (7).

_Experimental protocol._ Five to 6 days before the experiments, a 1-h session was held to familiarize the subjects with the equipment and the level of cold exposure faced in the experiments. For the actual experiments, subjects were exposed to cold on two separate occasions after following 1) a diet low in CHO and heavy exercise bouts (Lo) and 2) a diet high in CHO without exercise bouts (Hi). A detailed description of the diet and exercise regimen is given below. On the day of these experimental sessions, care was taken to minimize exercise or other thermal stresses between awakening and the start of the experiment (i.e., avoid exposure to hot or cold temperatures or exercise during transit from home to the laboratory). On their arrival in the laboratory (8:00 AM; 12 h postabsorptive), subjects were instrumented with thermal probes and an indwelling catheter (18 G, 32 mm, Medical, Arlington, TX) placed in an antecubital vein for blood sampling and were fitted with a liquid-conditioned suit (three-piece Delta Temax, Pembroke, ON). After voiding the bladder (time $t = 0 \text{ min}$), subjects remained seated comfortably for the next 2 h at $23.2 \pm 0.01°C$ (758 ± 2 mmHg, 39.8 ± 3.6% relative humidity). After this period, they were transferred to an environmental chamber identical for both diets and was calculated on the basis of the weight of each subject ($\sim 170 \text{ kJ/kg } -1\text{day}^{-1}$). Care was taken to avoid ingestion of CHO from plants naturally rich in $13C$ (C4 photosynthetic cycle) to maintain low $13C$ background enrichment in plasma glucose and expired $CO_2$. Three days before the Lo experiment, a glycogen-depletion phase began, with subjects performing upper and lower body exercises for 2 h at 65% $VO_{2\text{max}}$ on a cycloergometer and a hand-crank ergometer (alternating every 30 min between legs and arms). After these exercises, subjects were prescribed a Lo diet for the next 3 days until the evening of Lo experiment. During this phase, additional 1-h exercises were performed each day (days 2 and 3) with upper and lower body exercises (30 min each at 65% $VO_{2\text{max}}$). Immediately after the Lo experiment (day 4), subjects began a Hi diet (Table 2) for the next 2.5 days and were asked to refrain from exercising during this period. Hi experiment was performed on the morning of day 7. This experimental design was selected to minimize interindividual variability in the shivering response (i.e., same subjects for Lo and Hi) and to take advantage of the initial depletion in glycogen reserves to maximize glycogen loading on days 4 – 7. Using this nonrandomized design could potentially result in an order effect associated with muscle fatigue, leading to a reduction in shivering intensity. However, results from this and the next study (17) show that thermal responses, heat production (indirect calorimetry), and shivering intensity (EMG) were not different between Lo and Hi. This indicates that muscle fatigue was most likely minimal because glycogen-depletion exercise was performed at least 20 h before shivering measurements and muscle work for thermogenesis was always very low (only up to 25% $VO_{2\text{max}}$).

_Thermal response._ Whole body heat loss ($H_{\text{loss}}$, in watts) was estimated using the following equation:

$$H_{\text{loss}} = \frac{Q_{\text{met}} - Q_{\text{rad}} - Q_{\text{con}}}{\Delta T}$$

Table 1. Physical characteristics of subjects

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>22.2 ± 0.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, kg</td>
<td>70.6 ± 3.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174.1 ± 1.5</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>1.8 ± 0.04</td>
</tr>
<tr>
<td>Percent body fat, %</td>
<td>12.6 ± 1.0</td>
</tr>
<tr>
<td>$VO_{2\text{max}}$, ml·kg⁻¹·min⁻¹</td>
<td>37.1 ± 3.9</td>
</tr>
<tr>
<td>Upper body</td>
<td>51.7 ± 3.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 6$ subjects. $VO_{2\text{max}}$, maximal oxygen consumption.

Table 2. Macronutritional composition of low- and high-CHO diets

<table>
<thead>
<tr>
<th></th>
<th>Low CHO</th>
<th>High CHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO, g/day</td>
<td>67.9 ± 10.4</td>
<td>494.0 ± 54.8</td>
</tr>
<tr>
<td>Lipids, %</td>
<td>20.4 ± 19.7</td>
<td>38.4 ± 4.8</td>
</tr>
<tr>
<td>Protains, %</td>
<td>170.4 ± 20.5</td>
<td>111.5 ± 10.9</td>
</tr>
<tr>
<td>CHO, %</td>
<td>9.7 ± 0.5</td>
<td>71.5 ± 0.5</td>
</tr>
<tr>
<td>Lipids, %</td>
<td>65.8 ± 1.2</td>
<td>12.5 ± 0.5</td>
</tr>
<tr>
<td>Protains, %</td>
<td>24.3 ± 0.8</td>
<td>16.2 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 6$ subjects. CHO, carbohydrates.

Fig. 1. Seven-day diet and exercise protocol to decrease (Lo) and increase (Hi) glycogen reserves. Thick black lines represent duration of low- and high-carbohydrate (CHO) diets. Experiments for Lo and Hi were performed on the mornings of day 4 and day 7. Numbers in the shaded boxes (in exercise section) indicate the duration (min) of exercise bouts for upper and lower body. $VO_{2\text{max}}$, maximal oxygen consumption.
\[ \dot{V} \text{O}_2 = (\dot{R} + \dot{C}) + (\dot{E}_{\text{resp}} + \dot{C}_{\text{resp}}) \] (1)

where \( \dot{R} \) and \( \dot{C} \) represent rates of radiative and convective heat loss, respectively, and \( \dot{E}_{\text{resp}} \) and \( \dot{C}_{\text{resp}} \) are rates of evaporative and convective heat loss, respectively, by ventilation (2,411.3 J of heat per gram of evaporated water). \( \dot{R} \) and \( \dot{C} \) were estimated by using heat-flux transducers (Concept Engineering, Old Saybrook, CT) placed on the surface of the skin at 11 sites (i.e., forehead, chest, biceps, forearm, abdomen, lower and upper back, front and back calf, quadriceps, hamstrings) and calculated by using an area-weighed equation (12). Evaporative heat loss from the skin was assumed to be negligible at 23 and 10°C (24). \( H_{\text{res}} \) was calculated by indirect respiratory calorimetry corrected for protein oxidation (see below). Percent of shivering peak (Shivpeak) was determined by dividing oxygen consumption (V\( \dot{O}_2 \); ml·kg\(^{-1} \)·min\(^{-1} \)) values measured in the cold by the calculated Shivpeak (in ml·kg\(^{-1} \)·min\(^{-1} \)) estimated for each subject using the following equation (14)

\[ \text{Shivpeak} = 30.5 + (0.348 \times \dot{V} \text{O}_{2\text{max}}) - (0.909 \times \text{BMI}) - (0.233 \times \text{age}) \] (2)

where \( \dot{V} \text{O}_{2\text{max}} \) is in ml·kg\(^{-1} \)·min\(^{-1} \), BMI is the body mass index (kg/m\(^2 \)), and the age is in years.

Body temperature (\( T_{\text{b}} \)) was monitored continuously by using a pediatric esophageal probe (Mon-a-therm general purpose, Mallinckrodt Medical, St. Louis, MO). Mean skin temperature (\( T_{\text{sk}} \)) was averaged from 12 sites (i.e., fingertip plus the 11 heat transducer sites mentioned above for heat flux measurements) by using an area-weighed equation (12). Subjective thermal perception was determined every 30 min by asking the subjects to identify their comfort level on the basis of an 11-point Likert scale (16) (5 being the coldest and warmest ever experienced, respectively, with 0 feeling neither cold nor warm). This is a modification of the scale previously described by Thanasis et al. (32).

Metabolic rate and fuel utilization. Pulmonary ventilation, \( \dot{V} \text{O}_2 \), and carbon dioxide production (V\( \dot{C} \text{O}_2 \)) were determined by open-circuit spirometry (250 liters, chain-compensated gasometer, Warren Collins, Braintree, MA). All expired gas collections were made at ambient temperature outside the experimental chamber. A mouthpiece, a unidirectional valve (2700 series, Hans Rudolph, Kansas City, MO), and a 44-mm plastic tube were used to direct all expired gases to the collection tank. Expired gases were collected for 5 min every 30 min during the experiment. Isotopic composition of plasma glucose and expired CO\(_2\) were determined in blood and expired gas samples every 30 min before the ingestion of the next dose. On collection, blood samples were put on ice, spun in a refrigerated centrifuge, and separated, and the plasma was kept frozen at −20°C until analyzed.

Plasma glucose was isolated by double-bed ion exchange chromatography with superimposed columns (resins: AG 50W-X8 H\(^{+} \), 200–400 mesh, and AG 1-X8 chloride, 200–400 mesh). After evaporation, plasma glucose was combusted (60 min at 400°C) in the presence of CuO, 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 determined in a Prism mass spectrometer (VG, Manchester, UK). Isotopic enrichment was expressed as percent [\(^{13}\)C]/\(^{12}\)C.

The plasma glucose oxidation rate (R\(_{\text{ox-plasma}}\)) was calculated from [\(^{13}\)C]/\(^{12}\)C production at the mouth and plasma glucose isotopic enrichment (Fig. 2, A and B) by using the following equation (11, 42)

\[ \text{R}_{\text{ox-plasma}} = \frac{\dot{V} \text{C}_2 \text{O}_2 \left( R_{\text{exp}} - R_{\text{ref-exp}} \right) - R_{\text{glu}} \left( 1/1.1 \times 2 \right)}{1/1.1 \times 2} \] (6)

where V\( \dot{C}_2 \text{O}_2 \) is in liters per minute (STPD), R\(_{\text{ref-exp}}\) is the isotopic composition of expired CO\(_2\) before the ingestion of the first [\(^{13}\)C]glucose dose, R\(_{\text{ref-glu}}\) is the isotopic composition of plasma glucose before ingestion of the first [\(^{13}\)C]glucose dose, k1 (0.7426 l/g) is the volume of CO\(_2\) produced from the complete oxidation of glucose, and k2 is the fractional recovery at the mouth of CO\(_2\) produced in tissues (25). A fractional recovery of [\(^{13}\)C]CO\(_2\) at the mouth (k2) of 0.8 and 1 was used before and during cold exposure, respectively (42). Because of the large size of the bicarbonate pool, only values in the last 30 min before and during cold exposure were used in the calculation of R\(_{\text{ox-plasma}}\). In addition, R\(_{\text{ox-plasma}}\) was corrected to account for the fraction of plasma glucose oxidized from exogenous sources (18).

Oxidation of glucose derived from muscle glycogen stores (R\(_{\text{ox-mus}}\)....
g/min), directly or through the lactate shuttle (6), was calculated by subtracting $RG_{\text{ox-plasma}}$ from $RG_{\text{ox}}$

$$RG_{\text{ox-mus}} = RG_{\text{ox}} - RG_{\text{ox-plasma}} \quad (7)$$

**Blood analysis.** Plasma glucose and lactate concentrations were measured spectrophotometrically at 340 nm on a Beckman DU 640 (2), and total plasma nonesterified fatty acid (NEFA) and β-hydroxybutyrate (β-HB) concentration were determined by using an analytical assay kit (NEFA C, Wako Chemicals, Osaka, Japan and Sigma kit no. 310, Sigma-Aldrich Canada, Oakville, ON). Plasma insulin concentration was measured by using a radioimmunoassay (no. KTSP-11001, Medicorp, Montréal, QC).

**Statistical analyses.** Changes in $T_{es}$, $T_{\text{skin}}$, $H_{\text{loss}}$, $H_{\text{prod}}$, expired CO$_2$, and plasma glucose isotopic enrichments and gas exchange were assessed by two-way ANOVA for repeated measures. Differences in $H_{\text{prod}}$, fuel utilization for CHO ($RG_{\text{ox}}$, $RG_{\text{ox-plasma}}$, $RG_{\text{ox-mus}}$), lipids ($RF_{\text{ox}}$), and proteins ($RP_{\text{ox}}$) as well as plasma metabolite concentrations over the last 30 min before and during cold exposure were determined by using a one-way ANOVA to verify the main effect of diet (Lo vs. Hi). Statistical differences were considered significant when $P < 0.05$. The statistical power of our tests was calculated for key parameters (oxidation rates; relative contributions of plasma glucose, muscle glycogen, total CHO, and lipids to $H_{\text{prod}}$), and it ranged from 0.945 to 1.00. All values presented are means ± SE ($n = 6$), unless indicated otherwise.

**RESULTS**

**Thermal response.** Changes in absolute $H_{\text{loss}}$ and $H_{\text{prod}}$ before and during cold exposure for Lo and Hi are presented in Fig. 3. Absolute $H_{\text{loss}}$ and $H_{\text{prod}}$ increased as a result of cold exposure, but no significant difference was found between Lo and Hi. Maximal $H_{\text{loss}}$ was reached after 10 min of cold exposure (76.7 ± 3.6 W at 23°C to 220.5 ± 4.4 W at 10°C and 75.9 ± 4.7 to 210.8 ± 4.4 W for Lo and Hi, respectively) and decreased gradually by 11%, stabilizing in the last 60 min at 75.9 ± 4.7 and 76.7 ± 4.4 W for Lo and Hi, respectively. $H_{\text{prod}}$ averaged 94.5 ± 5.3 and 86.1 ± 5.9 W at 23°C and reached a maximum of 224.3 ± 17.4 and 209.1 ± 26.4 W after 120 min in the cold for Lo and Hi, respectively. Metabolic rate reached by the end of cold exposure was not different between Lo and Hi.

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Fig. 3. Changes in heat loss ($H_{\text{loss}}$; A) and heat production ($H_{\text{prod}}$; B) before and during cold exposure for Lo (●) and Hi (○). Values are means ± SE. *(Significantly different from control values before cold exposure, $P < 0.05$.

Fig. 4. Changes in esophageal ($T_{es}$; A) and mean skin ($T_{\text{skin}}$; B) temperature as well as subjective thermal comfort (C) before and during cold exposure for Lo (●) and Hi (○). Values are means ± SE. Arrows indicate times when the $[13C]$glucose solutions were ingested.

(34.4 ± 3.8% Shiv peak) and Hi (30.4 ± 2.0% Shiv peak). As shown in Fig. 4, changes in $T_{es}$, $T_{\text{skin}}$, and subjective thermal comfort were not different between Lo and Hi. Although $T_{es}$ did not change as a result of cold exposure (36.5 ± 0.3°C), $T_{\text{skin}}$ decreased from 33.6 ± 0.4°C at 23°C to 27.4 ± 0.5°C in the first 90 min of cold exposure and remained constant until the end of the experiment. Thermal comfort averaged +2 at 23°C and decreased continuously, reaching −3 at the end of the cold exposure.

**Plasma concentrations.** Average plasma concentrations of insulin, glucose, lactate, total NEFA, and β-HB calculated in the last 30 min before and during cold exposure are presented in Table 3. Before cold exposure, whereas insulin, glucose and lactate concentrations were significantly lower for Lo than Hi (−22, −4, and −46%, respectively), NEFA and β-HB were 4.3- and 10.3-fold higher for Lo than for Hi. Insulin and β-HB concentrations were not affected by the change in temperature, whereas glucose concentration increased 4 and 7.5% by the end of cold exposure for Lo and Hi, respectively. Cold exposure caused a 1.8-fold increase for Lo and 1.5-fold for Hi in lactate concentration over values at 23°C, and total NEFA concentration increased 1.5- and 2.5-fold over values before cold exposure for Lo and Hi, respectively.

**Metabolic fuel utilization.** Rates of total glucose ($RG_{\text{ox}}$) and lipid ($RF_{\text{ox}}$) oxidation for Lo and Hi are shown in Fig. 5. $RG_{\text{ox}}$ was significantly higher for Hi before (58.1 ± 20.0 mg/min and 240.6 ± 27.4 mg/min for Lo and Hi, respectively) as well as during cold exposure (Fig. 5A; maximum of 221.5 ± 45.0 and
Table 3. Plasma insulin, glucose, lactate, NEFA- and β-HB concentrations in men with low- and high-CHO reserves before (23°C) and during (10°C; time = 210–240 min) cold exposure

<table>
<thead>
<tr>
<th></th>
<th>Lo</th>
<th>Hi</th>
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<tbody>
<tr>
<td></td>
<td>23°C</td>
<td>10°C</td>
</tr>
<tr>
<td>Insulin, pm</td>
<td>77.0±1.7</td>
<td>82.0±2.4</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>4.7±0.1</td>
<td>4.9±0.1*</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>0.7±0.1</td>
<td>1.3±0.3*</td>
</tr>
<tr>
<td>NEFA, μM</td>
<td>431±34</td>
<td>651±33*</td>
</tr>
<tr>
<td>β-HB, μM</td>
<td>322±50</td>
<td>482±64</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. NEFA, nonesterified fatty acid; β-HB, β-hydroxybutyrate; Lo, low-CHO reserve condition; Hi, high-CHO reserve condition. *Significantly different from control values at 23°C; †Significantly different from control values at 23°C in Lo; ‡Significantly different from values at 10°C in Lo; P < 0.05.

469.9 ± 61.7 mg/min for Lo and Hi, respectively). Before cold exposure, RFox was not significantly different from zero for Hi (nonprotein respiratory exchange ratio was equal or larger than 1, indicating a stimulation of lipogenesis due to the high CHO uptake; Ref. 29) and averaged 58.2 ± 6.0 mg/min for Hi. In the cold, RFox increased continuously, reaching maximal values of 163.2 ± 21.6 mg/min for Lo and 68.4 ± 22.0 mg/min for Hi after 90 min in the cold (Fig. 5B). As shown in Table 4, a close to twofold difference in RFox was observed between Lo and Hi before cold exposure (111.5 ± 23.2 and 63.6 ± 6.0 mg/min for Lo and Hi, respectively) and in the cold (118.3 ± 12.9 and 65.5 ± 5.9 mg/min for Lo and Hi, respectively). Changes in the respective contributions of CHO (%RGox), lipid (%RFox), and protein (%RPox) oxidation to total Hprod under Lo and Hi conditions are presented in Fig. 6. Before cold exposure, %RGox was 4.3-fold lower for Lo (17.5 ± 5.7% Hprod) compared with Hi (75.7 ± 5.0% Hprod) (Fig. 6A). The subsequent decrease in environmental temperature had no effect on %RGox, with values averaging 24.1 ± 6.1% Hprod for Lo and 67.3 ± 6.0% Hprod for Hi. A continuous increase in %RFox was observed for Hi averaging a value not different from zero at 23°C and increasing to a maximum of 21.7 ± 5.3% Hprod in the last hour of cold exposure (Fig. 6B). For Lo, %RFox in the cold (58.2 ± 6.0% Hprod) was not different from control values at 23°C (52.5 ± 4.5% Hprod; Fig. 6B). As a result of cold exposure, a 2.2-fold decrease in %RPox was found for Lo (40.2 ± 7.8 at 23°C to 18.7 ± 1.5% Hprod at 10°C) and Hi (25.3 ± 3.6 at 23°C to 11.7 ± 1.5% Hprod at 10°C; Fig. 6C).

Table 4. Urinary urea excretion rate and absolute oxidation rate of protein in men with low- and high-CHO availability before (23°C) and during (10°C) cold exposure

<table>
<thead>
<tr>
<th></th>
<th>Lo</th>
<th>Hi</th>
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<tbody>
<tr>
<td></td>
<td>23°C</td>
<td>10°C</td>
</tr>
<tr>
<td>Urinary urea excretion, g/120 min</td>
<td>4.8±0.6</td>
<td>5.0±0.9</td>
</tr>
<tr>
<td>Protein oxidation rate, g/120 min</td>
<td>13.4±2.8</td>
<td>14.2±1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. *Significantly different from control values at 23°C in Lo; †Significantly different from values at 10°C in Lo; P < 0.05.

Fig. 5. Changes in absolute CHO (A) and lipid (B) oxidation rates before and during cold exposure for Lo (○) and Hi (●). Shaded circles represent previously published values in men with normal CHO reserves (18). Values are means ± SE. *Significantly different from control values before cold exposure, P < 0.05.

Fig. 6. Changes in CHO (A), lipid (B), and protein oxidation (C) before and during cold exposure for Lo (○) and Hi (●). Shaded circles represent previously published values for men with normal CHO reserves (18). Values are means ± SE. *Significantly different from control values before cold exposure, P < 0.05.
Table 5. Absolute oxidation rate and relative contributions of lipids, total CHO, plasma glucose, muscle glycogen, and proteins to total heat production in men with low- and high-CHO availability before (23°C; 90–120 min) and during (10°C; 210–240 min) cold exposure

<table>
<thead>
<tr>
<th></th>
<th>Lo 23°C</th>
<th>Hi 10°C</th>
<th>Lo 23°C</th>
<th>Hi 10°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hprod, kJ/min</td>
<td>5.5±0.2</td>
<td>12.6±0.8†</td>
<td>5.0±0.4</td>
<td>11.7±1.3†</td>
</tr>
<tr>
<td>Lipids mg/min</td>
<td>58.2±6.0</td>
<td>163.2±21.6†</td>
<td>0*</td>
<td>68.4±22.0†‡</td>
</tr>
<tr>
<td>%Hprod</td>
<td>41.6±3.6</td>
<td>53.4±4.5†</td>
<td>0*</td>
<td>23.0±5.2†</td>
</tr>
<tr>
<td>Total CHO</td>
<td>60.1±18.7</td>
<td>221.5±45.0†</td>
<td>248.6±27.9</td>
<td>469.9±61.7‡</td>
</tr>
<tr>
<td>%Hprod</td>
<td>18.1±5.4</td>
<td>27.7±5.2†</td>
<td>77.1±3.8‡</td>
<td>65.3±5.4‡</td>
</tr>
<tr>
<td>Liver (plasma)</td>
<td>29.3±2.4</td>
<td>53.8±6.1†</td>
<td>38.5±5.0</td>
<td>89.6±9.7‡</td>
</tr>
<tr>
<td>mg/min</td>
<td>8.9±0.8</td>
<td>7.3±0.9</td>
<td>12.3±1.2</td>
<td>13.1±1.4</td>
</tr>
<tr>
<td>Muscle glycogen</td>
<td>30.7±21.0</td>
<td>167.8±43.1†</td>
<td>210.1±24.0</td>
<td>380.3±58.3‡</td>
</tr>
<tr>
<td>%Hprod</td>
<td>9.3±5.9</td>
<td>20.4±4.6†</td>
<td>64.8±3.3‡</td>
<td>52.3±4.9‡</td>
</tr>
<tr>
<td>Proteins</td>
<td>111.5±23.2</td>
<td>118.3±12.9</td>
<td>63.6±6.0</td>
<td>65.5±5.9</td>
</tr>
<tr>
<td>mg/min</td>
<td>40.2±7.8</td>
<td>18.7±1.5†</td>
<td>25.3±3.6‡</td>
<td>11.7±1.5‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. Hprod, total heat production. *See text. †Significantly different from control values at 23°C; ‡significantly different from control values at 23°C in Lo; §significantly different from values at 10°C in Lo; P < 0.05.

Table 5 shows the absolute and relative contributions of CHO, lipids, and proteins to Hprod before and during cold exposure. Before cold exposure, RFox was not different from zero for Hi and averaged 58.2 ± 6.0 mg/min for Lo, whereas RGox and RPox showed a respective 4.1- and 1.8-fold difference between Lo and Hi. In addition, RGox-plasma was only 1.3-fold higher for Hi than for Lo, whereas RGox-mus showed a 6.8-fold difference between Lo and Hi. The relative contribution of proteins to Hprod was 1.6-fold higher for Lo than for Hi, whereas that of CHO was 4.3-fold higher for Hi than for Lo, and that of lipids was not different from 0 for Hi and averaged 41.6 ± 3.6% Hprod for Lo. Furthermore, RGox-plasma contributed significantly less heat for Lo than for Hi (40% less) and RGox-mus was 697% higher for Hi than for Lo. As a result of cold exposure, RGox increased respectively 4.7- and 2.0-fold for Lo and Hi, whereas RPox did not change. RFox increased 2.6-fold for Lo and from a value not different from zero at 23°C to 68.4 ± 22.0 mg/min by the end of cold exposure. Increases of 5.5- and 1.8-fold for RGox-mus as well as 1.9- and 1.4-fold for RGox-plasma were observed as a result of cold exposure for Lo and Hi, respectively. Although proteins contributed 1.6-fold more to total Hprod under Lo than Hi, %RGox was 2.4-fold higher for Hi than for Lo. In addition, the relative contribution of RGox-plasma and RGox-mus to Hprod in the cold was 179 and 256% higher for Hi than for Lo, respectively.

DISCUSSION

The purpose of this paper was to determine how glycogen depletion or loading would modify oxidative fuel selection during sustained low-intensity shivering, with a focus on the roles of plasma glucose and body proteins. The protocols used to try modifying glycogen stores caused major changes in total CHO oxidation before cold exposure (~65 vs. 27% Hprod for Hi and Lo) but had no effect on heat production in the cold. During shivering, we found that the relative role of plasma glucose oxidation remains minor under all conditions, falling to 7% Hprod in glycogen-depleted subjects. This study is the first to quantify the role of proteins for thermogenesis in individuals with altered CHO reserves. It shows that the relative contribution of protein oxidation is substantially increased in glycogen-depleted compared with glycogen-loaded subjects (19 vs. 12% Hprod). Therefore, adjusting plasma glucose oxidation to compensate for changes in glycogen availability is not a strategy used for maintaining heat production. Instead, proteins and lipids share responsibility for this compensation.

Blood glucose oxidation. Previous work in subjects with normal (N) CHO availability (18) and this study in glycogen-loaded and -depleted subjects (Table 5) show that the relative role of plasma glucose is always minor during sustained, low-intensity shivering (<13% Hprod). Cold exposure stimulates RGox-plasma in direct proportion to metabolic rate in Hi [2.3-fold; as previously observed in normal subjects, Haman et al. (18)], whereas a smaller increase is observed in Lo (1.8-fold). These results suggest that hepatic glucose production is lower in glycogen-depleted subjects, because gluconeogenesis does not make up for decreased liver glycogenolysis in this group. Plasma glucose oxidation does not compensate for the large decrease in muscle glycogen oxidation of Lo, and other fuels must be used to sustain heat production. Clearly, however, plasma glucose oxidation rates of our subjects were not limited by maximal hepatic glucose production, because much higher RGox-plasma have been reported during exercise (6–8 mg·kg⁻¹·min⁻¹ vs. 0.7–1.3 mg·kg⁻¹·min⁻¹ in the cold) (4–5a, 9, 15, 20, 27). Friedlander et al. (15) showed that RGox-plasma is stimulated by as much as fivefold during exercise at 45% VO₂max, the highest metabolic rate observed during maximal shivering (14). Considering the low metabolic rates reached in this study (2.3 times resting metabolic rate or 20% VO₂max), further research is needed to establish whether plasma glucose plays a more important role at higher shivering intensities.

Muscle glycogen oxidation. This study provides the first whole body measurements of glycogen utilization during sustained, low-intensity shivering. Muscle glycogen supplied 75–80% of total CHO oxidized (Table 5) and played an important role in Hprod (20 and 50% Hprod in Lo and Hi, respectively). The absolute rate of muscle glycogen utilization was two times higher in Hi (380.3 ± 58.3 mg/min) than in Lo (167.8 ± 43.1 mg/min), but the greatest relative change caused by cold exposure was observed in Lo (+445% for Lo vs. +80% for Hi). These results show that muscle glycogen reserves are always strongly mobilized for thermogenesis, even when they have been reduced before cold exposure.

Only two other papers have addressed the problem of oxidative fuel selection in relation to altered glycogen reserves (22, 43). They have reported much lower or higher rates of muscle glycogen utilization than observed here. The conclusions drawn from these studies were based on the direct measurement of glycogen concentration in vastus lateralis biopsies, and this experimental approach may be responsible for the large variability. Although their measurements were made at higher shivering intensities, Young et al. (43) reported no change in muscle glycogen levels in Lo or Hi. In the other study, Martineau and Jacobs (22) found an average glycogen

J Appl Physiol • VOL 96 • JANUARY 2004 • www.jap.org
utilization rate approximately four times higher (~20 mg·kg body wt\(^{-1}\)·min\(^{-1}\)) than values reported here for Hi, but no significant change for Lo. The exact reasons for these discrepancies are unclear, but methodological limitations may be responsible. For example, it is well known that estimating whole body glycogen utilization from muscle biopsies is extremely difficult because 1) glycogen concentration is variable within and among muscles and 2) the relative contribution of vastus lateralis to total shivering activity is not known.

**Protein oxidation.** Altering glycogen reserves through dietary and exercise manipulations had a major effect on the relative use of proteins before cold exposure. At 23°C, protein oxidation was responsible for 25% \(H_{\text{prod}}\) in Hi, and this value increased to 40% \(H_{\text{prod}}\) in Lo (Table 4). Absolute rates of protein oxidation were not affected by cold exposure, and, therefore, the 2.3-fold increase in metabolic rate observed in the cold was simply translated as a proportional decrease in the relative role of proteins for both groups (12% \(H_{\text{prod}}\) in Hi and 19% \(H_{\text{prod}}\) in Lo, Table 5). The contribution of protein oxidation to shivering thermogenesis has generally been assumed to be minor (~10% \(H_{\text{prod}}\)) and, therefore, rarely measured directly (18, 38, 39). Our results show that this assumption may still be true when glycogen reserves are artificially elevated but that the relative importance of proteins increases substantially when CHO reserves are reduced. In fact, the increased contribution of proteins plays a key role in compensating for the decrease in CHO use after glycogen depletion. Failing to take protein oxidation into account in fuel oxidation budgets during low-intensity shivering leads to a significant overestimation of CHO and lipid oxidation rates, particularly when glycogen reserves are depleted.

**Fuel metabolism before cold exposure.** It is clear that our dietary and exercise manipulations had the desired impact on glycogen reserves, but muscle glycogen concentrations were not directly measured in our study. Previous experiments in which muscle biopsies were taken provide strong evidence that our glycogen-loading and -depleting protocols affected muscle glycogen levels significantly (3, 10, 22, 23, 43). Using the same experimental protocol, Young et al. (43) found a fourfold difference in glycogen levels of the vastus lateralis between Lo and Hi (144 ± 14 vs. 543 ± 53 mmol glucose/kg dry muscle). Even when intense exercise was not used (in addition to diet) to deplete glycogen (22, 23), a twofold difference in muscle glycogen levels was still reported between Lo (247 ± 15 mmol glucose/kg dry muscle) and Hi (548 ± 42 mmol glucose/kg dry muscle). The various effects on fuel selection and plasma metabolite concentrations found in this study are also consistent with large changes in glycogen levels (Figs. 5 and 6, Tables 4 and 5). Before cold exposure, we show a complete shift from lipid and protein oxidation for Lo (40% \(H_{\text{prod}}\) each, for lipids and proteins) to CHO-based metabolism for Hi (80% \(H_{\text{prod}}\) accounted for by CHO). This large difference in total CHO oxidation (20 to 80% \(H_{\text{prod}}\)) was almost entirely caused by changes in \(R_{\text{ox-mus}}\) (30.7 ± 21.0 mg/min for Lo and 210.1 ± 24.0 mg/min for Hi) because \(R_{\text{ox-plasma}}\) remained minor in both groups. Together, these results show that the protocol selected for our study had the anticipated effects on glycogen reserves.

**Same heat production, very different fuel mixtures.** The contributions of all oxidative fuels to \(H_{\text{prod}}\) before and after cold exposure, as well as the percent changes caused by shivering, are summarized in Fig. 7 for Lo, N (18), and Hi. Altering glycogen reserves had a major effect on fuel selection before but also during shivering (Figs. 5 and 6, Table 5). In the cold, \(H_{\text{prod}}\) was unequally shared among lipids (53% \(H_{\text{prod}}\)), CHO (28% \(H_{\text{prod}}\)), and proteins (19% \(H_{\text{prod}}\)) in Lo, but the pattern of fuel selection was widely different for Hi: CHO (65% \(H_{\text{prod}}\)), lipids (23% \(H_{\text{prod}}\)), and proteins (12% \(H_{\text{prod}}\)). These drastic differences in oxidative fuel selection had no impact on \(H_{\text{prod}}\) because \(H_{\text{prod}}\) was the same in both groups. Similar results have been observed at higher shivering intensities (~3.5 times resting metabolic rate for men immersed at 18°C for up to 90 min) when large changes in substrate utilization only had a minor (22) or no effect (43) on \(H_{\text{prod}}\).

However, some discrepancies in the relative contributions of CHO and lipids between these studies and ours still remain. Because oxidation rates reported in these previous papers were not corrected for protein oxidation, we have recalculated our values using nonprotein respiratory exchange ratios for comparison. After this adjustment, CHO and lipid oxidation rates were found to be consistent in all studies for Lo, but not for Hi. A greater reliance on CHO was observed in Young et al. (43) and our study (65% \(H_{\text{prod}}\)) than in Martineau and Jacobs (22), respectively.

![Fig. 7. Relative effects of prolonged low-intensity shivering on the contribution of protein, lipid, plasma glucose, and muscle glycogen to total heat production in men with low, normal, and high CHO reserves. Relative changes from baseline were calculated from oxidation rates measured before and during the last 30 min of cold exposure. NC, no significant change. N, normal CHO subjects. *Values taken from Ref. 18.](http://www.jap.org)
observed in Hi, for which cold exposure caused RF ox to oxidation was observed by Tikuisis et al. during prolonged after 2 ho of shivering (in fact, a progressive increase in fat cant shift in fuel selection to spare glycogen takes place and, consequently, less muscle glycogen and less plasma glu
cose than men (see Ref. 31 for review). Similarly, during low-intensity shivering (~1.8 times resting metabolic rate), lipid oxidation was reported to be significantly higher in women than men (64 vs. 53% H_{prod}) (28), suggesting that women may show higher glycogen sparing and, possibly, longer survival in the cold. However, no such gender differ
cence in fuel use was found during high-intensity shivering (34). Future work should address whether plasma glucose and mus
cle glycogen oxidation are different in shivering males and females.

In conclusion, this study shows that large changes in glyco
gen reserves have no effect on thermogenesis during sustained shivering in men. Total CHO oxidation provides 65% of the heat in glycogen-depleted individuals (50% for muscle glycogen and 15% for plasma glucose) but only 27% in glycogen-depleted subjects (20% for glycogen and 7% for plasma glucose). We show that heat production of glycogen-depleted individuals is not compromised, because protein and lipid oxidation are both stimulated to compensate for the reduced contribution from CHO. Depletion of CHO reserves reduces the relative use of plasma glucose by shivering muscles even below that of subjects with normal CHO reserves. This study provides clear evidence that proteins can play a significant thermogenic role when CHO reserves are depleted (19% H_{prod}). Finally, EMG signals from shivering muscles were also collected in the present experiments, and the companion article investigates the patterns of muscle fiber recruitment in relation to the large changes in fuel selection reported here.

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