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TITLE

PLASMA GLUCOSE TURNOVER DURING COLD STRESS IN HUMANS

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Plasma glucose turnover during cold stress in humans

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Vallerand, André L., Jiri Zamecnik, and Ira Jacobs. Plasma glucose turnover during cold stress in humans. *J. Appl. Physiol.* 78(4): 1296–1302, 1995.—To clarify the source of increased carbohydrate oxidation during cold stress, six males rested for 3 h at 29 and 10°C dressed only in shorts. After priming the blood glucose and bicarbonate pools, [U-$^{13}C_6$]glucose was infused for 3 h in each condition to determine the plasma glucose rate of appearance (R_a) or turnover under relative steady-state conditions. Plasma enrichment (mol %excess) was determined by selective ion-monitoring gas chromatography-mass spectrometry. Cold exposure decreased rectal temperature and mean skin temperature and increased heat debt, metabolic rate, and whole body lipid and carbohydrate oxidation (CHO_{ox}) compared with the same subjects at thermal neutrality ($P < 0.05$). Cold exposure significantly increased R_a from 13.18 ± 0.70 to 16.22 ± 0.43 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ ($P < 0.05$). Plasma glucose clearance was elevated commensurately by the cold (from 2.68 ± 0.16 to 3.55 ± 0.14 $ml \cdot kg^{-1} \cdot min^{-1}$; $P < 0.05$). If we assume that R_a is completely oxidized (thus equivalent to maximum rates of plasma glucose oxidation) [J. A. Romijn, E. F. Coyle, L. S. Sidossis, A. Gastaldelli, J. F. Horowitz, E. Endert, and R. R. Wolfe. *Am. J. Physiol.* 265 (*Endocrinol. Metab.* 28): E380–E391, 1993], the minimum rates of glycogen and lactate oxidation in the cold would be the difference between CHO_{ox} and glucose oxidation (~14.0 ± 3.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). Therefore, under the present laboratory conditions, 54% of CHO_{ox} would be fueled by plasma glucose oxidation, whereas the remaining 46% would be derived from the combination of glycogen and lactate oxidation. The results of the present study demonstrate that cold exposure in humans enhances the turnover and metabolic clearance of plasma glucose. The results also suggest that carbohydrate oxidation during cold stress appears to be about equally divided between the oxidation of plasma glucose and the combination of lactate and intramuscular glycogen.

body temperatures; energy metabolism; energy substrates; heat balance; heat loss; heat production; shivering; stable isotope; [U-carbon-13]glucose

SURVIVAL DURING EXTREME COLD conditions depends on the ability to maintain the delicate balance between metabolic heat production and heat loss (3). Humans possess a remarkable capacity to dissipate heat, which is quite appropriate for hot environments but not advantageous in the cold. In the cold, heat loss poses an immense problem for humans. Preventing local or whole body failure of temperature-regulation mechanisms (frostbite or hypothermia, respectively) thus depends primarily on technological and behavioral thermoregulatory adjustments to enhance our microclimate (3). However, once the insulation provided by the microclimate and by peripheral vasoconstriction has been maximized, only thermoregulatory thermogenesis or the cold-induced increase in metabolic heat production can be used to offset an increasing heat debt. The energy transduced into heat production for thermoregulatory thermogenesis derives from metabolic substrate oxidation.

Carbohydrates (CHO) represent an important source of energy for thermoregulatory thermogenesis. Several animal studies have demonstrated that cold exposure greatly enhances plasma glucose tolerance and turnover as well as peripheral tissue glucose uptake and oxidation (11, 16, 28, 29). In cold-exposed humans, however, little is known about CHO metabolism, particularly with respect to plasma glucose turnover and the contribution of plasma glucose to whole body CHO oxidation (CHO_{ox}).

The goal of this study was therefore to determine the influence of cold stress on the turnover of plasma glucose in humans. It was also our intention to estimate the extent of the contribution to whole body CHO_{ox} from *1*) plasma glucose oxidation and *2*) the combination of glycogen and lactate oxidation. To accomplish these objectives, we combined two established techniques: tracer techniques with stable isotopes and indirect calorimetry.

METHODS

Subjects. The experimental protocol was approved by our institutional Human Ethics Committee. Each subject was examined by a physician who approved his participation. The nature, purpose, and possible risks of the study were carefully explained in detail to each individual before the subjects gave their consent to participate. Before any tests, subjects became familiar with the cold stress as well as other details of the protocol during a 1.5-h familiarization trial. Six healthy male (Caucasian) volunteers participated in the present study. Maximal aerobic power ($\dot{V}O_{2\,max}$) was determined on the treadmill using an incremental protocol to exhaustion (Bruce protocol). Body fat was assessed via underwater weighing, as previously described (25). Their mean physical characteristics were 31.1 ± 1.7 (SE) yr, 1.72 ± 0.01 m, 75.6 ± 2.2 kg, 51 ± 6 $ml \cdot kg^{-1} \cdot min^{-1}$ $\dot{V}O_{2\,max}$, and 12 ± 1% body fat.

Protocol. Two tests were performed ~1 wk apart in each subject who served as his own control (repeated-measures design). Subjects were asked to avoid alcohol for ≥48 h before the test, to avoid exercise for 24 h before, and to report to the laboratory in a 12- to 14-h postabsorptive state. The order of treatments was balanced.

Early in the morning, subjects (wearing only jogging shorts and foam slippers) were instrumented with a rectal probe (APC 400 series, American Pharmaseal, Valencia, CA), 12 recalibrated heat flux transducers (Concept Engineering, Old Saybrook, NJ), a heart rate monitor (Polar Vantage XL, Polar USA, Stamford, CT), and two indwelling intravenous catheters. Then they rested quietly while sitting on the experimental chair (a modified nylon-webbed lawn chair that was used to wheel the subjects in and out of the chamber) at ~22°C and 50% relative humidity for ~0.5 h before the test. The cold test consisted of exposing seminude subjects for 3 h at 10.0 ± 0.1°C air. The test at thermal neutrality was identical to the above but was performed at 29.2 ± 0.1°C; all subjects were able to complete both tests.

Measurements. During the tests, rectal temperature (T_{re}) was monitored using a thin thermistor probe inserted and secured 10 cm beyond the anus. Skin temperature (T_{sk}) and dry heat losses were measured with 12 recalibrated heat-

TABLE 1. *Influence of cold on several important thermal parameters*

	T_{re} (0 min), °C	T_{re} (180 min), °C	ΔT_{re}, °C	$\bar{T}_{sk}$ (0 min), °C	$\bar{T}_{sk}$ (180 min), °C	$\Delta \bar{T}_{sk}$, °C	S, kJ/kg
Thermal neutrality	36.99±0.10	37.04±0.07	0.04±0.09	32.82±0.24	33.57±0.11	0.75±0.21	0.34±0.62
Cold	36.97±0.07	36.67±0.13*	−0.31±0.12*	31.02±0.24	24.05±0.46*	−6.97±0.38*	−15.51±0.72*

Values are means ± SE. T_{re}, rectal temperature; ΔT_{re}, change in T_{re}; $\bar{T}_{sk}$, mean skin temperature; $\Delta \bar{T}_{sk}$, change in $\bar{T}_{sk}$; S, cumulative heat debt. * Significantly different from thermal neutrality, $P \leq 0.05$.

flux transducers taped to the skin. Using a 12-point area-weighted system, mean T_{sk} ($\bar{T}_{sk}$) and mean dry heat loss were calculated as described elsewhere (27). All thermal data were continuously recorded with a computerized Hewlett-Packard 236 data-acquisition system.

O_2 uptake ($\dot{V}O_2$) and CO_2 production (in l/min STPD) were measured from expired air using a computerized metabolic cart consisting of an IBM PC, a polarographic O_2 analyzer and an infrared CO_2 analyzer (Applied Electrochemistry, Pittsburgh, PA), a ventilation module (Interface Associates, Irvine, CA), and a Turbofit analog-to-digital computer interface (Vacumetrics, Ventura, CA). Because a face mask was used (Hans Rudolph, Kansas City, MO), it was possible to measure respiratory gas exchanges for the entire 3-h period, except for 5 min beginning at *minute 85*. Analyzers were recalibrated at that point (if required) as well as before any test. Urine collection took place after the tests for the determination of urinary urea nitrogen, used as an index of protein oxidation (30).

Isotope infusion and blood sampling. Indwelling catheters (Insyte, Deseret Medicals, Sandy, UT) were inserted in an antecubital vein of each arm. One was used for the isotope infusion and the other for blood sampling. After the collection of a baseline blood sample, a primed (7.25 μmol/kg; 93.6% mol %excess; MSD Isotopes, Montreal, Quebec) constant infusion (0.081 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) of [U-$^{13}C_6$]glucose was started and was maintained for 180 min with a calibrated syringe pump (Harvard Apparatus, Framingham, MA) (6). The plasma CO_2 pool was also primed with a bolus injection of NaH[U-^{13}C]O_3 (16.52 μmol/kg; 90 atom %excess; ICONS, Summit, NJ) (32). An independent laboratory tested each batch of solutions before use, and they were all found to be sterile and pyrogen free.

Venous blood was drawn ~15 min before each trial while subjects were sitting at a comfortable ambient temperature (22°C) as well as in the climatic chamber at *minutes 30, 60, 90, 120, 135, 150, 165*, and *180*. To ensure catheter patency without a heparin lock, we used a slow infusion of warm isotonic saline (~1 ml/min) coupled with regular flushes of warm saline; the total volume of saline infused amounted to ~200 ml. A temperature controller maintained the insulated saline bag and insulated intravenous line at ~35–37°C. Blood samples were collected into chilled heparinized tubes (for the [U-$^{13}C_6$]glucose samples) and EDTA tubes (for all other analyses) at the specified times. Thereafter, the plasma was promptly separated by centrifugation and kept frozen at −70°C until assayed for glycerol (1), free fatty acids (Wako Chemicals kit, Dallas, TX), glucose (Yellow Springs Instruments glucose analyzer, Yellow Springs, OH), insulin (Pharmacia Diagnostics kit, Uppsala, Sweden), and glucagon (Diagnostic Products, Los Angeles, CA). Plasma values were corrected for changes in plasma volume according to changes in hemoglobin (Sigma Chemical kit) and hematocrit, as before (24, 26).

[U-$^{13}C_6$]glucose enrichment was determined using gas chromatography-mass spectrometry (model MAT TSQ-70, Finnigan, Sunnyvale, CA) as described elsewhere (4), except for the following modifications. The cyclic methylboronate-pentafluoropropinionate derivative of glucose was prepared with D-[5,6,6-$^2H_3$]glucose as an internal standard. The gas chromatography conditions were as follows: injector in split mode (1:30) at 280°C, gas chromatography oven of 160–260°C at 15°C/min, and separator of 280°C. The ion source was at 110°C, the filament current was at 200 μA, and the electron multiplier was at 1,600 V, scanning a single quadrupole in an selective ion-monitoring mode using methane as chemical ionization gas in negative mode at masses (m/z) 374 (m+0), 377 (m+3), and 380 (m+6), which represented the unlabeled glucose, [$^2H_3$]glucose, and [U-$^{13}C_6$]glucose, respectively. All between and within coefficients of variation were <5%.

Calculations. The heat balance equation summarizes whole body heat exchange in terms of heat production and the various avenues of heat loss (all variables in W/m^2) (8, 26, 30). The equation is

$$\dot{S} = \dot{M} - (\dot{R} + \dot{C}) - \dot{E}_{persp} - \dot{C}_{resp} - \dot{E}_{resp} \quad (1)$$

where $\dot{M}$ is the metabolic rate calculated from measured values of $\dot{V}O_2$ (in l/min STPD) and the respiratory exchange ratio (see below); $\dot{R} + \dot{C}$ is the measured rate of dry heat exchange by radiation and convection corrected for the thermal resistance of the transducer itself (26, 30); $\dot{E}_{persp}$ is the calculated rate of evaporative heat loss from the skin; $\dot{C}_{resp}$ and $\dot{E}_{resp}$ are the calculated rates of convective and evaporative heat loss, respectively, by the respiratory tract (17); and $\dot{S}$ is the rate of heat storage determined as the minute-by-minute balance of heat gains and heat losses. A negative value of $\dot{S}$ signifies a heat debt, whereas a positive value signifies a heat gain. The cumulative heat debt in kilojoules per kilogram (S) was then easily obtained by the summation of the $\dot{S}$ values over the entire trial ($\dot{S}$ in W/m^2, as in *Eq. 1*), after taking into account the subject's body surface area and body mass.

The rates of CHO_{ox} and lipid substrate oxidation ($lipid_{ox}$) (both in g/min) were calculated using the nonprotein $\dot{V}O_2$ ($\dot{V}O_{2\,np}$) and the nonprotein respiratory exchange ratio (NPRER). Protein oxidation ($prot_{ox}$) was assessed by analyzing the urinary urea nitrogen excretion ($urea_{corr}$) (oxidation from the ~4-h urine collection was converted to g/min) and correcting it for changes in the blood urea nitrogen, as previously described (kit 640, Sigma Chemical) (12). The calculations were performed as

$$term = (NPRER - 0.707)/0.293 \quad (2)$$

$$CHO_{ox} = term \cdot \dot{V}O_{2\,np} \cdot 0.788^{-1} \quad (3)$$

$$lipid_{ox} = (1 - term) \cdot \dot{V}O_{2\,np} \cdot 2.0193^{-1} \quad (4)$$

$$prot_{ox} = urea_{corr} \cdot 6.25 \quad (5)$$

$$\dot{M} = [(19.61 + term \cdot 1.51) \cdot \dot{V}O_{2\,np} + (18.66 \cdot \dot{V}O_{2\,prot})] \cdot 0.06^{-1} \cdot BSA^{-1} \quad (6)$$

In *Eqs. 2–5*, term is the ratio of CHO-to-nonprotein oxidation; 0.293 is the difference between NPRER of 1.000 and 0.707; $\dot{V}O_{2\,prot}$ is the $\dot{V}O_2$ due to protein oxidation determined using 0.966 and 0.782 liter of O_2 and CO_2 per gram of protein, respectively; 0.788 is the average $\dot{V}O_2$ required for the

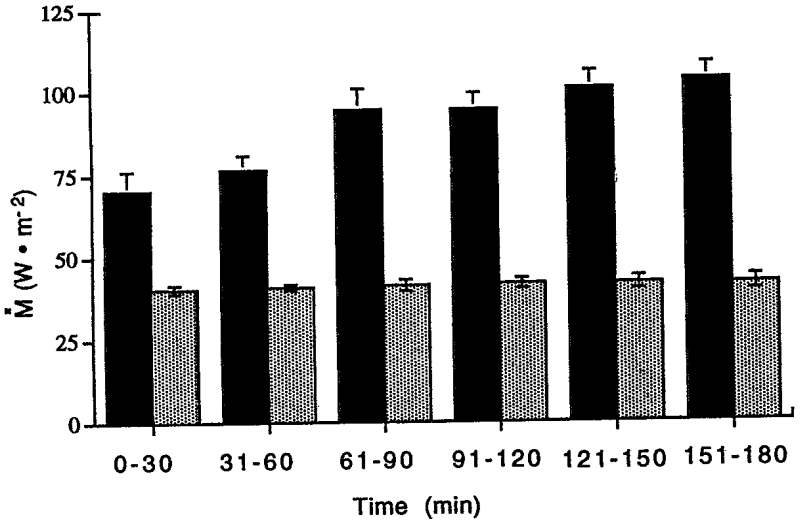

FIG. 1. Metabolic rate ($\dot{M}$) during 3-h exposure to either thermal neutrality (29°C; stippled bars) or mild cold (10°C; solid bars). Data are means ± SE. Fasting subjects sat at rest in climatic chamber, wearing only jogging shorts. Results were calculated from oxygen uptake and nonprotein respiratory exchange ratio as in METHODS, and each data point represents mean of previous 30 min. Repeated-measures analysis of variance indicated that $\dot{M}$ was affected by significant main effect of cold ($P < 0.01$).

oxidation of 1 g of glycogen (0.829 l/g) or 1 g of glucose (0.744 l/g); 2.0193 is $\dot{V}O_2$ per gram of triglyceride (l/g); 6.25 is the conversion factor for urinary urea nitrogen to protein; 19.61 and 18.66 are the energy equivalents in kilojoules per liter O_2 when lipid and protein are oxidized, respectively; 1.51 is the difference between the energy equivalent of 1 liter O_2 at an NPRER of 1.000 and 0.707; 0.06 is a conversion factor to joules per second, or watts; and BSA is the body surface area in square meters. The relative proportions of the average energy expenditure derived from CHO (average of glucose and glycogen), lipids, and protein were determined using energy equivalents of 16.7, 39.3, and 18.0 kJ/g, respectively.

After having established that relative steady-state enrichments were obtained in the last hour of experimentation (i.e., between *minutes 120* and *180*), the true (nonrecycling) rate of appearance (R_a) of plasma glucose (in $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was calculated during this time period using steady-state kinetics modified for use with stable isotopes (4, 32)

$$R_a = [(IE_{inf}/IE_{plasma}) - 1] \cdot F \quad (7)$$

where IE_{inf} and IE_{plasma} are the isotopic enrichment (in mol%) of the infusate and plasma samples, respectively, and F is the infusion rate in micromoles per kilogram per minute. Under relative steady-state conditions, the rate of disappearance of plasma glucose (R_d) and plasma glucose turnover were assumed to be equal to R_a (4, 16, 19, 32). The rate of [U-$^{13}C_6$]glucose infusion was calculated by multiplying the exact infusion pump rate by the measured infusate [U-$^{13}C_6$]glucose concentration. The clearance rate of plasma glucose [metabolic clearance (MC); in $ml \cdot kg^{-1} \cdot min^{-1}$] was calculated by dividing R_d by the plasma glucose concentration (32).

If we assume that R_a is completely oxidized (4, 21) and thus equivalent to maximum rates of plasma glucose oxidation (R_{ox}; in $\mu mol \cdot kg^{-1} \cdot min^{-1}$), the minimum rates of glycogen and lactate oxidation were calculated as the difference between CHO_{ox} and R_{ox} (2, 9, 21). As stated earlier, all calculations related to the [U-$^{13}C_6$]glucose pertain to the steady-state conditions in the last hour of exposure.

Statistics. The main effects of time and treatments (thermoneutral vs. cold) as well as time-treatment interactions were tested by repeated-measures analysis of variance (ANOVA) on all thermal and metabolic data (Biomedical Computer Programs, BMDP-90, Los Angeles, CA). ANOVA was corrected by the Huynh-Feldt epsilon-adjusted degrees of freedom when the sphericity test was significant (BMDP-90). Paired t-tests, adjusted for multiple comparisons when required (7), were used to identify the location of significant differences ($P \leq 0.05$) when ANOVA yielded a significant F ratio for the interaction. Results are expressed as means ± SE.

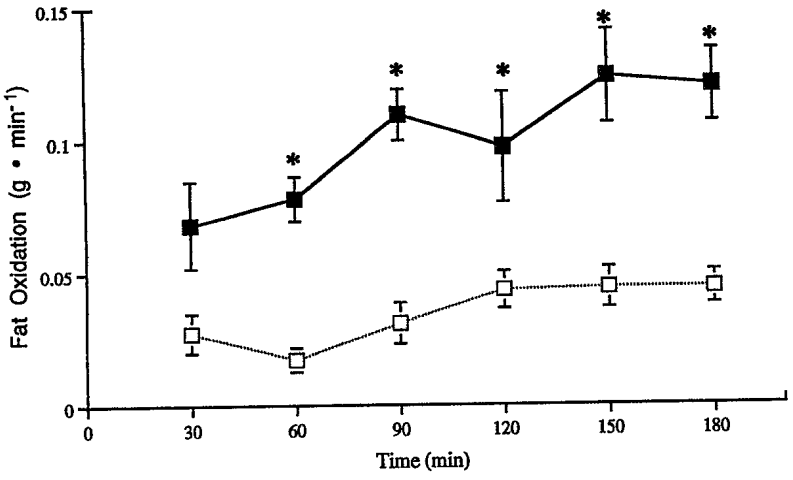

FIG. 2. Rates of fat or lipid oxidation during 3-h test at thermal neutrality (□) or in cold (■). Each data point represents mean of previous 30 min. * Significantly different from thermal neutrality, $P \leq 0.05$.

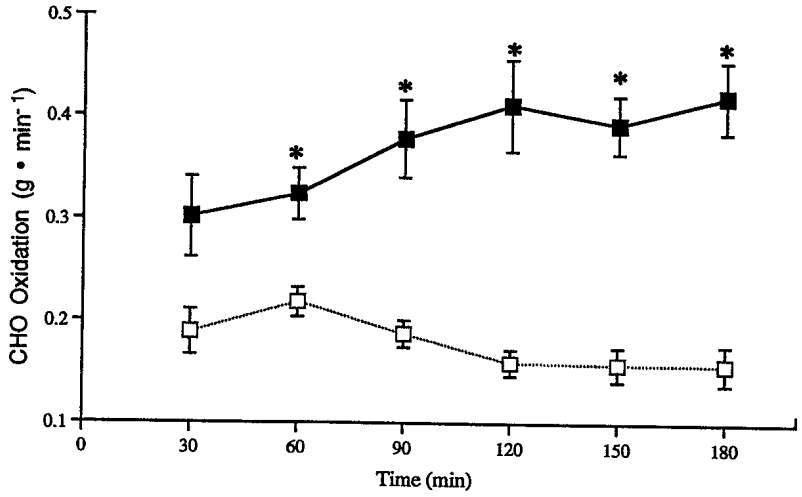

FIG. 3. Rates of carbohydrate (CHO) oxidation during 3-h test at thermal neutrality (□) or in cold (■). Each data point represents mean of previous 30 min. * Significantly different from thermal neutrality, $P \leq 0.05$.

RESULTS

The influence of the mild cold stress on selected thermal parameters is shown in Table 1. As expected, cold exposure reduced $\bar{T}_{sk}$ by almost 7°C, whereas the drop in T_{re} was of a much smaller magnitude. Both temperature decreases were significantly different ($P < 0.05$) from the trial at thermoneutrality. The rates of heat loss increased in the cold, particularly dry heat losses ($\dot{R} + \dot{C}$; final values at *minute 180* of thermoneutral vs. cold tests were 37.6 ± 1.1 vs. 116.0 ± 2.6 W/m^2, respectively, $P < 0.01$), as expected. Although final wet ($\dot{E}_{persp}$ at *minute 180*: 3.7 ± 0.2 vs. 5.4 ± 0.2 W/m^2, $P < 0.01$) and respiratory ($\dot{C}_{resp}$ at *minute 180*: 0.3 ± 0.0 vs. 3.5 ± 0.2 W/m^2, $P < 0.01$; $\dot{E}_{resp}$ at *minute 180*: 1.6 ± 0.1 vs. 8.5 ± 0.4 W/m^2, $P < 0.01$) heat losses were also increased by the cold, they were much less important quantitatively. Therefore, it is not surprising that the cold test produced a large overall heat debt, whereas a very small heat gain was observed at thermal neutrality ($P < 0.01$, Table 1).

$\dot{M}$ was 38.9 ± 1.8 W/m^2 at rest before the climatic suite was entered. $\dot{M}$ was significantly affected by the cold ($P < 0.01$), increasing gradually to 2.5 times resting values at *minute 120*, where it stayed relatively stable at ~100 W/m (Fig. 1). At thermal neutrality, there was no significant change in $\dot{M}$ during the 3-h exposure. The cold-induced increase in $\dot{M}$ was in part due to an increase in whole body lipid oxidation (Fig. 2). There was a significant trial × time interaction that indicated that the effect of time changed as a function of environmental conditions; the rates of lipid oxidation were higher in the cold than at thermal neutrality at all points in time, except for *minute 30* ($P < 0.05$). When averaged over the entire 3-h period, cold exposure increased lipid oxidation by ~2.9-fold compared with thermal neutrality (0.099 ± 0.012 vs. 0.034 ± 0.005 g/min, $P < 0.05$).

The cold-induced increase in $\dot{M}$ was also the result of an increase in whole body CHO_{ox}. Similar to lipid oxidation above, CHO_{ox} was also affected by a significant interaction of effects. Figure 3 shows that the rates of CHO_{ox} in the cold were significantly greater at all points in time ($P < 0.05$) except at *minute 30*. Taken over the 3-h period, CHO_{ox} was increased by more than twofold due to cold (0.370 ± 0.027 vs. 0.177 ± 0.013 g/min; $P < 0.01$). CHO_{ox} in the cold amounted to 6.2 ± 0.4 kJ/min, accounting for 57% of the average energy expenditure of 10.9 ± 0.5 kJ/min. The rest of the heat production was attributed to lipid oxidation (36%) and protein oxidation (7%).

Figure 4 describes the plasma enrichment of [U-$^{13}C_6$]-

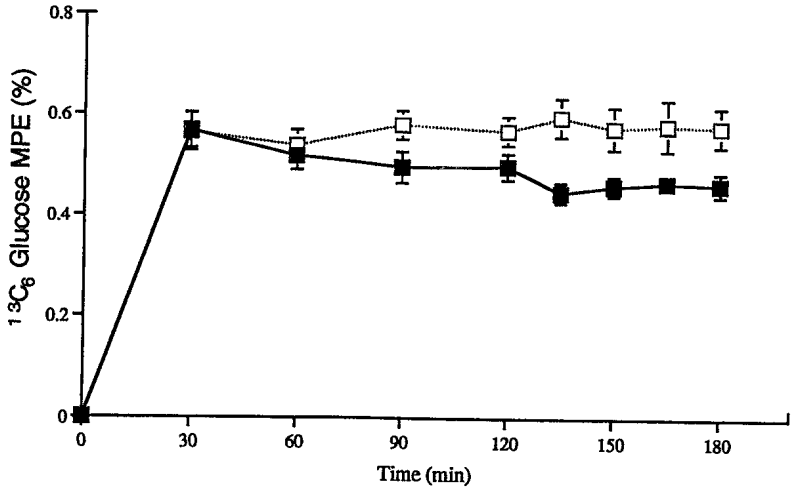

FIG. 4. Plasma [U-$^{13}C_6$]glucose enrichment [mol %excess (MPE%)] as function of time during test at thermal neutrality (□) or in cold (■). A significant main effect of cold is reported in RESULTS.

TABLE 2. *Influence of cold on plasma glucose kinetics and CHO_{ox}*

	MC, $ml \cdot kg^{-1} \cdot min^{-1}$	R_a, $\mu mol \cdot kg^{-1} \cdot min^{-1}$	CHO_{ox}, $\mu mol \cdot kg^{-1} \cdot min^{-1}$
Thermal neutrality	2.68±0.16	13.18±0.70	11.76±1.45
Cold	3.55±0.14*	16.22±0.43*	30.24±2.9*

Values are means ± SE. CHO_{ox}, carbohydrate oxidation (derived from indirect calorimetry); R_a, glucose appearance rate; MC, metabolic clearance rate. See METHODS for calculations, which apply to relative steady-state period of last hour of testing. * Significantly different from thermal neutrality, $P \leq 0.05$.

glucose as a function of time. In both physiological conditions, relative steady states were obtained in the last hour of testing. The enrichment profile, from *minutes 120–180*, was reduced by a significant main effect of cold exposure ($P < 0.05$). Table 2 summarizes the calculations related to the steady-state kinetics of the [U-$^{13}C_6$]glucose as well as rates of whole body CHO_{ox}. Cold increased the rate of MC of glucose by 32% and R_a by 23% ($P < 0.05$). If we assume that R_a is completely oxidized in the cold (equivalent to R_{ox}), the minimum rates of glycogen and lactate oxidation would be the difference between CHO_{ox} and R_{ox} (~14.0 ± 3.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). Under the present laboratory conditions, the oxidation of plasma glucose would thus account for a maximum of ~54% of the whole body CHO_{ox}, whereas a minimum of 46% could be attributed to the combination of glycogen and lactate oxidation.

Table 3 summarizes the effect of cold exposure on plasma levels of selected metabolites and hormones. Although mild in nature, the cold stress did influence several parameters. It reduced plasma glucose and increased plasma free fatty acids (2-fold) and glycerol levels (2.8-fold) ($P < 0.05$). Plasma glucagon and insulin levels were not significantly affected by the treatments.

DISCUSSION

The results of the present study demonstrate that cold exposure in humans enhances the R_d and the MC of plasma glucose (Table 2). These new observations corroborate in humans previous animal experiments that documented similar cold-induced increases in R_a (16, 20, 31), cold-induced improvements in glucose tolerance (i.e., an index of more rapid utilization of injected glucose), and skeletal muscle glucose uptake (28, 29). The results are also consistent with other human studies describing increases in whole body CHO_{ox} and glucose tolerance with cold stress (24, 25). Table 2 not only summarizes the relative increase due to cold but it also shows the absolute resting R_a values at thermal neutrality. These values are similar in magnitude to the R_a values reported in several other studies (13, 22, 23).

Although the increases in R_a and MC were significant, it is worthwhile to explore why they were limited to ~23–32%. Were our subjects sufficiently cold stressed? Although the cold test was intended to be relatively mild in nature, reflected by the very small drop in T_{re} (Table 1), it was nevertheless sufficient to cause a large decrease in $\bar{T}_{sk}$; a large elevation in dry and wet heat losses; a relatively large heat debt; as well as significant shivering activity that was associated with an increase in CHO_{ox}, lipid oxidation; and a 2.5-fold increase in $\dot{M}$. Thus these subjects were cold stressed, although not severely (30). This could be an important aspect because, as early as 1972, Pernod et al. (19) suggested that cold exposure in dogs produces a nonlinear increase in R_a with increasing $\dot{M}$, similar to aerobic exercise (5). This concept seems to be supported by some (16, 20, 31) but not all (18) glucose turnover studies in cold-exposed animals. Whether a more severe cold stress in humans would further enhance R_a or the reliance on plasma glucose as a source of energy for thermoregulatory thermogenesis can only be assessed through further testing.

Although the fate of the increased disappearance of plasma glucose is not clear, it is established that there are two major pathways of glucose disposal: oxidative and nonoxidative. Oxidative pathways refer to the complete oxidation of glucose to CO_2 and the net loss of CHO from the CHO pool, whereas nonoxidative glucose disposal encompasses glycogenesis, anaerobic glycolysis, and lipogenesis (from glucose-derived carbons) (12, 32). Under the present conditions for fasting subjects resting at thermal neutrality, it is quite reasonable to assume that oxidative pathways predominate and that R_{ox} becomes maximal when it matches R_a. The data in Table 2 indicate that at thermal neutrality CHO_{ox} would slightly exceed R_{ox} (when $R_{ox} = R_a$) by ~1.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, an amount that could be attributed to gluconeogenesis, since muscle glycogen oxidation should be practically negligible under these conditions. It should be mentioned that via indirect calorimetry gluconeogenesis (from amino acids) proceeds as protein and not CHO_{ox}. If we assume that $R_a = R_{ox}$ in the cold, we can estimate the oxidation rate of glycogen and lactate (2, 9, 21), which would amount to ~14.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, or ~46% of CHO_{ox} (see RESULTS). Such an estimate has proven difficult to obtain in the past, although it is only dependent on two factors: *1*) the average rate of glycogen utilization in cold-exposed humans and *2*) the active shivering muscle mass utilizing glycogen at that rate.

TABLE 3. *Effect of cold on selected plasma metabolites and hormones*

	Glucose, mM	FFA, mM	Glycerol, mM	Insulin, pmol/l	Glucagon, ng/l
Minute 0	5.18±0.24	0.378±0.042	0.052±0.006	65.29±25.11	110.8±11.8
Minute 180	4.60±0.17*	0.833±0.085*	0.146±0.024*	32.29±3.59	111.8±13.4

Values are means ± SE. FFA, free fatty acid. * Significantly different from *minute 0*, $P \leq 0.05$.

Is our estimate consistent with available information? At the average muscle glycogen utilization rate reported for shivering humans of ~179 μmol · kg wet muscle wt^{-1} · min^{-1} (14, 15) (equivalent to 2.36 μmol · kg wet muscle wt^{-1} · min^{-1} · kg body mass^{-1} for our body mass of 76 kg), an active muscle mass of ~6 kg would be required to be shivering to match the estimated whole body glycogen oxidation rate of 14.0 μmol · kg^{-1} · min^{-1}. This calculation seems reasonable considering the lean body mass of our subjects (~66 kg) and the fact that $\dot{M}$ is increased because of the combined shivering activity of several muscles contracting at <15% of their capacity (11) and that an active muscle mass of 8 kg has been suggested for cycling males at a $\dot{V}O_2$ of ~2 l/min (10).

Because CHO_{ox} appears to be fueled equally by the oxidation of plasma glucose (54%) and the combination of glycogen and lactate (46%; see RESULTS), it seems important to determine whether alterations in *1*) glycemia and *2*) glycogen availability would alter CHO_{ox}, thermoregulatory thermogenesis, and body temperatures. Lower initial muscle glycogen levels per se have been associated with a lower CHO_{ox}, a lower thermoregulatory thermogenesis, and a greater drop in T_{re} (14). Similarly, it is established that hypoglycemia per se impairs thermoregulation, but it appears to be primarily the result of central nervous system effects (see Ref. 11). The opposite, enhancing the availability of plasma glucose (with CHO-rich supplements) or muscle glycogen (by supercompensation protocols), has also been tested in the cold. Although such treatments were found to increase CHO_{ox}, they did not significantly improve thermoregulatory thermogenesis and body temperatures (14, 26, 30). It should be emphasized here that the above conclusions are based on data obtained through cold-air exposures of ~3 h and cold-water immersions of ~1.5 h. It is hypothesized that during much longer trials (i.e., 6–24 h in cold air or survival-like conditions of continuous mild cold), hypoglycemia, and/or the depletion of muscle intramuscular glycogen could negatively affect CHO_{ox}, compromise thermoregulatory thermogenesis, and accelerate the drop in core temperature or the heat debt. It is thus recommended that additional studies proceed within this framework. Future work is also required to fill in gaps of knowledge with respect to the effect of cold on gluconeogenesis. Similarly, lipid metabolism in the cold should be better characterized with respect to the importance of various sources of fatty acids to whole body lipid oxidation, particularly under energy deficiency conditions usually associated with survival in the cold. Although it is clear that lipid metabolism is increased by the cold, it remains to be determined exactly why CHOs are the preferred fuel for thermoregulatory thermogenesis, similarly to physical exertion.

In summary, the results of this study demonstrate that cold exposure in humans enhances plasma glucose turnover, MC of glucose, and whole body CHO_{ox}. These results also suggest that the source of CHO_{ox} in the cold appears to be about equally divided between plasma glucose and the combination of lactate and intramuscular glycogen, at least under the present experimental conditions. Direct measurements of plasma glucose oxidation are required to confirm this suggestion. We recommend that the present results are extended first to a more severe cold exposure and then to a slightly milder but much longer exposure to approximate survival conditions.

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