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Effect of cold exposure on fuel utilization in humans: plasma glucose, muscle glycogen, and lipids

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Haman, François, François Péronnet, Glen P. Kenny, Denis Massicotte, Carole Lavoie, Chris Scott, and Jean-Michel Weber. Effect of cold exposure on fuel utilization in humans: plasma glucose, muscle glycogen, and lipids. *J Appl Physiol* 93: 77–84, 2002. First published March 1, 2002; 10.1152/japplphysiol.00773.2001.—The relative roles of circulatory glucose, muscle glycogen, and lipids in shivering thermogenesis are unclear. Using a combination of indirect calorimetry and stable isotope methodology ([U-^{13}C]glucose ingestion), we have quantified the oxidation rates of these substrates in men acutely exposed to cold for 2 h (liquid conditioned suit perfused with 10°C water). Cold exposure stimulated heat production by 2.6-fold and increased the oxidation of plasma glucose from 39.4 ± 2.4 to 93.9 ± 5.5 mg/min (+138%), of muscle glycogen from 126.6 ± 7.8 to 264.2 ± 36.9 mg glucosyl units/min (+109%), and of lipids from 46.9 ± 3.2 to 176.5 ± 17.3 mg/min (+376%). Despite the observed increase in plasma glucose oxidation, this fuel only supplied 10% of the energy for heat generation. The major source of carbohydrate was muscle glycogen (75% of all glucose oxidized), and lipids produced as much heat as all other fuels combined. During prolonged, low-intensity shivering, we conclude that total heat production is unequally shared among lipids (50%), muscle glycogen (30%), plasma glucose (10%), and proteins (10%). Therefore, future research should focus on lipids and muscle glycogen that provide most of the energy for heat production.

energy metabolism; shivering thermogenesis; heat loss; plasma glucose oxidation; stable isotopes

DURING ENVIRONMENTAL COLD exposure in adult humans, a decrease in core temperature is prevented by increasing heat production ($\dot{H}_{prod}$) via shivering thermogenesis. Involuntary muscle contractions during shivering are mainly fueled by carbohydrates (CHO) and lipids, whereas the contribution of protein oxidation remains minor (~10%) (16, 36, 42). However, the respective importance of CHO and lipid oxidation has not been clearly established. For example, some researchers imply that CHO is the preferred fuel in the cold (~60% of total $\dot{H}_{prod}$) (12, 13, 20, 36, 37, 39, 41–43), whereas others show a greater reliance on lipids (~60% of total $\dot{H}_{prod}$) (21–23, 33, 45). Possible reasons for such discrepancies between studies are differences in shivering intensity (but see DISCUSSION), cooling protocol, and/or nutritional state. Over the last decade, most studies of fuel selection during shivering have focused on CHO metabolism as a probable limiting factor for $\dot{H}_{prod}$. However, two important issues remain unresolved: *1*) the relative contributions of hepatic glucose and muscle glycogen to total CHO oxidation have not been quantified, and *2*) the potentially important role of triacylglycerol stores (adipose tissue, liver, and muscle) has often been underrated, particularly during prolonged, low-intensity shivering.

Plasma glucose and muscle glycogen have both been shown to play significant roles in $\dot{H}_{prod}$ during cold exposure (16). Vallerand et al. (42, 43) calculated that plasma glucose and muscle glycogen would contribute equally to total CHO oxidation, assuming that 100% of hepatic glucose production (R_a Glu) is oxidized. However, as pointed out by these authors, at such low rates of oxygen consumption ($\dot{V}O_2$), this assumption is probably not met because nonoxidative glucose disposal could be important. Studies in which measurements of R_a Glu and plasma glucose oxidation were carried out simultaneously indicate that nonoxidative disposal ranges between 25% R_a Glu during submaximal exercise [45% maximal $\dot{V}O_2$ ($\dot{V}O_{2\,max}$)] and 70% R_a Glu at rest (11).

A series of studies have shown that 90 min of cold-water immersion cause a reduction of glycogen concentration in biopsies from the vastus lateralis (17, 21–24, 47). Unfortunately, estimating total use of muscle glycogen, at the whole organism level, from small biopsy samples is inaccurate at best, because *1*) glycogen content varies greatly between and within individual muscles and *2*) the specific muscles involved in heat

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generation and their level of recruitment are unknown. Therefore, the exact contributions of plasma glucose and muscle glycogen are presently unclear because their rates of oxidation have never been measured directly during shivering.

The purpose of this study was to quantify the respective contributions of plasma glucose, muscle glycogen, and lipid oxidation to total $\dot{H}_{prod}$ during prolonged, low-intensity shivering by using a combination of stable isotope and indirect calorimetry methods. In human subjects exposed to low-intensity shivering [10°C for 2 h with a liquid conditioned suit (LCS)], we hypothesize that plasma glucose oxidation will play a lesser role than previously suggested (42, 43) and that lipid oxidation will be a major pathway for heat generation because of the small change in metabolic rate observed in the cold.

METHODS

Subjects. Six healthy and trained men volunteered for this study, which was approved by the Health Sciences Ethics Committee of the University of Ottawa, and written consent was obtained from the participants. Percent body fat [underwater weighing; Brosek et al. (5)] and $\dot{V}O_{2\,max}$ were measured with a progressive treadmill protocol 5–7 days before the experiments. Physical characteristics of the subjects are presented in Table 1.

Experimental protocol. Experiments were conducted between 900 and 1300 after 36 h without heavy physical activity. The last evening meal was standardized (~988 kcal, ~52% CHO, ~18% lipids, ~30% proteins), and subjects were asked to report to the laboratory the next morning (900) after a 12- to 14-h fast. Ingestion of CHO from plants naturally rich in ^{13}C (C_4 photosynthetic cycle) was avoided to maintain low ^{13}C background enrichment in plasma glucose and expired CO_2. Care was taken to minimize thermal stimuli between awakening and the start of the experiment (i.e., avoid exposure to hot or cold temperatures, very-low-intensity exercise during transit from home to the laboratory). On their arrival in the laboratory, subjects were instrumented with thermal probes and an indwelling catheter (18-gauge, 32 mm, Medical, Arlington, TX) placed in an antecubital vein (left arm) for blood sampling and were fitted with a LCS (Three Piece Delta Temax, Pembroke, ON). Subjects were then asked to empty their bladder [time (t) = 0 min] and sit quietly for 2 h at 28.1 ± 0.3°C (758 ± 4 mmHg, 20–30% relative humidity). After this habituation period, they were transferred to an environmental chamber (11.1 ± 0.1°C, 760 ± 4 mmHg, 40–57% relative humidity), and a 10°C water perfusion was started through the LCS by using a temperature-controlled circulation bath (Endocal, NESLAB; and model 200–00, Micropump, Vancouver, WA). Thermal response, metabolic rate, and fuel utilization were measured at 28°C and during the subsequent 2-h cold exposure.

Table 1. *Physical characteristics of subjects*

Age, yr	24.7 ± 1.5
Body mass, kg	78.1 ± 4.8
Height, cm	178.2 ± 2.5
Body surface area, m^2	2.0 ± 0.07
Body fat, %	13.3 ± 1.9
$\dot{V}O_{2\,max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	56.4 ± 2.9

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

Thermal response. Central body temperature [esophageal temperature (T_{es})] was monitored continuously by using a pediatric T_{es} probe (Mon-a-therm general purpose, Mallinckrodt Medical, St. Louis, MO), which was inserted through the nose to a depth placing the tip of the thermocouple at the level of the left atrium, or one-fourth of the standing height of the subject (25). Heat flux transducers (Concept Engineering, Old Saybrook, CT) were used to estimate skin temperature and nonevaporative heat flux from the forehead, chest, biceps, forearm, abdomen, lower and upper back, front and back calf, quadriceps, hamstrings, and finger. Mean skin temperature (T_{sk}) and mean heat flux were calculated with an area-weighted equation (8). Heat flux measurements were used to calculate whole body radiative ($\dot{R}$) and convective heat exchange ($\dot{C}$). Respiratory evaporative ($\dot{E}_{resp}$) and convective heat exchanges ($\dot{C}_{resp}$) were determined from ventilation ($\dot{V}E$) measurements by estimating water loss via the respiratory tract (2,411.3 J heat/g evaporated water) (4). It was assumed that evaporative heat loss ($\dot{H}_{loss}$) from the skin was negligible under the LCS. Whole body $\dot{H}_{loss}$ (in watts) was calculated as follows

$$\dot{H}_{loss} = (\dot{R} + \dot{C}) + (\dot{E}_{resp} + \dot{C}_{resp}) \quad (1)$$

Metabolic rate and fuel utilization. $\dot{V}E$, $\dot{V}O_2$, and carbon dioxide production ($\dot{V}CO_2$) 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 at 28°C and during cold exposure. $\dot{V}E$ (l/min, BTPS) was calculated from the displacement of the cylinder and corrected for temperature and pressure. Oxygen and carbon dioxide concentrations in dry expired gases ($CaSO_4$, Drierite, 8 mesh, Fisher Scientific, Ottawa, ON) were determined directly from the spirometer by using calibrated electrochemical gas analyzers (AMETEK model S-3A/1 and CD 3A, Applied Electrochemistry, Pittsburgh, PA).

Total CHO (RG_{ox}), lipid (RF_{ox}), and protein oxidation rates (RP_{ox}) were calculated with the following equations (19)

$$RG_{ox}\,(g/min) = 4.59\,\dot{V}CO_2\,(l/min) - 3.23\,\dot{V}O_2\,(l/min) \quad (2)$$

$$RF_{ox}\,(g/min) = -1.70\,\dot{V}CO_2\,(l/min) + 1.70\,\dot{V}O_2\,(l/min) \quad (3)$$

$$RP_{ox}\,(g/min) = 2.9 \times Urea_{urine}\,(g/min) \quad (4)$$

where $\dot{V}CO_2$ and $\dot{V}O_2$ (*Eqs. 2* and *3*) were corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1.010 and 0.843 l/g, respectively), and $Urea_{urine}$ is urinary urea excretion.

Estimates of RP_{ox} (*Eq. 4*) were made by measuring $Urea_{urine}$ from urine samples collected for a period of 120 min at 28 and 10°C. A correction for urea accumulation in plasma was not required because plasma levels did not change during cold exposure (P = 0.28; paired t-test) (18). Urinary and plasma urea concentrations were determined on a Synchron Clinical System (CX7, Beckman, Anaheim, CA). Respective contributions of glucose, lipid, and protein oxidation to total $\dot{H}_{prod}$ were calculated by using energy potentials of 16.3, 40.8, and 19.7 kJ/g, respectively (9, 28).

Plasma glucose oxidation. For the measurement of plasma glucose oxidation, the subjects ingested 10 g of glucose [7 × 1.4 g in 100 ml of water; corn sugar, with a ratio of ^{13}C to C

($^{13}C/C$) = 0.01098] artificially enriched with ^{13}C ([U-^{13}C]glucose, $^{13}C/C$ >99%, Isotec, Miamisburg, OH) to obtain a final $^{13}C/C$ of 0.0476 isotopic composition of exogenous glucose solution (R_{exo}).

After baseline $^{13}C/^{12}C$ in plasma and expired CO_2 (t = 30 min) were measured, subjects ingested the first dose of [^{13}C]glucose. Subsequent doses were then taken every 30 min until the end of 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 analysis.

The isotopic composition of plasma glucose (R_{glu}) was measured as previously described (29). Briefly, plasma samples (1 ml) were deproteinized (BaOH: 1.5 ml, 0.3 N; and $ZnSO_4$: 1.5 ml, 0.3 N) and centrifuged to precipitate the proteins. Double-bed ion exchange chromatography with superimposed columns (resins: AG 50W-X8 H^+, 200–400 mesh, and AG 1-X8 chloride, 200–400 mesh) was used to isolate plasma glucose. After evaporation, 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 and in CO_2 obtained from glucose combustion were determined in a prism mass spectrometer (VG, Manchester, UK). Isotopic composition was expressed as ‰ difference (δ) compared with Pee Dee Belemnitella-1 (PDB-1) Chicago standard with the equation of Craig (6)

$$\delta^{13}C‰ = \{[(^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{standard})] - 1\} \times 10^3 \quad (5)$$

The rate of exogenous glucose oxidation ($RG_{ox\text{-}exo}$, g/min) was estimated from isotopic composition of expired CO_2 (R_{exp}) and R_{exo} as follows (26)

$$RG_{ox\text{-}exo}\ (g/min) = \dot{V}CO_2 \times (R_{exp} - R_{ref}/R_{exo} - R_{ref})(1/k1 \cdot k2) \quad (6)$$

where $\dot{V}CO_2$ is in l/min (STPD), R_{ref} is the isotopic composition of expired CO_2 at 28°C 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 (28), and $k2$ is the fractional recovery at the mouth of CO_2 produced in tissues. A fractional recovery of $^{13}CO_2$ at the mouth ($k2$) of 0.8 and 1 was used at 28°C and during cold exposure, respectively (46). Because of the large size of the bicarbonate pool, only values in the last 30 min at 28°C and at 10°C were used in the calculation of plasma glucose oxidation rate ($RG_{ox\text{-}plasma}$). This delay allows sufficient time for equilibrium of the $^{13}C/^{12}C$ to be attained in the bicarbonate pool (27).

Plasma glucose oxidation was calculated from $^{13}CO_2$ excretion and the isotopic enrichment of plasma glucose by using the following equation (7, 46)

$$RG_{ox\text{-}plasma} = \dot{V}CO_2\ (R_{exp} - R_{ref}/R_{glu} - R_{ref})(1/k1 \cdot k2) \quad (7)$$

Oxidation of glucose released from the liver ($RG_{ox\text{-}liver}$) was estimated by subtracting the low rate of $RG_{ox\text{-}exo}$ from $RG_{ox\text{-}plasma}$. Calculation of glucose oxidation derived from glycogen stores ($RG_{ox\text{-}mus}$; g/min) in the tissues, either directly or through the lactate shuttle (3), was calculated by subtracting $RG_{ox\text{-}plasma}$ (*Eq. 8*) from RG_{ox} (*Eq. 2*)

$$RG_{ox\text{-}mus} = RG_{ox} - RG_{ox\text{-}plasma} \quad (8)$$

Blood analysis. Plasma glucose and lactate concentrations were measured spectrophotometrically at 340 nm on a Beckman DU 640 (2), whereas total plasma nonesterified fatty acid (NEFA) concentration was determined by using an analytic assay kit (NEFA C, Wako Chemicals, Osaka, Japan). Insulin concentration was measured by using a radioimmunoassay (#KTSP-11001, Medicorp, Montréal, PQ).

Statistical analyses. Overall changes in T_{es}, $\bar{T}_{sk}$, $\dot{H}_{loss}$, $\dot{H}_{prod}$, blood metabolite concentrations, expired CO_2 and plasma glucose isotopic enrichments, and gas exchange over time were assessed by using a one-way ANOVA with replication. For each sampling time, a Bonferroni t-test was used to detect potential differences with control values observed at 28°C. Differences in metabolic fuel utilization for CHO (RG_{ox}, $RG_{ox\text{-}exo}$, $RG_{ox\text{-}plasma}$, $RG_{ox\text{-}mus}$, $RG_{ox\text{-}liver}$), lipids (RF_{ox}), and proteins (RP_{ox}) over the last 30 min at 28°C and during cold exposure were determined by using two-tailed paired t-tests. Statistical differences were considered significant when $P \leq 0.05$. All values given are means ± SE (n = 6).

RESULTS

Thermal response. Changes in T_{es} and $\bar{T}_{sk}$ are presented in Fig. 1. Whereas T_{es} remained constant at 36.4 ± 0.1°C throughout the experiment, $\bar{T}_{sk}$ decreased from 34.0 ± 0.02 to 27.2 ± 0.02°C in the initial 90 min of cold exposure and did not change for the last 30 min. Absolute $\dot{H}_{loss}$ and $\dot{H}_{prod}$ increased by a maximum of 3.3-fold (77.7 ± 0.6 to 258.4 ± 10.6 W) and 2.6-fold (95.3 ± 2.2 to 243.8 ± 4.2 W), respectively (Fig. 2). After reaching a maximum 20 min after the onset of cold exposure (t = 140 min), $\dot{H}_{loss}$ decreased by 16% over the next 100 min (238.3 ± 0.6 W). Maximal $\dot{H}_{prod}$ was reached after 90 min of cold exposure and stayed constant for the remainder of the experiment. Observed shivering activity appeared minimal over the first 60 min of cold exposure but increased progressively in the last hour.

Metabolic response and fuel utilization. Changes in $\dot{V}E$, $\dot{V}O_2$, and respiratory exchange ratio (RER) are shown in Fig. 3. $\dot{V}E$ and $\dot{V}O_2$ increased by 2.4- and 2.6-fold, respectively (Fig. 3, *A* and *B*). A small decrease in RER was observed during cold exposure, but it did not reach overall statistical significance (one-way ANOVA with replication; P = 0.075), averaging 0.84 ±

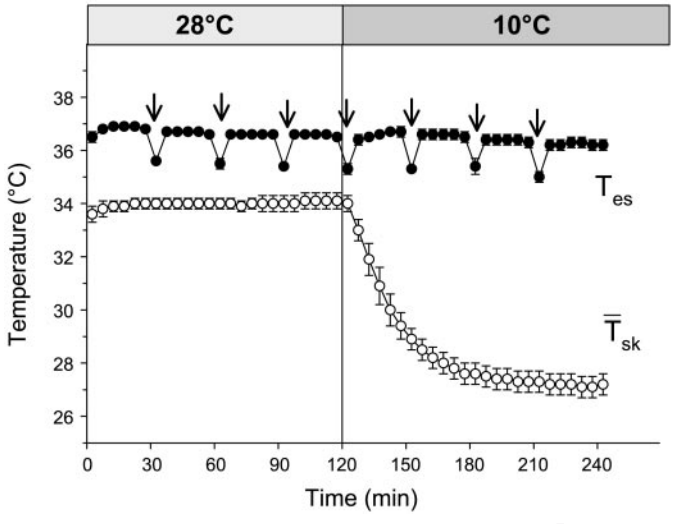

Fig. 1. Esophageal (T_{es}) and mean skin temperature ($\bar{T}_{sk}$) at 28°C and during whole body 10°C cold exposure. Arrows indicate the times at which [^{13}C]glucose solutions were ingested. Values are means ± SE.

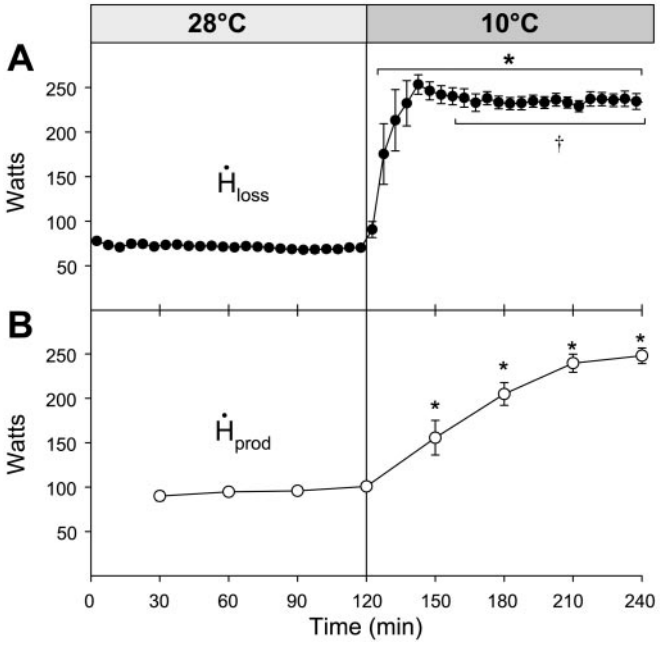

Fig. 2. Absolute heat loss ($\dot{H}_{loss}$; *Eq. 1*; *A*) and heat production ($\dot{H}_{prod}$; *B*) at 28°C and during whole body 10°C cold exposure. Values are means ± SE. *Significantly different from values at 28°C, $P \leq 0.05$. †Significantly different from maximal value reached during cold exposure, $P \leq 0.05$.

0.01 throughout the experiment (Fig. 3*C*; 0.86 ± 0.01 at 28°C and 0.83 ± 0.02 between 150 and 180 min at 10°C).

RF_{ox}, RG_{ox}, and RP_{ox} and their respective contributions to total $\dot{H}_{prod}$ throughout cold exposure are plotted in Fig. 4. Total lipid and CHO utilization increased 3.8-fold (Fig. 4*A*; 39 ± 2 mg fatty acids/min at 28°C to 177 ± 17 mg fatty acids/min at 10°C) and 2.2-fold (Fig. 4*B*; 165 ± 9 mg glucose/min at 28°C to 358 ± 41 mg glucose/min at 10°C), respectively. Protein utilization was not affected significantly by the change in temperature and averaged 62.1 ± 3.1 mg/min at 28°C and 77.7 ± 5.0 mg/min at 10°C. A trend toward an increase in the relative contribution of lipids and a decrease in the relative contribution of CHO during cold exposure was noticed in the cold. However, these observed changes failed to reach statistical significance (Fig. 4*B*; $P = 0.10$). In contrast, the relative contribution of protein oxidation to total $\dot{H}_{prod}$ decreased significantly throughout cold exposure, from 21.2 ± 0.8% at 28°C to 10.7 ± 0.8% at 10°C.

Plasma concentrations. Changes in plasma concentrations of insulin, glucose, lactate, and NEFA during cold exposure are presented in Fig. 5. Insulin and glucose concentrations were not affected by the change in temperature (Fig. 5, *A* and *B*). After 90 min of cold exposure, plasma lactate and NEFA concentrations were increased 1.8-fold (0.90 ± 0.11 to 1.61 ± 0.15 mM, Fig. 5*C*) and 2.5-fold (0.21 ± 0.03 to 0.52 ± 0.01 mM, $P < 0.001$; Fig. 5*D*), respectively, over control values at 28°C.

CHO oxidation: plasma glucose vs. muscle glycogen. The amount of [^{13}C]glucose administered provided a strong signal in CO_2 and plasma glucose to quantify circulatory glucose oxidation (Fig. 6, *A* and *B*). The change in isotopic enrichment of expired CO_2 and plasma glucose ([δ-^{13}C]PDB-1), as well as the calculated values of $RG_{ox\text{-}plasma}$ and $RG_{ox\text{-}exo}$ obtained throughout the experiment are plotted in Fig. 6. At 28°C, 60 min after glucose ingestion, $RG_{ox\text{-}plasma}$ averaged 39.4 ± 2.4 mg/min ($RG_{ox\text{-}liver}$ was 37.7 ± 2.3 mg/min and $RG_{ox\text{-}exo}$ only 2.1 ± 0.4 mg/min) and increased progressively throughout cold exposure to reach a maximal value of 107.3 ± 6.1 mg/min ($RG_{ox\text{-}liv}$ was 84.0 ± 6.0 mg/min and $RG_{ox\text{-}exo}$ only 9.6 ± 5.5 mg/min; Fig. 6*C*).

Table 2 summarizes average values measured at 28°C (90–120 min) and 10°C (210–240 min) for all of the parameters of fuel utilization estimated in this study ($RG_{ox\text{-}plasma}$, $RG_{ox\text{-}mus}$, RF_{ox}, and RP_{ox}). Expired CO_2 and plasma glucose isotopic composition did not change significantly over the last 30 min at 28°C (t = 90 and 120 min) and over the last 30 min at 10°C (t = 210 and 240 min) (ANOVA, $P > 0.05$). However, Fig. 6, *A* and *B*, suggest that priming of the bicarbonate pool

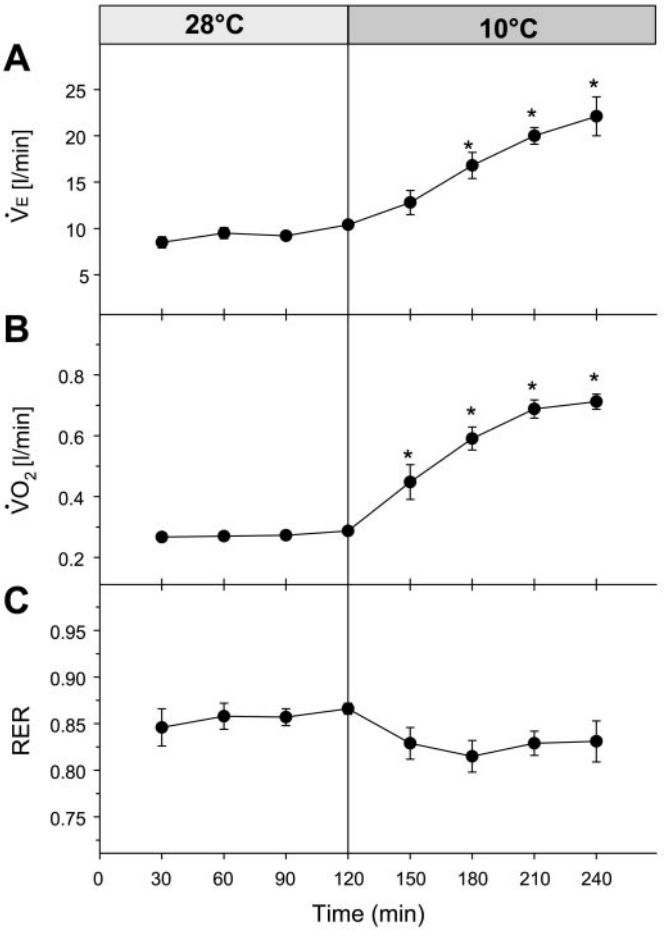

Fig. 3. Ventilation ($\dot{V}_E$, in l/min, BTPS; *A*), absolute oxygen consumption ($\dot{V}O_2$, in l O_2/min, STPD; *B*), and respiratory exchange ratio (RER; *C*) at 28°C and during whole body 10°C cold exposure. Values are means ± SE. *Significantly different from values at 28°C, $P \leq 0.05$.

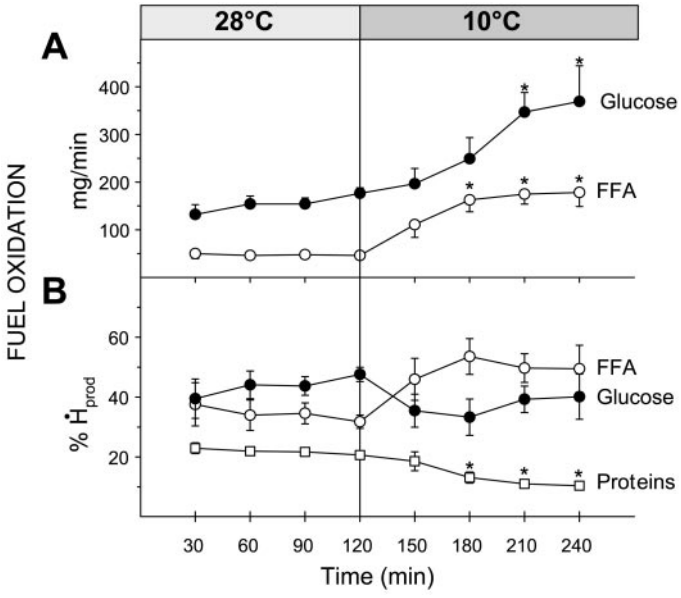

Fig. 4. Glucose (●, *Eq. 2*) and lipid (○, *Eq. 3*) utilization rates (protein oxidation was constant at 62.1 ± 3.1 mg/min at 28°C and 77.7 ± 5.0 mg/min at 10°C; *A*), as well as their relative contribution to total $\dot{H}_{prod}$ (%$\dot{H}_{prod}$; proteins, □; *B*) at 28°C and during whole body 10°C. FFA, free fatty acid. Values are means ± SE. *Significantly different from values at 28°C, $P \leq 0.05$.

with $NaH^{13}CO_3$ would have produced a better isotopic steady state. It is, therefore, possible that $RG_{ox\text{-}plasma}$ was slightly underestimated. As a result of cold exposure, $RG_{ox\text{-}plasma}$ and $RG_{ox\text{-}mus}$ increased by 2.1- and 2.4-fold, respectively. Although RF_{ox} increased by as much as 3.8-fold at 10°C, RP_{ox} remained constant throughout the experiment. The relative contributions of $RG_{ox\text{-}plasma}$ and $RG_{ox\text{-}mus}$ to total $\dot{H}_{prod}$ did not change when subjects were exposed to the cold, whereas that of RF_{ox} increased 1.5-fold and that of RP_{ox} decreased twofold.

DISCUSSION

Even though plasma glucose oxidation is strongly stimulated during low-intensity shivering (+138%), we show that this fuel only plays a minor role in total $\dot{H}_{prod}$ (10% $\dot{H}_{prod}$). Also, muscle glycogen oxidation doubled during mild cold exposure, providing 75% of total CHO oxidized. Interestingly, lipids are the most important fuel, showing close to a fourfold increase in oxidation rate and accounting for the production of as much heat as all other metabolic substrates combined.

This study quantifies the $RG_{ox\text{-}plasma}$ during cold exposure. It shows that $RG_{ox\text{-}plasma}$ is stimulated in direct proportion to metabolic rate (Table 2, Fig. 3*B*) and that its relative contribution to total $\dot{H}_{prod}$ remains constant and low. During low-intensity shivering, the contribution of plasma glucose is as minor as that of proteins (Table 2). In contrast, muscle glycogen stores play a more prominent role, providing three times more glucose units for oxidation than the circulation (Table 2).

The [^{13}C]glucose ingestion technique selected for this study allowed us to quantify the role of circulatory glucose as an oxidative fuel to support shivering. Results show that, during mild cold exposure, circulatory glucose plays a more minor role than previously suggested when oxidation was estimated from measurements of R_a Glu (42, 43). As anticipated, neglecting to subtract nonoxidative glucose disposal from R_a Glu caused a significant overestimation of glucose oxidation rates. Under conditions of steady state (i.e., when plasma glucose concentration remains constant over time), R_a Glu and glucose disposal were matched. However, the disposal of glucose can take place through two distinct metabolic pathways: oxidation ($RG_{ox\text{-}plasma}$) and storage (or nonoxidative disposal). At low metabolic rates (rest, mild exercise, or low-intensity shivering), nonoxidative disposal represents a significant fraction of R_a Glu, and, therefore, a direct measurement of oxidation is necessary to quantify the role of plasma glucose as an oxidative fuel.

The stimulation of circulatory glucose utilization in the cold was not accompanied by changes in plasma glucose or insulin concentrations (Fig. 5, *A* and *B*), as previously observed in several other studies (23, 34, 38, 42, 43). Constant glycemia shows that R_a Glu and glucose disposal were both increased in parallel. The increase in glucose uptake taking place during cold exposure is, therefore, not dependent on changes in plasma insulin. However, it may be the consequence of a cold-induced increase in insulin sensitivity and/or

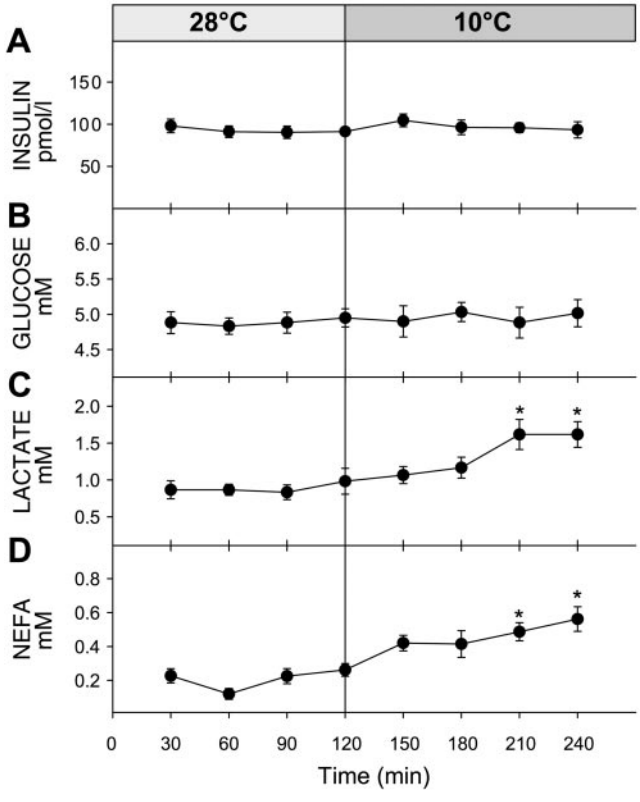

Fig. 5. Plasma insulin (*A*), glucose (*B*), lactate (*C*), and nonesterified fatty acid (*D*) concentrations at 28°C and during whole body 10°C cold exposure. Values are means ± SE. *Significantly different from values at 28°C, $P \leq 0.05$.

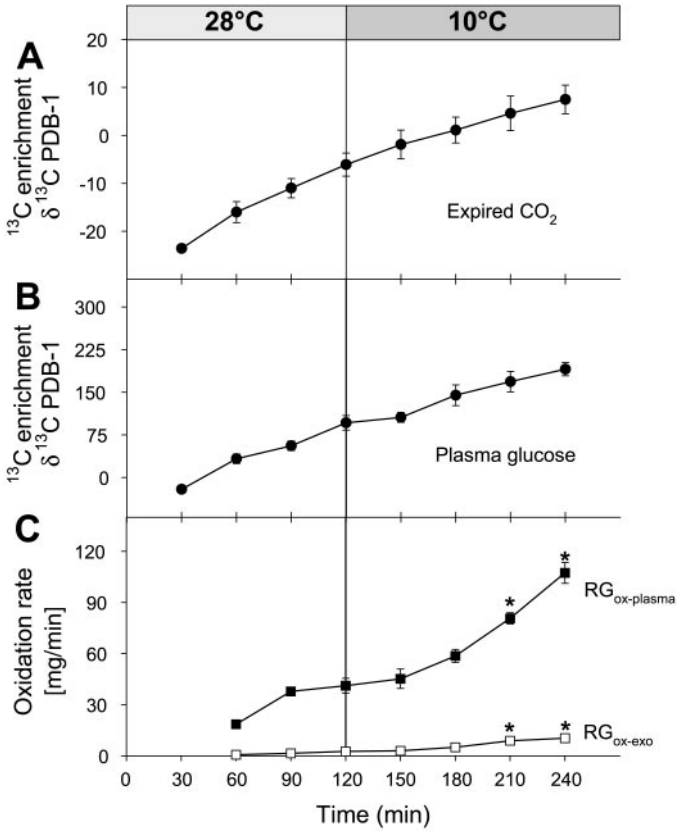

Fig. 6. Expired CO_2 (*A*) and plasma glucose (*B*) ^{13}C enrichment [δ^{13}C-to-^{12}C ratio, Pee Dee Belemnitella-1 (PDB-1)] and calculated plasma glucose ($RG_{ox\text{-}plasma}$) and exogenous glucose oxidation rates ($RG_{ox\text{-}exo}$) (*C*) measured at 28°C and during whole body 10°C cold exposure. Values are means ± SE. *Significantly different from values at 28°C, $P \leq 0.05$.

GLUT translocation, as previously proposed for humans (35, 40) as well as animals (32, 44). An insulin-independent control of glucose uptake may allow an increase in glucose delivery specifically to shivering muscles rather than indiscriminately to all insulin-sensitive tissues.

The major source of CHO was muscle glycogen, providing three-fourths of all of the glucose oxidized in the cold (Table 2). Even though whole body glycogen stores only represent 1% of total energy stores, shivering studies in humans have shown that glycogen availability, modified through diet or exercise, affects fuel selection (22, 33, 47) and possibly body cooling rate (22). In these studies, whereas $\dot{H}_{prod}$ was the same among depleted glycogen, loaded glycogen, and normal glycogen controls immersed in 18°C water, RER values were significantly lower for depleted glycogen than for loaded glycogen and glycogen controls, indicating a compensatory shift to a greater relative use of lipids when glycogen reserves are depleted (22, 33, 47). However, these studies do not provide information on the effect of glycogen availability on the relative importance of $RG_{ox\text{-}plasma}$ and $RG_{ox\text{-}mus}$ to total CHO oxidation.

Oxidizing lipids to generate heat. The dual role of lipids as a heat insulation layer and as a large, energy-dense metabolic fuel (>95% of total energy stored) has been recognized for a long time (31). However, the quantitative importance of lipids as a substrate to support prolonged, low-intensity shivering has been somewhat neglected because, over the last decade, most studies have focused on CHO-dependent $\dot{H}_{prod}$ (16). Our results show that RF_{ox} provides 50% of all of the heat produced (Table 2). The relative importance of lipid oxidation measured here is consistent with several studies (15, 21–23, 33, 45), whereas many others found that CHO oxidation is dominant (12, 13, 20, 36, 37, 39, 41–43). In all of these studies, it is very interesting to note that the reported dominance of either CHO or lipids has no clear link with differences in shivering intensity but seem to be correlated with the cooling protocol. Whereas subjects exposed to cool air used CHO preferentially (~60% of $\dot{H}_{prod}$; Refs. 12, 13, 20, 36, 37, 39–43), those cooled by water immersion or by LCS favored lipid utilization (~60% of $\dot{H}_{prod}$; Refs. 15, 21–23, 33, 45). Physiological reasons for such a difference are unclear, and further research will be needed to explain it.

If one considers the relatively low metabolic rates reached here during mild shivering (15–20% $\dot{V}O_{2\,max}$), the observed utilization of lipids is not that surprising. Exercise studies reveal that lipid oxidation predominates for prolonged work at all intensities <50% $\dot{V}O_{2\,max}$ (1, 4, 30). Therefore, even at the highest possible metabolic rates reached during maximum shivering [~5 times resting metabolic rate or ~40% $\dot{V}O_{2\,max}$ (10)], lipids may still play a significant role in heat generation, if fuel selection patterns are identical between exercise and shivering.

The large increase in lipid utilization observed here during low-intensity shivering (Table 2) is a strategy to spare limited CHO reserves. Any increase in the relative use of lipids allows the maintenance of $\dot{H}_{prod}$ for longer and, therefore, improves chances of survival in

Table 2. *Absolute oxidation and relative contributions of plasma glucose, muscle glycogen, lipid, and protein oxidation to total heat production at 28°C (90–120 min) and quasi-steady state at 10°C (210–240 min)*

	28°C	10°C
Plasma glucose ($RG_{ox\text{-}plasma}$)		
mg/min	39.4 ± 2.4	93.9 ± 5.5*
$\%\dot{H}_{prod}$	10.7 ± 0.5	10.5 ± 0.9
Muscle glycogen ($RG_{ox\text{-}mus}$)		
mg/min	126.6 ± 7.8	264.2 ± 36.9*
$\%\dot{H}_{prod}$	35.0 ± 1.9	29.2 ± 3.8
Lipids (RF_{ox})		
mg/min	46.9 ± 3.2	176.5 ± 17.3*
$\%\dot{H}_{prod}$	33.2 ± 2.0	49.6 ± 4.4*
Proteins (RP_{ox})		
mg/min	62.1 ± 3.1	77.7 ± 5.0
$\%\dot{H}_{prod}$	21.0 ± 0.8	10.6 ± 0.8*

Values are means ± SE. Absolute oxidation values are in mg/min; relative contributions are in percent heat production ($\%\dot{H}_{prod}$). $RG_{ox\text{-}plasma}$, plasma glucose oxidation rate (*Eq. 7*); $RG_{ox\text{-}mus}$, rate of glucose oxidation derived from muscle glycogen (*Eq. 8*); RF_{ox}, lipid oxidation rate (*Eq. 3*); RP_{ox}, protein oxidation rate (*Eq. 4*). *Significantly different from values at 28°C, paired *t*-test, $P \leq 0.05$.

the cold. We can estimate theoretical values for maximum cold endurance under the conditions of our experiments, assuming that the relative use of the different fuels remains the same as measured after 2 h of mild shivering. An average adult man would be able to shiver at 245 W (Fig. 2) for ~20 h under the specific conditions of the present study before depleting muscle glycogen reserves (assuming that 80% of muscle glycogen is available for oxidation; mean muscle glycogen concentration = 100 mmol glucosyl units/kg wet mass; actively shivering muscle mass = 70% of 36 kg; $RG_{ox\text{-}mus}$ = 18.4 $\mu mol \cdot kg\ body\ mass^{-1} \cdot min^{-1}$; Table 2).

The stimulation of RF_{ox} (Table 2) found here was accompanied by a twofold increase in circulatory NEFA levels (Fig. 5*D*) as observed in other studies (34, 37, 38, 42, 43, 45). Vallerand et al. (43) found that men exposed to 5°C for 3 h showed a parallel increase in NEFA concentration and NEFA disappearance rate. Together, these observations suggest that the oxidation of circulatory NEFA increased throughout cold exposure. However, the relative contributions of NEFA from the circulation (adipose and liver) and from muscle triacylglycerols to the fourfold increase in total fat oxidation remain to be established.

In conclusion, this study shows that total $\dot{H}_{prod}$ during prolonged, low-intensity shivering is unequally shared among lipids (50%), muscle glycogen (30%), circulatory glucose (10%), and proteins (10%). Therefore, the importance of plasma glucose oxidation is only minor, and future research should focus on lipid and muscle glycogen stores that provide most of the energy for $\dot{H}_{prod}$.

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REFERENCES

1. **Bergman BC and Brooks GA.** Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *J Appl Physiol* 86: 479–487, 1999.
2. **Bergmeyer HU** (Editor). *Methods of Enzymatic Analysis*. Weinheim, Germany: VCH, vol. 8, 1985.
3. **Brooks GA.** The lactate shuttle during exercise and recovery. *Med Sci Sports Exerc* 18: 360–368, 1986.
4. **Brooks GA, Fahey TD, White TP, and Baldwin KM.** *Exercise Physiology–Human Bioenergetics and Its Applications*. Toronto: Mayfield, 1999.
5. **Brosek JF, Grande JT, Andersen JT, and Keys A.** Densiometric analysis of body composition: review of some quantitative assumptions. *Ann NY Acad Sci* 110: 113–140, 1963.
6. **Craig H.** Isotopic standards for carbon and oxygen and correction factors. *Geochim Cosmochim Acta* 12: 133–149, 1957.
7. **Derman KD, Hawley JA, Noakes TD, and Dennis SC.** Fuel kinetics during intense running and cycling when fed carbohydrate. *Eur J Appl Physiol* 74: 36–43, 1996.
8. **Dubois D and Dubois EF.** A formula to estimate the approximate surface area if height and weight be known. *Arch Intern Med* 17: 863–871, 1916.
9. **Elia M.** Energy equivalents of CO_2 and their importance in assessing energy expenditure when using tracer techniques. *Am J Physiol Endocrinol Metab* 260: E75–E88, 1991.
10. **Eyolfson DA, Tikuisis P, Xu X, Weseen G, and Giesbrecht GG.** Measurement and prediction of peak shivering intensity in humans. *Eur J Appl Physiol* 84: 100–106, 2001.
11. **Friedlander AL, Casazza GA, Huie MJ, Horning MA, and Brooks GA.** Endurance training alters glucose kinetics in response to the same absolute, but not the same relative workload. *J Appl Physiol* 82: 1360–1369, 1997.
12. **Glickman-Weiss EL, Nelson AG, Hearon CM, Vasanthakumar SR, and Stringer BT.** Does feeding regime affect physiologic and thermal responses during exposure to 8, 20, and 27°C? *Eur J Appl Physiol* 67: 30–34, 1993.
13. **Glickman-Weiss EL, Nelson AG, Hearon CM, Windhauser M, and Heltz D.** The thermogenic effect of carbohydrate feeding during exposure to 8, 12 and 27°C. *Eur J Appl Physiol* 68: 291–297, 1994.
14. **Hagenfeldt L.** Turnover of individual free fatty acids in man. *Fed Proc* 34: 2236–2240, 1975.
15. **Jacobs I.** *Energy Metabolism in Cold-stressed Females: Implications for Predictive Modeling.* North York, Ontario: Defense and Civil Institute of Environmental Medicine, 1997.
16. **Jacobs I, Martineau L, and Vallerand AL.** Thermoregulatory thermogenesis in humans during cold stress. In: *Exercise Sport Science Review*, edited by Hollosky JO. Baltimore, MD: Williams and Wilkins, 1994, vol. 22, p. 221–250.
17. **Jacobs I, Romet TT, and Kerrigan-Brown D.** Muscle glycogen depletion during exercise at 9°C and 21°C. *Eur J Appl Physiol* 54: 35–39, 1985.
18. **Jéquier E, Acheson K, and Schutz Y.** Assessment of energy expenditure and fuel utilization in man. *Annu Rev Nutr* 7: 187–208, 1987.
19. **Livesey G and Elia M.** Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. *Am J Clin Nutr* 47: 608–628, 1988.
20. **MacNaughton K, Sathasivam P, Vallerand A, and Graham T.** Influence of caffeine on metabolic responses of men at rest in 28 and 5°C. *J Appl Physiol* 68: 1889–1895, 1990.
21. **Martineau L and Jacobs I.** Muscle glycogen utilization during shivering thermogenesis in humans. *J Appl Physiol* 65: 2046–2050, 1988.
22. **Martineau L and Jacobs I.** Muscle glycogen availability and temperature regulation in humans. *J Appl Physiol* 66: 72–78, 1989.
23. **Martineau L and Jacobs I.** Free fatty acid availability and temperature regulation in cold water. *J Appl Physiol* 67: 2466–2472, 1989.
24. **Martineau L and Jacobs I.** Effects of muscle glycogen and plasma FFA availability on human metabolism responses in cold water. *J Appl Physiol* 71: 1331–1339, 1991.
25. **Mekjavic IB and Rempel ME.** Determination of esophageal probe insertion length based on standing and sitting height. *J Appl Physiol* 69: 376–379, 1990.
26. **Mosora F, Lefèbvre PJ, Pirnay F, Lacroix M, Luyckx AS, and Duchesne J.** Quantitative evaluation of the oxidation of an exogenous glucose load using naturally labeled ^{13}C-glucose. *Metabolism* 25: 1575–1582, 1976.
27. **Pallikarakis N, Sphiris N, and Lefèbvre P.** Influence of the bicarbonate pool on the occurrence of $^{13}CO_2$ in exhaled air. *Eur J Appl Physiol* 63: 179–183, 1991.
28. **Péronnet F and Massicotte D.** Table of nonprotein respiratory quotient: an update. *Can J Sport Sci* 16: 23–29, 1991.
29. **Péronnet F, Rhéaume N, Lavoie C, Hillaire-Marcel C, and Massicotte D.** Oral [^{13}C]glucose oxidation during prolonged exercise after high- and low-carbohydrate diets. *J Appl Physiol* 85: 723–730, 1998.
30. **Roberts TJ, Weber JM, Hoppeler H, Weibel ER, and Taylor CR.** Design of the oxygen and substrate pathways. II. Defining the upper limits of carbohydrate and fat oxidation. *J Exp Biol* 199: 1651–1658, 1996.
31. **Schmidt-Nielsen K.** *Animal Physiology.* New York: Cambridge University Press, 1990.
32. **Smith OLK and Davidson SB.** Shivering thermogenesis and glucose uptake by muscles of normal and diabetic rats. *Am J Physiol Regulatory Integrative Comp Physiol* 242: R109–R115, 1982.

33. **Tikuisis P, Jacobs I, Moroz D, Vallerand AL, and Martineau L.** Comparison of thermoregulatory responses between men and women immersed in cold water. *J Appl Physiol* 89: 1403–1411, 2000.
34. **Tipton MJ, Franks GM, Meneilly GS, and Mekjavic IB.** Substrate utilisation during exercise and shivering. *Eur J Appl Physiol* 76: 103–108, 1997.
35. **Vallerand AL, Frim J, and Kavanagh F.** Plasma glucose and insulin responses to oral and intravenous glucose in cold-exposed humans. *J Appl Physiol* 65: 2395–2399, 1988.
36. **Vallerand AL and Jacobs I.** Rates of energy substrates utilization during human cold exposure. *Eur J Appl Physiol* 58: 873–878, 1989.
37. **Vallerand AL and Jacobs I.** Influence of cold exposure on plasma triglyceride clearance in humans. *Metabolism* 39: 1211–1218, 1990.
38. **Vallerand AL and Jacobs I.** Energy metabolism during cold exposure. *Int J Sports Med* 13: S191–S193, 1992.
39. **Vallerand AL, Jacobs I, and Kavanagh MF.** Mechanism of enhanced cold tolerance by an ephedrine-caffeine mixture in humans. *J Appl Physiol* 67: 438–444, 1989.
40. **Vallerand AL, Pérusse LF, and Bukowiecki LJ.** Cold exposure potentiates the effect of insulin on in vivo glucose uptake. *Am J Physiol Endocrinol Metab* 253: E179–E186, 1987.
41. **Vallerand AL, Tikuisis P, Ducharme MB, and Jacobs I.** Is energy substrate mobilization a limiting factor for cold thermogenesis? *Eur J Appl Physiol* 67: 239–244, 1993.
42. **Vallerand AL, Zamecnik J, and Jacobs I.** Plasma glucose turnover during cold stress in humans. *J Appl Physiol* 78: 1296–1302, 1995.
43. **Vallerand AL, Zamecnik J, Jones PJH, and Jacobs I.** Cold stress increases lipolysis, FFA R_a and TG/FFA cycling in humans. *Aviat Space Environ Med* 70: 42–50, 1999.
44. **Weekes TEC, Sasaki Y, and Tsuda T.** Enhanced responsiveness to insulin in sheep exposed to cold. *Am J Physiol Endocrinol Metab* 244: E335–E345, 1983.
45. **Weller AS, Greenhaff PL, and Macdonald IA.** Physiological responses to moderate cold stress in man and the influence of prior prolonged exhaustive exercise. *Exp Physiol* 83: 679–695, 1998.
46. **Wolfe RR.** *Radioactive and Stable Isotope Tracers in Biomedicine—Principles and Practice of Kinetic Analysis*. New York: Wiley-Liss, 1992.
47. **Young AJ, Sawka MN, Neufer PD, Muza SR, Askew EW, and Pandolf KB.** Thermoregulation during cold water immersion is impaired by low glycogen levels. *J Appl Physiol* 66: 1806–1816, 1989.