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POSTDOCTORAL STUDIES**

Isabelle Dépault

AUTEUR DE LA THÈSE / AUTHOR OF THESIS

M.Sc. (Human Kinetics)

GRADE / DEGREE

School of Human Kinetics

FACULTE, ÉCOLE, DÉPARTEMENT / FACULTY, SCHOOL, DEPARTMENT

Effects of Glucose Ingestion on Fuel Selection During Cold Exposure

TITRE DE LA THÈSE / TITLE OF THESIS

Dr. Pascal Imbeault

DIRECTEUR (DIRECTRICE) DE LA THÈSE / THESIS SUPERVISOR

Dr. François Haman

CO-DIRECTEUR (CO-DIRECTRICE) DE LA THÈSE / THESIS CO-SUPERVISOR

EXAMINATEURS (EXAMINATRICES) DE LA THÈSE / THESIS EXAMINERS

Dr. Denis Prud'homme

Dr. Eric Doucet

Gary W. Slater

Le Doyen de la Faculté des études supérieures et postdoctorales / Dean of the Faculty of Graduate and Postdoctoral Studies

EFFECTS OF GLUCOSE INGESTION ON FUEL SELECTION DURING COLD EXPOSURE

Isabelle Dépault

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Faculty of Health Sciences
University of Ottawa



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ABSTRACT

Recent studies of cold exposure have focused on the nutritional status of shivering individuals, studying the importance of carbohydrate stores for shivering muscles. These studies have illustrated that during moderate cold stress, muscle glycogen is the main substrate for heat production. Consequently, it is thought that shivering will cease and hypothermia will set in when muscle glycogen stores gets compromised or exhausted. In spite of this, no studies to date have investigated the effects glucose ingestion on fuel selection during cold exposure. Using a combination of indirect calorimetry and isotopic methods, the aims of this thesis were to determine the effects of glucose ingestion on whole body heat production and oxidative fuel selection to 1) confirm that heat production and core temperature will not change with glucose feedings during shivering, 2) quantify the effects of glucose feedings on whole body fuel selection, 3) quantify the effects of glucose feedings on the oxidation of CHO stores (muscle glycogen), and 4) quantify the oxidation of the ingested exogenous glucose. Six healthy men were exposed to cold for 120 min (liquid conditioned suit perfused with 4°C water) on three randomized occasions during which 500ml of a glucose solution was ingested to supply: 0.04 g•min⁻¹ (C), 0.4 g•min⁻¹ (Lo) or 0.8 g•min⁻¹ (Hi) of glucose. We observed that 1) glucose ingestion has no effects on whole body thermal response. 2) Absolute and relative CHO and lipids oxidation rates did not differ significantly between conditions. 3) Muscle glycogen oxidation was not affected by glucose ingestion, but hepatic glucose production decreased with 0.8 g•min⁻¹ of glucose ingestion. 4) The maximal rate of exogenous glucose oxidation observed was 0.2 g•min⁻¹. No difference was seen between exogenous glucose oxidation rate during the Lo and Hi conditions. The result of this thesis provides the first estimates of exogenous glucose oxidation rate in the cold and

quantifies the effects of glucose ingestion on whole-body energy demands. This information may contribute to improve survival strategies for human exposed to cold environments.

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LIST OF ABBREVIATIONS AND ACRONYMS

ATP	adenosine triphosphate
C	control condition
CHO	carbohydrates
DEXA	dual energy X-ray absorptiometry
%GLU	relative contribution to total carbohydrate oxidation
%GLU _{liver}	relative contribution of liver glycogen to total carbohydrate oxidation
%GLU _{muscle}	relative contribution of muscle glycogen to total carbohydrate oxidation
Hi	condition where participants ingested 0.8 g/min of glucose
H_{loss}	heat loss
H_{prod}	heat production
LCS	liquid conditioned suit
Lo	condition where participants ingested 0.4 g/min of glucose
mM	millimolar
NEFA	non esterified fatty acids
PDB	Pee Dee Belemnite
Rexo	isotopic composition of exogenous glucose solution
Rexp	isotopic composition of expired CO ₂
RFox	total lipid oxidation
%RFox	relative contribution of lipid oxidation to total heat production
RGexo	rate of exogenous glucose oxidation
RGliver	oxidation of glucose released from the liver
Rglu	isotopic composition of plasma glucose
RG _{muscle}	glucose oxidation derived from glycogen stores
RGox	total carbohydrate oxidation
%RGox	relative contribution of carbohydrate oxidation to total heat production
RG _{plasma}	plasma glucose oxidation
rpm	revolutions per minute
RPox	total protein oxidation
%RPox	relative contribution of protein oxidation to total heat production
R _{ref-exp}	¹³ C/ ¹² C ratio of expired CO ₂ at baseline
R _{spl}	¹³ C/ ¹² C ratio of the sample
R _{std}	¹³ C/ ¹² C ratio of the standard
SE	standard error
Shiv _{peak}	maximal shivering intensity
%Shiv _{peak}	relative shivering intensity
STPD	standard temperature and pressure dry

T_{skin}	mean skin temperature
T_{tymp}	tympanic temperature
$\text{Urea}_{\text{urine}}$	urinary urea excretion
VCO_2	carbon dioxide production
V_E	ventilation
VO_2	oxygen consumption
$\text{VO}_{2\text{max}}$	maximal oxygen consumption
$\delta^{13}\text{C PDB}^{-1}$	‰ difference compared with Pee Dee Belemnite-1 (PDB-1) Chicago standard

INTRODUCTION

Humans have for centuries been faced with a variety of environmental challenges. Heat transfers in or out of the body through conductive, convective, radiative and evaporative heat exchange (Crawshaw LI, 2007). Consequently, humans beings exposed to a cold environment for a prolonged period of time, whether it be cold air, cold surfaces or cold water will loose heat. If the heat loss is not compensated by heat production (H_{prod}), core body temperature decreases. Unfortunately, large decreases in core temperature in humans can ultimately be fatal, as many enzymatic reactions have a very specific operating range of temperatures. A human's core temperature must therefore remain within a specific range of temperatures. Fortunately, the human body has the capacity to maintain a viable body temperature under many climatic conditions by producing or releasing body heat (Crawshaw LI, 2007). Controlled by the central receptors in the hypothalamus and peripheral receptors located in the dermis and epidermis (Crawshaw LI, 2007), humans produce heat by two main classes of mechanisms: non-shivering and shivering thermogenesis (Jacobs, Martineau, & Vallerand, 1994). Non-shivering thermogenesis in adults includes increased thyroxin, and catecholamine secretion and free fatty acid release, which increase metabolic heat production (G. A. Brooks, Fahey, & Baldwin, 2005). Shivering consists of a form of involuntary rhythmic contractions of skeletal muscle, where no useful work is performed, and is the main mechanism for increasing heat production during negative heat balance (G. A. Brooks et al., 2005). . These muscular contractions produce heat, because conversion of chemical energy to mechanical work is inefficient, and looses about 75% of energy as heat (Jacobs et al., 1994). In extreme cold environmental conditions, when the rate of heat loss is too great to be compensated, shivering only delays the onset of critical hypothermia (F. Haman, 2006). If the cold is sufficiently less stressful, heat loss is balanced with

heat produced from shivering, and survival is dependent on how long shivering can be sustained (Tikuisis, Eyolfson, Xu, & Giesbrecht, 2002). Shivering can reach intensities equivalent to ~40% of maximal oxygen consumption or 5 times the resting metabolic rate (Eyolfson, Tikuisis, Xu, Weseen, & Giesbrecht, 2001). Just as any muscle contraction, shivering muscles require continual substrates to produce energy.

Substrates provided to shivering muscles can come from exogenous sources or whole body energy stores. Whether from exogenous or endogenous sources, shivering muscles use adenosine triphosphate (ATP), the basic energy resource of cell work (G. A. Brooks et al., 2005). ATP can be supplied from the oxidation of carbohydrates (CHO), lipids, and proteins. The proportion of energy provided from each of these macronutrients depends on shivering intensity and on the availability of each of these macronutrients. A number of studies have looked at fuel selection during shivering in the fasted state. Lipids, CHO and proteins each contribute varying amounts of energy to shivering muscles.

Only 4 studies have investigated the effects of giving exogenous food sources to shivering humans (Glickman-Weiss et al., 1993; Glickman-Weiss, Nelson, Hearon, Windhauser, & Heltz, 1994; Vallerand, Frim, & Kavanagh, 1988; Vallerand, Tikuisis, Ducharme, & Jacobs, 1993) and only 3 of them measured fuel selection during the experiments (Glickman-Weiss et al., 1993; Glickman-Weiss et al., 1994; Vallerand et al., 1993). Only one of these studies measured protein oxidation (Vallerand et al., 1993), and none of these studies used isotope tracers to accurately determine the proportion and sources of lipids and CHO oxidized. Of the three studies that have provided an exogenous source of energy to shivering humans and looked at heat production (Glickman-Weiss et al., 1993; Glickman-Weiss et al., 1994; Vallerand et al., 1993), none

observed differences in heat production, heat loss, or core temperatures with feeding compared to the fasted state or placebo. **Consequently, the aims of this study were to determine the effect of exogenous glucose ingestion in hivering men to 1) confirm that heat production and core temperature will be stable with glucose feedings during shivering, 2) quantify the effects of glucose feedings on whole body fuel selection, 3) quantify the effects of glucose feedings on the oxidation of CHO stores (muscle glycogen), and 4) quantify the oxidation of the ingested exogenous glucose.**

Though few studies have looked at the effects of exogenous food sources on fuel selection during shivering, a great number of studies have looked at fuel selection in the fasted state during shivering (F. Haman, Peronnet et al., 2004; F. Haman et al., 2002; F. Haman et al., 2005; Francois Haman, Scott, & Kenny, 2007; MacNaughton, Sathasivam, Vallerand, & Graham, 1990; Martineau & Jacobs, 1988, 1989b; Pettit, Marchand, & Graham, 1999; Tikuisis, Bell, & Jacobs, 1991; Tikuisis, Jacobs, Moroz, Vallerand, & Martineau, 2000; Vallerand & Jacobs, 1989; Vallerand, Zamecnik, & Jacobs, 1995; Vallerand, Zamecnik, Jones, & Jacobs, 1999; Weller, Greenhaff, & Macdonald, 1998; Young et al., 1989). It was for a long time discordant as to whether lipids (F. Haman et al., 2002; Martineau & Jacobs, 1988, 1989a, 1989b; Pettit et al., 1999; Tikuisis et al., 2000; Weller et al., 1998) or CHO (Glickman-Weiss et al., 1993; Glickman-Weiss et al., 1994; Vallerand & Jacobs, 1989, 1990; Vallerand et al., 1995) were the main substrate used by shivering muscles. Recently however, Haman et al. determined that in the fasted state, lipids are the preferred substrate at low shivering intensity, while CHO the preferred substrate at high shivering intensity (F. Haman et al., 2005; Francois Haman et al., 2007). The exception to this observation seems to be when glycogen reserves are depleted by heavy exercise or nutritional status, in which circumstances lipids are the preferred substrate for shivering, even

at high shivering intensity (F. Haman, Peronnet et al., 2004; Martineau & Jacobs, 1989b; Young et al., 1989). Of the four previously mentioned studies that provided exogenous substrates to shivering individuals (Glickman-Weiss et al., 1993; Glickman-Weiss et al., 1994; Vallerand et al., 1988; Vallerand et al., 1993), two of these studies observed an increase in both CHO and lipid oxidation with high CHO feedings (Glickman-Weiss et al., 1993; Glickman-Weiss et al., 1994), however these studies failed to measure protein oxidation and estimate substrate partitioning from the respiratory exchange ratio only. A study evaluating the effects of an oral glucose tolerance test in the cold found a 650% increase in CHO oxidation, but the effects on lipids is not mentioned (Vallerand et al., 1988). When looking at studies that did take into account protein oxidation, it was observed both at thermoneutrality (Tappy et al., 1986) and in the cold (Vallerand et al., 1993) that high CHO feeding increases CHO oxidation and decreases fat oxidation compared to placebo. **Consequently, one purpose of the current thesis was to determine and quantify the effects of two different doses of glucose feedings compared to placebo on whole body protein, lipid and CHO oxidation. Based on Vallerand's observations (Vallerand et al., 1993), we hypothesized that CHO oxidation would increase and lipid oxidation would decrease during cold exposure.** However, the main sources of CHO during the shivering process remains to be elucidated.

Substrates can be provided from intramuscular reserves or from other tissues via the circulation. Therefore, two major sources of CHO can fuel shivering muscles: muscle glycogen and plasma glucose (discussed below). To date, no shivering studies have focused on muscle glycogen oxidation in CHO fed individuals. It has been known for some time that muscle glycogen is a major source of substrate in shivering. Early studies doing muscle biopsies to quantify muscle glycogen before and after cold exposure have reported confounding results (Martineau & Jacobs,

1988, 1989b; Young et al., 1989). Nonetheless 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 (F. Haman, Peronnet et al., 2004). In recent years, studies have quantified the contribution of muscle glycogen to heat production in fasted shivering individuals by using stable isotope tracers. It is now known that in the fasted state, muscle glycogen alone can provide 20 to 50% of substrate for heat production, depending on the intensity of shivering and on muscle glycogen stores (F. Haman, Peronnet et al., 2004; F. Haman et al., 2002; F. Haman et al., 2005; Francois Haman et al., 2007). **Consequently, we hypothesize that CHO feeding during shivering could provide an immediately available source of CHO for oxidation and possibly reduce muscle glycogen oxidation.**

Plasma glucose may come from the liver, or, when CHO are ingested, glucose may be absorbed from the gastrointestinal system (A. E. Jeukendrup, Wagenmakers et al., 1999). Early studies often estimated plasma glucose oxidation by measuring the rate of glucose appearance in blood (Vallerand et al., 1995; Vallerand et al., 1999), assuming that the rate of glucose appearance and rate of glucose disposal are matched. However, neglecting to subtract nonoxidative glucose disposal (storage) from the rates of appearance/disposal of glucose causes a significant overestimation of glucose oxidation rates, especially at low metabolic rates where it can represent a large portion of disposal (F. Haman et al., 2002). Recent studies using stable isotopes have shown that even at moderate intensity shivering, where plasma glucose oxidation more than doubles compared to low-intensity shivering and CHO become the dominant thermogenic fuel, the relative contribution of plasma glucose to total heat generation always remains minor (<15% H_{prod}) (F. Haman et al., 2005). Therefore, even if hepatic glucose production is strongly

stimulated during low-intensity shivering (~138%), this fuel only mildly contributes to the survival process (F. Haman et al., 2002). The fact that plasma glucose remains a minor contributor to total heat production during moderate shivering suggests that maintaining a low thermogenic contribution for plasma glucose may be necessary to prevent hypoglycaemia (F. Haman et al., 2005). Both animal (Cassidy, 1925; Dworkin & Finney, 1927; Silva & Bouland, 1984) and human studies (Gale, Bennett, Green, & MacDonald, 1981; Haight & Keatinge, 1973; Passias, Meneilly, & Mekjavic, 1996) suggest that hypoglycaemia inhibits shivering thermogenesis. For example, Passias *et al.* (1996) (Passias et al., 1996) showed that decreasing plasma glucose concentration to 2.8mM by hyperinsulinaemic, hypoglycaemic clamp decreased heat production by 20%. In another study, a complete inhibition of shivering was observed below 2.5mM plasma glucose (Gale et al., 1981). However, this inhibitory effect seems to occur centrally (inhibition of cold-sensitive neurons within the preoptic anterior hypothalamus), rather than peripherally by lack of substrate to support shivering (Gale et al., 1981). To date, however, even in the study monitoring the most prolonged and intense cold stress (3–4 hours at 60–70% Shivpeak) there was no evidence that cold exposure alone can elicit hypoglycaemia in humans (Tikusis et al., 2002). No studies to date have observed the effects of CHO feeding on plasma glucose oxidation in shivering individuals. **If preventing hypoglycemia is the main reason for the minor contribution of plasma glucose to heat production, then we hypothesized that giving exogenous glucose during shivering may once again increase plasma glucose availability, and consequently increase plasma glucose oxidation.** This, however, is based on the presumption that the exogenous glucose will be absorbed and oxidized in lieu of other CHO sources.

At exercise, the human body is able to absorb and use exogenous glucose (A. E. Jeukendrup, 2008). The number of studies concluding that CHO feedings during exercise improve exercise capacity or exercise performance is so large that, from a scientific point of view, this relationship can be considered as true (A. E. Jeukendrup & Jentjens, 2000). It is possible to compare glucose ingestion during shivering with glucose ingestion at exercise, as both shivering and exercise involve skeletal muscle contractions. Also, when shivering intensity is plotted as means of %VO₂max, and shivering plotted as relative shivering intensity (%Shiv peak), patterns of fuel selection at exercise and shivering are similar (F. Haman et al., 2005). Unlike at shivering, CHO ingestion at exercise has been widely studied and there is a good quantity of literature available. It has been shown that CHO feeding during exercise can increase exogenous CHO oxidation and decrease reliance on endogenous reserves (van Hamont et al., 2005). Most studies observed reduced liver glycogen use (Bosch, Dennis, & Noakes, 1994; Coyle, Coggan, Hemmert, & Ivy, 1986; A. E. Jeukendrup, Wagenmakers et al., 1999; McConell, Canny, Daddo, Nance, & Snow, 2000; Wallis, Dawson, Achten, Webber, & Jeukendrup, 2006), but some others did not (Couture, Massicotte, Lavoie, Hillaire-Marcel, & Peronnet, 2002; van Hamont et al., 2005). Some studies observed reduced muscle glycogen use (Couture et al., 2002; O. K. Tsintzas, Williams, Boobis, & Greenhaff, 1995, 1996; van Hamont et al., 2005) whereas others did not (Bosch et al., 1994; Coyle et al., 1986; A. E. Jeukendrup, Raben et al., 1999; A. E. Jeukendrup, Wagenmakers et al., 1999; McConell et al., 2000; Wallis et al., 2006). Exercise intensity and type, CHO dose and dosing schedule, can all affect glycogen use and could explain the differences observed by different investigators. Most important, methodological differences as mentioned previously; 1) using muscle biopsies versus isotope tracers to determine muscle glycogen use, or 2) not correcting for background isotope enrichment, can also explain the varying results.

Consequently, this study will use isotope tracers and correct for background isotope enrichment to quantify exogenous glucose oxidation in shivering individuals.

OBJECTIVES

In this thesis we will feed shivering men a $0.04 \text{ g}\cdot\text{min}^{-1}$ (control), $0.4 \text{ g}\cdot\text{min}^{-1}$ or $0.8 \text{ g}\cdot\text{min}^{-1}$ of exogenous glucose in a random cross-over design in order to compare the metabolic responses to each condition. Our objectives are 1) to confirm that heat production and core temperature during shivering will be stable with glucose feedings 2) to quantify the effects of glucose feedings on whole body fuel selection during shivering 3) to quantify the effects of glucose feedings on the oxidation of carbohydrate stores (muscle and liver glycogen) and 4) to quantify the oxidation of the ingested exogenous glucose during shivering.

HYPOTHESES

We hypothesize that 1) exogenous glucose ingestion will not alter total body heat production and core temperature 2) glucose ingestion will cause relative carbohydrate oxidation to increase and lipid and protein oxidation to decrease 3) providing exogenous glucose during shivering will increase plasma glucose oxidation in a dose-dependent matter and reduce muscle glycogen oxidation 4) the exogenous glucose ingested will be oxidized instead of oxidizing muscle glycogen.

METHODOLOGY

Subjects

Six healthy, lean young men volunteered for this study, approved by the Health Sciences Ethical Committee of the University of Ottawa. All subjects gave their informed written consent to participate in the study. The participants were not acclimatized to cold exposure. Body composition was measured using dual energy X-ray absorptiometry (DEXA) (Lunar Prodigy, GE Medical Systems). Maximal oxygen consumption (VO_2max) ($\text{ml}\cdot\text{Kg}^{-1}\cdot\text{min}^{-1}$) was measured using a progressive treadmill protocol.

Experimental sessions

The experiments were conducted between 7:30am and 12:00pm following 48h without strenuous exercise (activities of daily living only). The last evening meal was standardized (3213 kJ, 42% CHO, 28% fat and 30% protein) and subjects were asked to report to the research unit the next morning (7:30am) after a 12hour fast.

On their arrival to the laboratory, subjects were instrumented with thermal probes and a nurse placed an indwelling catheter (22-gauge, 25.4 mm, Medex, CA) in an antecubital vein for blood sampling. Subjects were fitted with a Liquid Conditioned Suit (LCS), a snug fitting suit with multiple circuits of tubing through which chilled water is circulated to cool the skin (Three Piece, Med-Eng, Ottawa, ON). Subjects were then asked to empty their bladder [time (t) _ 0 min] and lie quietly for 2 hours at ambient temperature (approx. 21°C). After this habituation period, they were transferred to the experimental room, and a 4°C water perfusion was started through the LCS by using a temperature-controlled

circulation bath (Koolant Coolers, model JT-500, MI, USA). Thermal response, metabolic rate, and fuel utilization were measured at ambient temperature and during the subsequent 2 hour cold exposure.

During each of the experiments, the subjects ingested 500 ml of water containing 5g (C), 50g (Lo) or 100g (Hi) of ^{13}C labeled glucose (Table 1). A splash of lemon juice was added to the drinks of all conditions, and Splenda© sweetener to the C and Lo preparations to blind the drinks to the participants. The drink was given in 8 equal volumes of 50ml taken every 30 min as of time (t)₀ min. A double dose was given at the start of the cold exposure [time (t)₁₂₀ min] (Figure 1). An aliquot of the ingested drink was frozen for later measurement of glucose concentration and ^{13}C labeled glucose concentration.

Thermal response

Central body temperature [tympenic temperature (T_{tymp})] was monitored continuously by using a Tympanic probe (Mon-a-therm Tympanic, Mallinckrodt Medical, St. Louis, MO). Skin temperature probes (Physitemp Instruments, Inc., NJ, USA) were also affixed to the forehead, chest, triceps, forearm, abdomen, lower and upper back, *tibialis* anterior, quadriceps, hamstrings, and finger of the right side of the body.

Whole body heat loss (H_{loss}) (in $\text{kJ}\cdot\text{min}^{-1}$) for the last 30 minutes of ambient and cold exposure was calculated as follows: (*where 60 000 converts $\text{ml}\cdot\text{min}^{-1}$ to Kg/sec)

$$H_{\text{loss}} = \text{flow rate (ml}\cdot\text{min}^{-1}) / 60000 * 4180(\text{J}/\text{kg}\cdot^{\circ}\text{C}) * (\Delta T^{\circ}) * 0.06 \text{ kJ}\cdot\text{W}\cdot\text{min}^{-1} \quad (1)$$

Table 1. Isotopic enrichment and dose of oral exogenous glucose solutions

Condition	Quantity of glucose (g)	Isotopic enrichment ($\delta^{13}\text{C PDB}^{-1}$)
Control	5	+200
Low	50	+50
High	100	+50

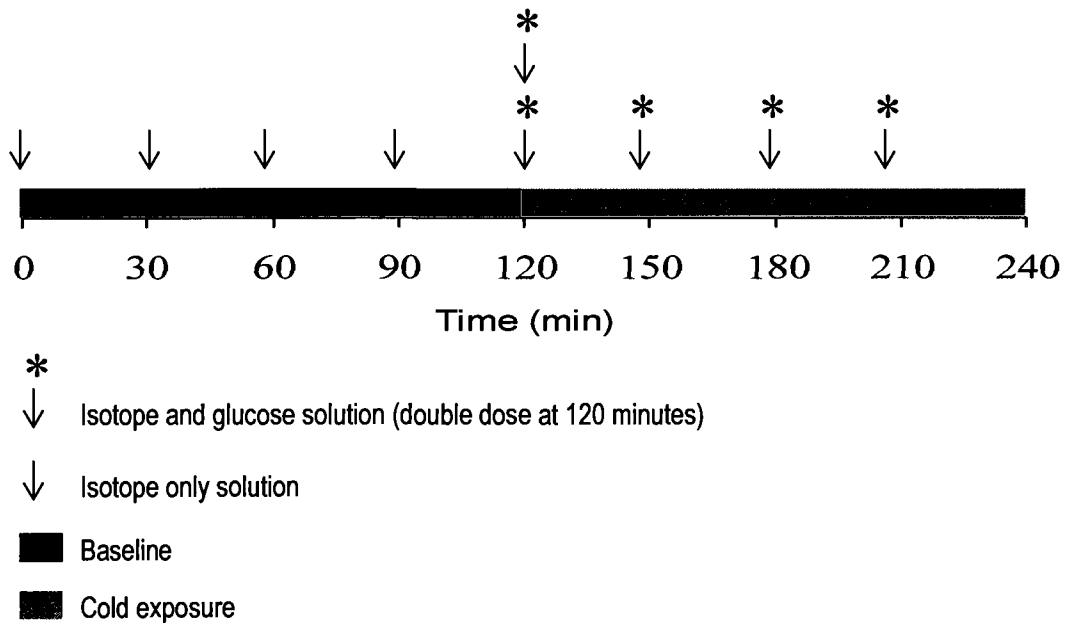


Figure 1. Protocol of a cold exposure session

Metabolic rate and fuel utilization

Ventilation (V_E), oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were determined using a calibrated metabolic cart (MOXUS, Applied Electrochemistry Inc., PA, USA). 10 ml samples of expired gases were collected from the metabolic cart's mixing chamber and stored in vacutainers (Becton Dickinson, Franklin Lakes, NJ). This was done every 15 minutes for the last 30 min of ambient and cold exposure and every 30 minutes for the remaining period of cold exposure. Total CHO (RGox), lipid (RFox), and protein (RPox) oxidation rates were calculated using the following equations (Livesey, 1988):

$$RGox (g \cdot \text{min}^{-1}) = 4.59VCO_2 (l \cdot \text{min}^{-1}) - 3.23VO_2(l \cdot \text{min}^{-1}) \quad (2)$$

$$RFox (g \cdot \text{min}^{-1}) = -1.70 VCO_2 (l \cdot \text{min}^{-1}) + 1.70 VO_2 (l \cdot \text{min}^{-1}) \quad (3)$$

$$RPox (g \cdot \text{min}^{-1}) = 2.9 * Urea_{\text{urine}} (g \cdot \text{min}^{-1}) \quad (4)$$

where VCO_2 and VO_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 $Urea_{\text{urine}}$ is urinary urea excretion. Estimates of RPox (Eq. 4) were made by measuring $Urea_{\text{urine}}$ from urine samples collected for a period of 120 min of ambient and cold exposure. A correction for urea accumulation in plasma is not required as plasma levels do not change during cold exposure (Jequier, Acheson, & Schutz, 1987). Urinary urea concentrations were determined using a commercial urine assay kit (QuantiChrom™ Urea Assay Kit, DIUR-500, BioAssay Systems, CA, USA). Respective contributions of glucose, lipid, and protein oxidation to total H_{prod} were calculated by using energy potentials of 16.3, 40.8, and 19.7 kJ/g, respectively (Elia, 1991). For the measurement of plasma glucose oxidation, the oral glucose solutions ingested by subjects was prepared from a combination of corn sugar and ^{13}C glucose, ($^{13}C/^{12}C$ 99%, Cambridge Isotopes, MA, USA) to obtain a final $^{13}C/^{12}C$ of 0.0476 isotopic composition of exogenous

glucose solution (Rexo). After baseline $^{13}\text{C}/^{12}\text{C}$ in plasma and expired CO_2 [(t)_0 min] were measured, subjects ingested the first dose of exogenous glucose solution. Isotopic composition of plasma glucose and expired CO_2 were determined in blood and expired gas samples every 15 minutes for the last 30 min of ambient and cold exposure and every 30 minutes for the remaining period of cold exposure before the ingestion of the next exogenous glucose dose. Immediately after collection, blood samples were put on ice and spun in a refrigerated centrifuge (4°C at 3000rpm for 10 minutes). Plasma was then collected, and kept frozen at -80°C until analysis.

Isotopic composition of plasma glucose (Rglu) was measured as previously described (Peronnet, Rheume, Lavoie, Hillaire-Marcel, & Massicotte, 1998). Plasma samples (800 μl) were deproteinized by incubating 20 minutes at 4°C with 1.5 ml each barium hydroxide 0.3 N and zinc sulfate 0.3 N. The soluble phase was separated from the protein precipitate by centrifugation 20 minutes at 4°C (3000rpm). Glucose was then isolated from the solution by double-bed ion exchange chromatography using glass columns prepared with approximately 3ml each AG 50W-X8 H^+ and AG1-X8 chloride resins [200–400 mesh (Bio-Rad Laboratories, Mississauga, Canada)] equilibrated and eluted with deionized water. Collected glucose eluents were then freeze dried in glass vials, reconstituted with 200 μl deionized water and 25 μl of the reconstituted solution transferred to pre-cleaned tin capsule and evaporated overnight in an incubator (37°C). Glucose and tin was combusted (60 min at 400°C) in the presence of copper oxide, and CO_2 was recovered. Measurements of $^{13}\text{C}/^{12}\text{C}$ in expired CO_2 and in CO_2 obtained from glucose combustion were determined in a prism mass spectrometer (VG, Manchester, UK). This procedure was validated by Wolfe et al. (Wolfe, Allsop, & Burke, 1979), and the material obtained after evaporation is not significantly contaminated by non-glucose carbons (Peronnet, Burelle, Massicotte, Lavoie, & Hillaire-Marcel, 1997).

Isotopic composition was expressed as ‰ difference compared with Pee Dee Belemnite-1 (PDB-1) Chicago standard with the equation of Craig: *Where R_{spl} and R_{std} are the $^{13}C/^{12}C$ ratio of the sample and standard respectively.

$$\text{‰}\delta^{13}C \text{ PDB-1} = [R_{spl}/R_{std} - 1] \times 1000 \quad (5)$$

The rate of exogenous glucose oxidation (RG_{exo} , $g \cdot \text{min}^{-1}$) was estimated from the isotopic composition of expired CO_2 (R_{exp}) and R_{exo} as follows:

$$RG_{exo} = VCO_2 [(R_{exp} - R_{ref-exp})/(R_{exo} - R_{ref-exp})] * 1/K_1 * K_2 \quad (6)$$

Where VCO_2 is in $l \cdot \text{min}^{-1}$ corrected for protein oxidation (STPD), $R_{ref-exp}$ is the $^{13}C/^{12}C$ ratio of expired CO_2 at baseline, K_1 (0.7426 l/g) is the volume of CO_2 produced from the complete oxidation of glucose (Peronnet, Massicotte, Brisson, & Hillaire-Marcel, 1990), and K_2 is the fractional recovery at the mouth of CO_2 produced in tissues. A fractional recovery of $^{13}CO_2$ at the mouth (K_2) of 0.8 and 1 were used at ambient and cold exposure, respectively (Wolfe, 1992).

Plasma glucose oxidation was calculated from $^{13}CO_2$ excretion and the isotopic enrichment of plasma glucose by using the following equation (Derman, Hawley, Noakes, & Dennis, 1996; Wolfe, 1992).

$$RG_{plasma} = VCO_2 [(R_{exp} - R_{ref-exp})/(R_{glu} - R_{ref-exp})] * 1/K_1 * K_2 \quad (7)$$

Oxidation of glucose released from the liver (RGliver) was estimated by subtracting the low rate of RGexo (Eq. 6) from RGplasma (Eq. 7). Calculation of glucose oxidation derived from glycogen stores (RGmuscle) in the tissues, either directly or through the lactate shuttle (G. Brooks, 1986), was calculated by subtracting RGplasma (Eq. 7) from RGox (Eq. 2)

Blood analysis

All plasma samples from the same individuals were analyzed in duplicate, within the same kit for each analysis. Plasma glucose concentrations were assayed using spectrophotometric analysis after conversion of glucose to glucose 6-phosphate by hexokinase. Laboratory-grade reagents (Sigma-Aldrich Canada Ltd., Oakville, Ont; Fisher Scientific Ltd., Nepean Ont.) were used for preparing a standard hexokinase reaction, and after 30 min incubation of prepared samples at room temperature, spectrophotometric analysis of resultant NADH light absorbance was performed in duplicate using a Synergy HT Series Multi-Detection Reader (Bio-Tek Instruments Inc., Highland Park, Winoosi, Vt.), with absorbance readings of 340 nm wavelength emissions. Samples collected from the three experimental sessions were analysed on the same plate. The intrassay coefficient of variation for glucose analyses was 3.4%. A 2-site ELISA immunoassay using 2 monoclonal antibodies (LINCO Research, St-Louis, Mo.) was used to measure plasma insulin levels with intraassay coefficient of variation of 3.5%. Plasma glucose concentration was measured spectrophotometrically at 340 nm on a Bio-Tek Synergy HT plate-reader (See Appendix I for assay protocol). Insulin concentration was measured by using a commercial human insulin ELISA kit (# EZHI-14K, Linco, Missouri, USA).

Statistical analyses

Statistical differences were considered significant when $p < 0.05$. All values presented are means \pm SE ($n = 6$), unless indicated otherwise. Changes in H_{prod} , T_{tymp} and T_{skin} , absolute and relative CHO, lipid and protein utilization, as well as plasma metabolite concentrations (insulin, glucose, and NEFA), were assessed by General Linear Model (one-way ANOVA) for repeated measures (effects of time, effects of condition and time x condition interactions). All General Linear Model significant differences have an observed power greater than 0.75. Differences in heat loss, as well as relative contributions of muscle glycogen, liver glycogen and exogenous glucose to total carbohydrate oxidation and total heat production were determined using paired sample t-tests. The Effect Size (d) of all significant paired sample t-test analyses is greater than 0.98.

RESULTS

Physical characteristics of the subjects are presented in Table 2.

Thermal response

Heat loss (H_{loss}), heat production (H_{prod}), tympanic temperature and mean skin temperature at baseline and during cold exposure are presented in Figure 2. Mean heat loss during cold exposure was 16.8 ± 0.4 kJ•min⁻¹, 17.9 ± 0.3 kJ•min⁻¹ and 17.4 ± 0.5 kJ•min⁻¹ for C, Lo and Hi conditions respectively (Figure 2A). There was no significant difference in heat loss (kJ•min⁻¹) between conditions. There was a significant increase ($p < 0.05$) in H_{prod} (kJ•min⁻¹) with time in all conditions, but glucose ingestion had no effect on heat production. Mean H_{prod} increased 2.8 fold during cold exposure, starting at 5.9 ± 0.2 kJ•min⁻¹ and rising to 16.8 ± 0.4 kJ•min⁻¹ (Figure 2B).

Tympanic temperature (T_{tymp}) did not significantly vary in any condition during cold exposure (Figure 2C). Mean skin temperature (T_{skin}) significantly decreased ($p < 0.05$) with time in all conditions, with no difference between conditions. Mean T_{skin} dropped 28% from 33.2°C at baseline to 23.9°C during cold exposure. Glucose ingestion had no significant effect on body temperature.

Table 2. Physical characteristics of subject. Values are means \pm SE; n = 6 subjects. VO₂max, maximal oxygen consumption.

Age (years)	24.0 \pm 2.1
Body mass (kg)	71.7 \pm 2.2
Height (cm)	177.7 \pm 1.9
Percent body fat (%)	12.1 \pm 1.4
VO ₂ max (ml · Kg ⁻¹ · min ⁻¹)	57.7 \pm 3.0

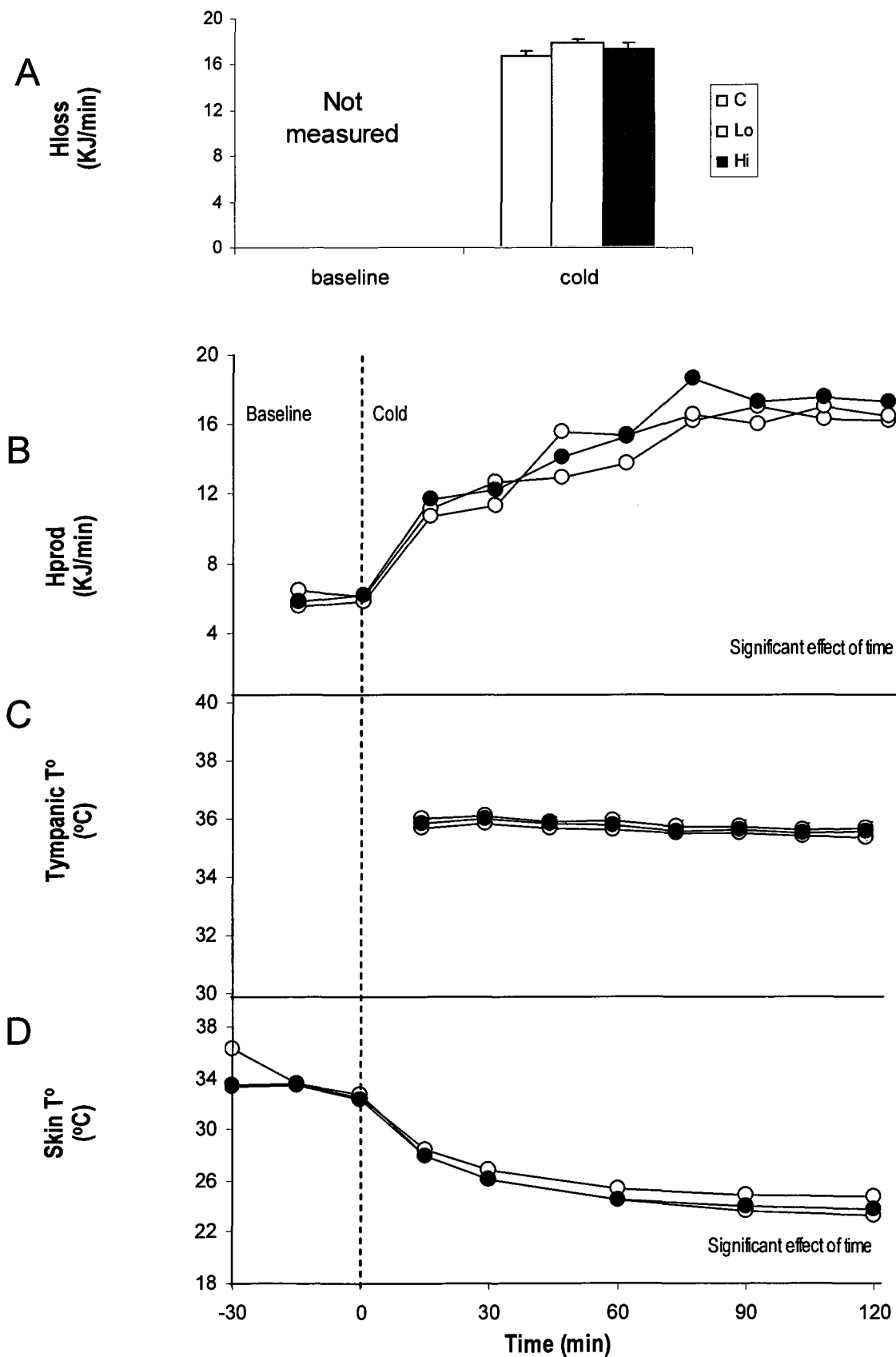


Figure 2. Changes in heat loss (Hloss, A), heat production (Hprod, B), tympanic (C) and mean skin (D) temperatures at baseline and during cold exposure for C (white) Lo (shaded) and Hi (black). Values are means \pm SE. Significantly different at $P < 0.05$.

Whole body fuel selection

Figures 3 and 4 and Table 4 present absolute and relative carbohydrate (RG_{ox}), lipid (RF_{ox}) and protein (RP_{ox}) oxidation. Absolute rates of utilization, RG_{ox} significantly increased an average of 3.3 times with time in all conditions (Figure 3A and Table 4). RF_{ox} increased 3 fold during cold exposure independently of condition, from $61.4 \pm 9.1 \text{ mg}\cdot\text{min}^{-1}$, $48.0 \pm 8.6 \text{ mg}\cdot\text{min}^{-1}$ and $44.1 \pm 11.3 \text{ mg}\cdot\text{min}^{-1}$ to $203.5 \pm 14.8 \text{ mg}\cdot\text{min}^{-1}$, $152.1 \pm 15.9 \text{ mg}\cdot\text{min}^{-1}$ and $155.0 \pm 16.8 \text{ mg}\cdot\text{min}^{-1}$ for C, Lo and Hi conditions respectively ($p < 0.05$) (Figure 3B). A small but significant effect of temperature was seen for absolute protein oxidation rates (Table 3), and a small significant effect of condition was seen at baseline only. No effect of condition was seen in the cold. (Figure 3C and Table 3).

Changes in the relative contributions of CHO ($\%RG_{ox}$), lipids ($\%RF_{ox}$), and proteins ($\%RP_{ox}$) to total heat production are shown in Figure 4. The relative contributions of carbohydrate and lipid oxidation to total heat production remain constant from baseline and through cold exposure with no statistically significant difference between conditions. (Figure 4).

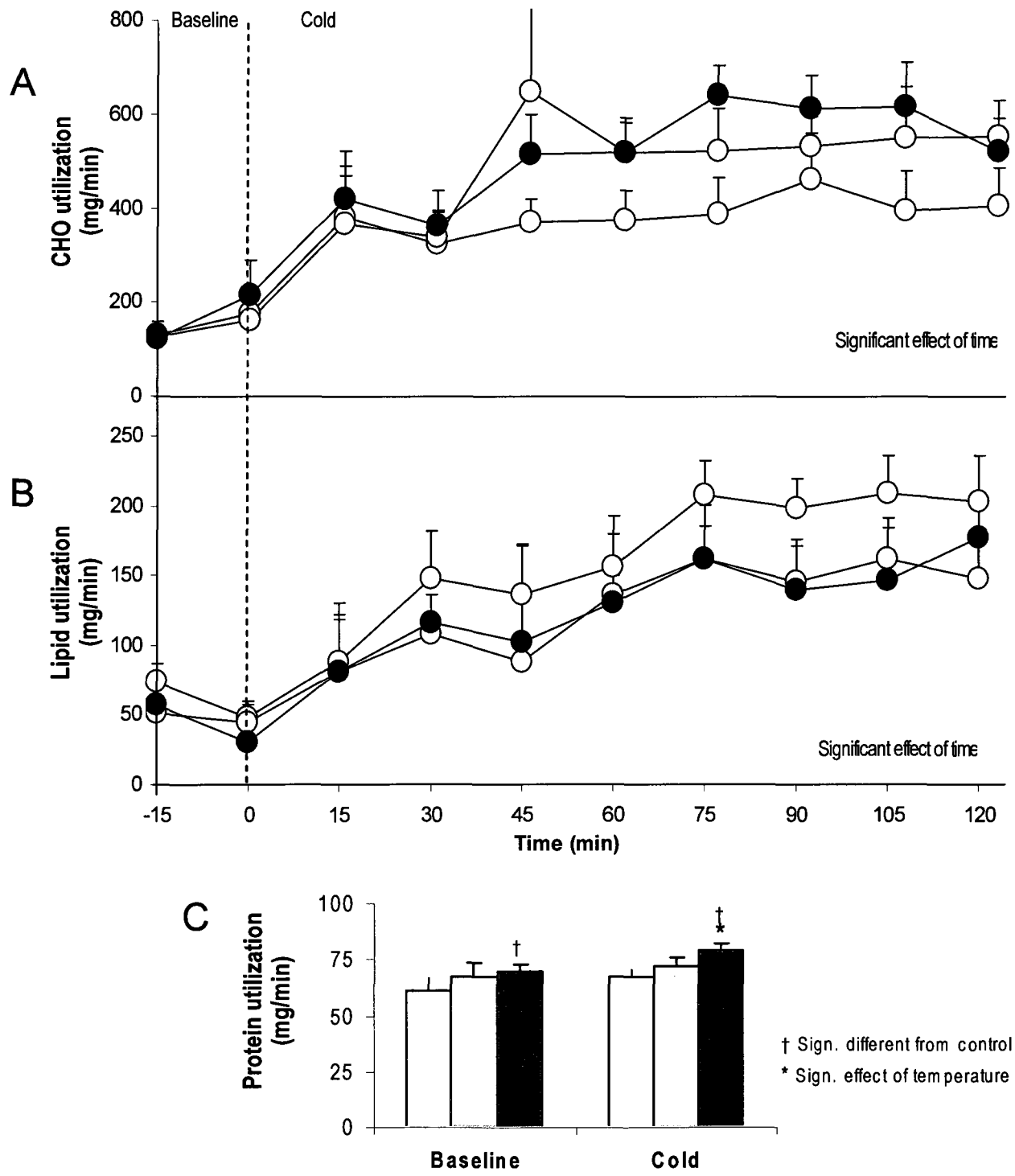


Figure 3. Absolute carbohydrate (A, CHO), lipid (B) and protein (C) utilization rates before (Baseline) and during cold exposure for C (white) Lo (shaded) and Hi (black). Values are means \pm SE. $P < 0.05$.

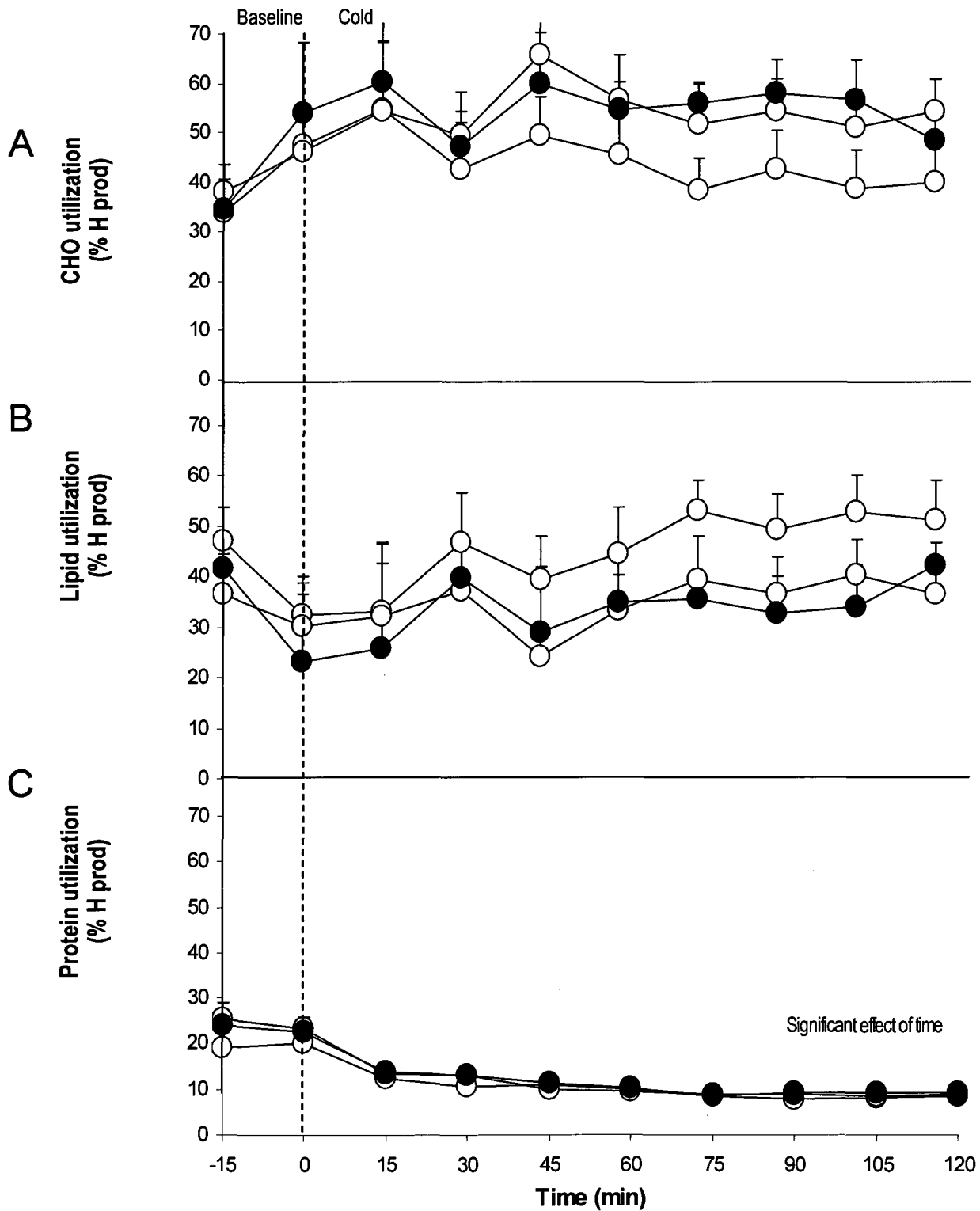


Figure 4. Relative contributions of carbohydrates (A, CHO), lipids (B) and proteins* (C) to total heat production before (Baseline) and during cold exposure for C (white) Lo (shaded) and Hi (black). Values are means \pm SE. Significantly different at $P < 0.05$.

* Protein utilization values calculated from total urea production over 120mins. Urea production is assumed to be constant throughout 120mins.

Table 3. Urinary excretion rate and absolute oxidation rate of protein in men orally ingesting traces (Control), 0.4 g•min⁻¹ (Lo) and 0.8 g•min⁻¹ (Hi) of glucose before (baseline) and during cold exposure (cold). Values are means ± SE; n = 6 subjects.

	Control		Low		Hi	
	Baseline	Cold	Baseline	Cold	Baseline	Cold
Urinary urea excretion, (g/120min)	2.6 ± 0.2	2.8 ± 0.1	2.8 ± 0.2	3.0 ± 0.2	2.9 ± 0.1	3.3 ± 0.1
Protein oxidation rate, (g/120 min)	7.4 ± 0.6	8.1 ± 0.4	8.1 ± 0.7	8.6 ± 0.4	8.4 ± 0.4	9.5 ± 0.4

Table 4. Absolute oxidation rate (mg•min⁻¹) and relative contributions to total heat production (% H_{prod}) of lipids, total CHO, liver CHO, muscle glycogen, and proteins in men orally ingesting traces (Control), 0.4 g•min⁻¹ (Lo) and 0.8 g•min⁻¹ (Hi) of glucose before (baseline; 90-120 min) and during (cold; 90-120 min) cold exposure. Values are means ± SE; n = 6 subjects. * Significantly different than Baseline; P<0.05. † Significantly different from Control; P<0.05. †† Significantly different from Control and Lo; P<0.05.

	Control			Lo			Hi		
	Baseline	Cold		Baseline	Cold		Baseline	Cold	
<i>H</i> _{prod} , kJ•min ⁻¹	6.2 ± 0.2	16.5 ± 0.5 *		5.6 ± 0.2	16.5 ± 0.6 *		6.0 ± 0.2	17.4 ± 0.6 *	
<i>H</i> _{loss} , kJ•min ⁻¹		16.8 ± 0.4			18.0 ± 0.3			17.4 ± 0.5	
Lipids									
mg•min ⁻¹	61.4 ± 9.1	203.5 ± 14.8 *		48.0 ± 8.6	152.1 ± 15.9 *		44.1 ± 11.3	155.0 ± 16.8 *	
% <i>H</i> _{prod}	39.7 ± 5.3	51.2 ± 4.1 *		33.5 ± 5.6	37.9 ± 3.9		32.4 ± 7.2	36.4 ± 3.9	
Total CHO									
mg•min ⁻¹	154.0 ± 19.8	421.5 ± 47.9 *		143.7 ± 14.7	544.8 ± 46.5 *		170.4 ± 38.1	582.5 ± 45.0 *	
% <i>H</i> _{prod}	40.8 ± 5.4	40.6 ± 4.3		42.1 ± 4.4	53.3 ± 3.7 *		44.2 ± 7.7	54.4 ± 3.8	
Liver CHO									
mg•min ⁻¹	43.6 ± 2.8	146.2 ± 11.5 *		36.9 ± 5.3	103.6 ± 5.8 *		35.6 ± 2.6	84.0 ± 6.0 * †	
% <i>H</i> _{prod}	11.4 ± 0.74	14.2 ± 1.0		10.6 ± 1.4	10.0 ± 0.4 †		9.9 ± 0.6	7.7 ± 0.5 ††	
Muscle glycogen									
mg•min ⁻¹	106.4 ± 19.4	247.7 ± 39.4 *		114.3 ± 16.3	285.2 ± 44.4 *		98.2 ± 13.8	326.8 ± 48.4 *	
% <i>H</i> _{prod}	28.3 ± 5.2	30.8 ± 5.4		33.6 ± 4.8	30.6 ± 5.2		26.7 ± 3.4	36.2 ± 6.2	
Exogenous glucose									
mg•min ⁻¹	3.7 ± 0.3	27.7 ± 1.2 *		3.3 ± 0.4	178.0 ± 17.7 * †		2.7 ± 0.3	180.5 ± 10.5 * †	
% <i>H</i> _{prod}	1.1 ± 0.1	2.7 ± 0.1 *		1.0 ± 0.2	16.9 ± 1.4 * †		0.9 ± 0.1	16.5 ± 0.5 * †	
Proteins									
mg•min ⁻¹	61.6 ± 5.4	67.1 ± 3.1		67.6 ± 5.8	72.0 ± 3.7		70.0 ± 3.1	79.2 ± 3.3 *	
% <i>H</i> _{prod}	19.6 ± 1.2	8.2 ± 0.4 *		24.3 ± 2.2	8.8 ± 0.4 *		23.4 ± 0.9	9.2 ± 0.4 *	

Assuming that urea is produced at a constant rate at during cold exposure, we calculated the relative contribution of protein oxidation to total heat production. A significant effect of time was observed, as the relative contribution of proteins to total heat production decreased significantly in all conditions from 19.6 ± 1.2 , 24.3 ± 2.2 , and 23.4 ± 0.9 % H_{prod} at baseline for C, Lo and Hi respectively to 8.2 ± 0.4 , 8.8 ± 0.4 , and 9.2 ± 0.4 % H_{prod} during cold exposure for C, Lo and Hi respectively ($p < 0.05$) (Figure 4C).

Oxidation of carbohydrate stores

Absolute and relative contributions of muscle glycogen (%GLU_{muscle}) and liver glycogen (%GLU_{liver}) are presented in Figures 5 and 6. Glucose ingestion had no effect on muscle glycogen oxidation. No significant effect of condition was observed for absolute muscle glycogen contribution (247.66 ± 71.00 mg•min⁻¹ for C, 256.21 ± 72.79 mg•min⁻¹ for Lo and 320.89 ± 70.09 mg•min⁻¹ for Hi) (Figure 6A) or relative contribution of muscle glycogen to total CHO oxidation (47.93 ± 6.56 %GLU for C, 43.59 ± 4.00 %GLU for Lo and 51.749 ± 2.99 %GLU for Hi) (Figure 6B). Muscle glycogen contribution to heat production was also not significantly different from one condition to another: 30.79 ± 9.44 % H_{prod} for C, 27.41 ± 8.42 % H_{prod} for Lo and 34.65 ± 8.53 % H_{prod} for Hi (Figure 6C).

Absolute liver glycogen oxidation was significantly lower with glucose ingestion compared to the Control condition, but only in the Hi condition (146.15 ± 20.69 mg•min⁻¹ for C vs 84.28 ± 8.85 mg•min⁻¹ for Hi and 112.59 ± 12.24 mg•min⁻¹ for Lo) (Figure 6A).

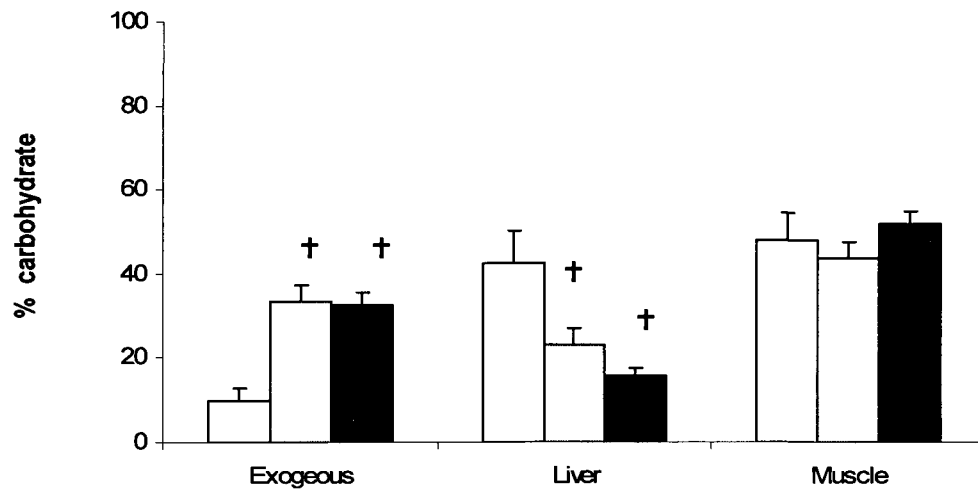


Figure 5. Relative contributions of exogenous glucose, liver glycogen and muscle glycogen to total carbohydrate utilization during cold exposure for C (white), Lo (shaded) and HI (black). Values are means \pm SE. † Significantly different from Control, $P < 0.05$.

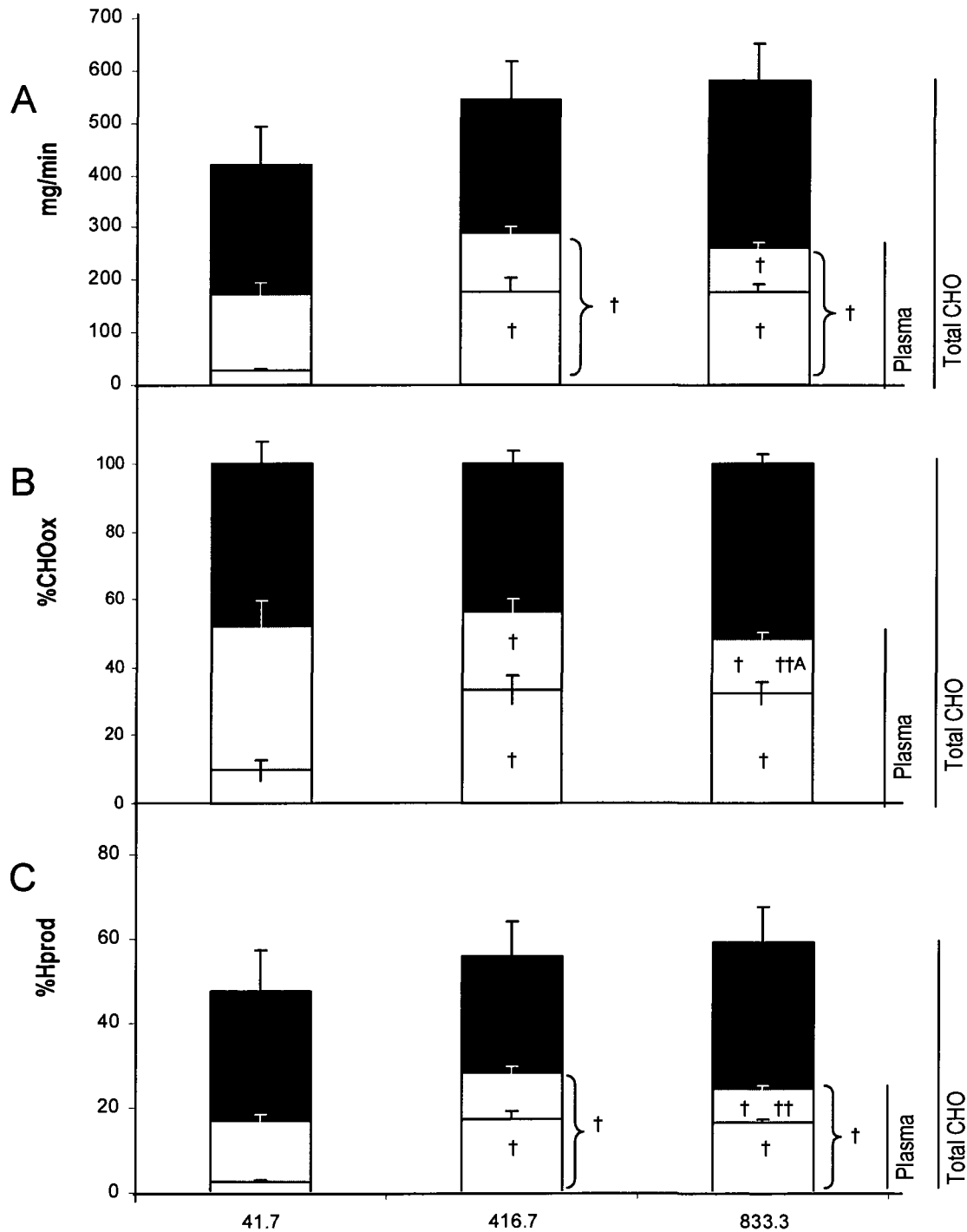


Figure 6. Absolute (A) and relative (B) contributions of exogenous glucose (*White*), liver glycogen (*Grey*) and muscle glycogen (*Black*) to carbohydrate oxidation and to total heat production (C) during cold exposure in subjects ingesting different doses of exogenous glucose. Values are means \pm SE. † Significant effect of condition compared to control ($p < 0.05$) †† Significant effect of condition compared to Lo ($p < 0.05$). A ($p < 0.1$).

However, looking at the relative contribution of liver glycogen to carbohydrate oxidation, the liver's contribution was significantly lower in both glucose ingestion conditions (23.04 ± 3.84 %GLU for Lo and 15.73 ± 2.04 %GLU for Hi) compared to the fasted state (42.39 ± 7.82 %GLU for C) ($p < 0.05$). A significant difference was also observed between Lo and Hi ($p < 0.1$) (Figure 6B). Contribution of liver glycogen to heat production (Figure 6C) was significantly lower in the Hi condition (7.91 ± 0.72 % H_{prod}) compared to both the Control and Lo conditions (14.20 ± 1.70 and 11.29 ± 1.43 % H_{prod} respectively) ($p < 0.05$). The difference between C and Lo was not significant.

Exogenous carbohydrate oxidation

A significant effect of condition for absolute contribution of exogenous glucose to carbohydrate oxidation was observed ($p < 0.05$). Absolute contribution of exogenous glucose was 6.3 times higher for Lo (176.03 ± 26.66 mg•min⁻¹) and Hi (177.29 ± 15.60 mg•min⁻¹) compared to C (27.69 ± 2.17 mg•min⁻¹) (Figure 6A). Contribution of exogenous glucose to carbohydrate oxidation was also significantly higher for Lo (33.37 ± 4.18 %GLU) and Hi (32.52 ± 3.27 %GLU) compared to C (9.68 ± 3.24 %GLU) ($p < 0.05$) (Figure 6B). Exogenous glucose contribution to heat production was 6 times higher during the Lo and Hi conditions (17.19 ± 2.08 % H_{prod} and 16.55 ± 0.75 % H_{prod} respectively) than during the Control condition (2.73 ± 0.16 % H_{prod}) ($p < 0.05$). However, it is interesting to see that no significant differences were observed, whether absolute or relative to carbohydrate oxidation or heat production, between the Lo and Hi conditions.

Plasma concentrations

Plasma concentrations of glucose, insulin and NEFA were also measured before and during shivering. Figure 7 presents changes in plasma concentrations of insulin, glucose, and NEFA. A significant condition x time interaction was observed for plasma glucose levels, in that plasma glucose concentrations increased with time for Hi, but not for C or Lo (Figure 7B). A significant condition x time interaction was observed for insulin levels. Also, a significant increase over time was observed for C, Lo and Hi. The increase in insulin was greater in Lo and Hi over time as compared to C, with no difference between the Lo and Hi conditions (Figure 7A). There was no significant change in NEFA concentrations over time and between conditions.

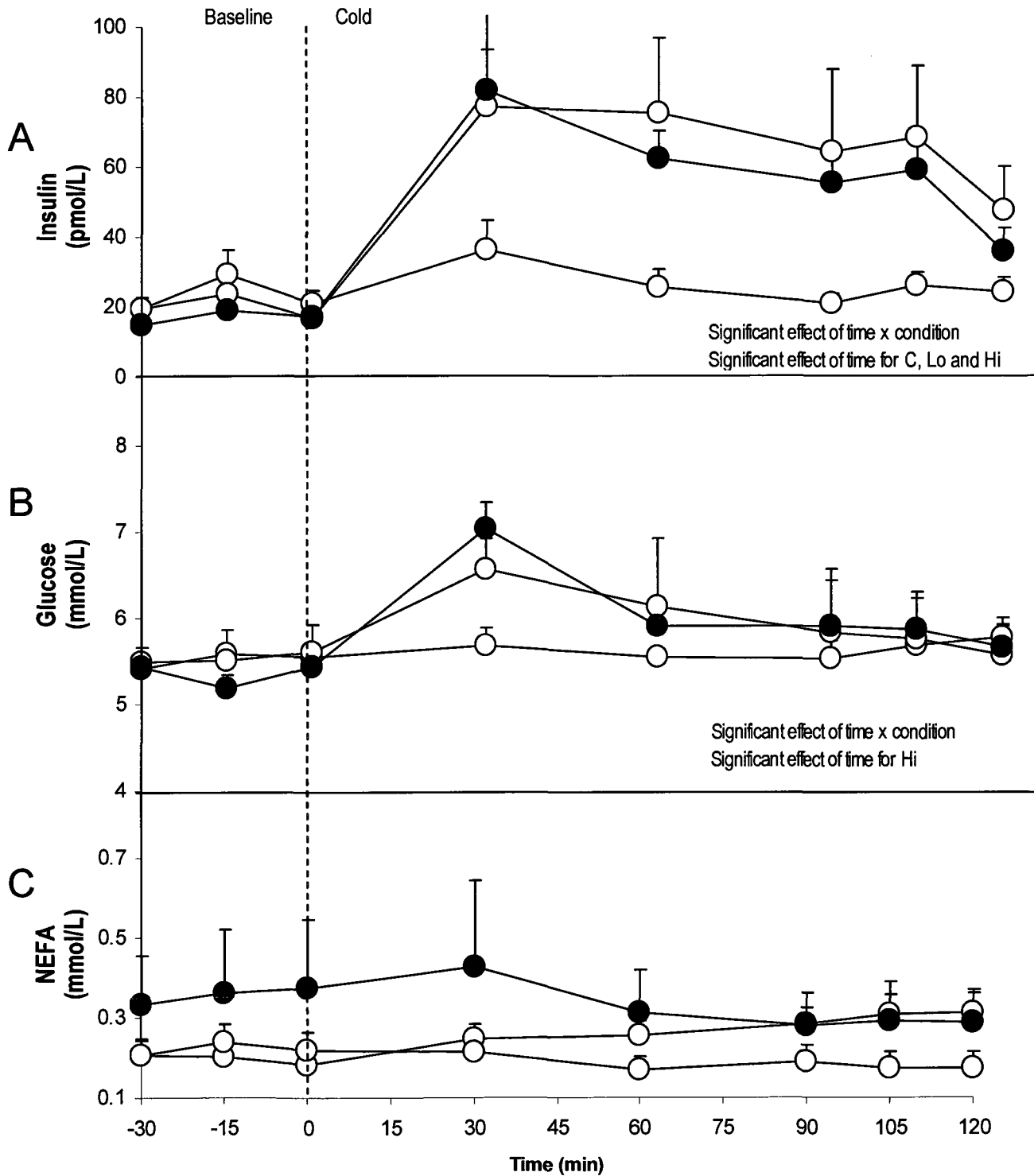


Figure 7. Plasma insulin (A), glucose (B), and non-esterified fatty acid (C, NEFA) concentrations before (Baseline) and during cold exposure for C (white) Lo (shaded) and Hi (black). Values are means \pm SE. Significantly different at $P < 0.05$.

DISCUSSION

To date, no studies have looked at the effects of glucose ingestion on fuel selection and fuel sources during cold exposure. A few shivering studies looking at fuel selection and fuel sources have emerged in the literature in the past few years (F. Haman, Legault, Rakobowchuk, Ducharme, & Weber, 2004; F. Haman, Peronnet et al., 2004; F. Haman et al., 2002; F. Haman et al., 2005). However, these studies have focused on individuals shivering with normal glycogen stores or depleted glycogen stores in the fasted state. Therefore, using a combination of indirect calorimetry and isotopic methods, the aims of this thesis were to determine the effects of glucose ingestion on whole body heat production and oxidative fuel selection. Results from this study suggest that glucose ingestion has no effects on whole body thermal response in lean, healthy shivering men. Looking at whole body fuel selection, absolute CHO and lipids oxidation rates did not change significantly, but an increase in the relative contribution of CHO to heat production was seen with glucose ingestion. Muscle glycogen oxidation was not affected by glucose ingestion, but hepatic glucose production decreased with glucose ingestion. Finally, the maximal rate of exogenous glucose oxidation observed was $0.2 \text{ g}\cdot\text{min}^{-1}$. Interestingly, no difference was seen between exogenous glucose oxidation rate of the Lo and Hi conditions.

Thermal response

Only three previous studies have quantified the effects of the ingestion of an exogenous source of energy on the energy demands of shivering humans (Glickman-Weiss et al., 1993; Glickman-Weiss et al., 1994; Vallerand et al., 1993). None have observed a change in heat production, heat loss, or core body temperatures with feeding compared to the fasted state or placebo. Other

studies investigating the thermal response in fasted shivering individuals with normal, low or high glycogen stores (F. Haman, Peronnet et al., 2004; Martineau & Jacobs, 1989b; Young et al., 1989) also observed no effects on thermal response. Consequently, we hypothesized that giving exogenous glucose would not alter total body heat production and core temperature. Results from this study confirm this hypothesis, showing no difference in heat production, heat loss, or tympanic temperature between the control and glucose feeding conditions. Subsequently, we looked at which of the macronutrients were oxidized to maintain this stable level of heat production.

Fuel selection

As previously mentioned, it has been observed that high CHO feeding increases CHO oxidation and decreases fat oxidation compared to placebo both at thermoneutrality (Tappy et al., 1986) and in the cold (Vallerand et al., 1993). In light of this, the purpose of this study was to observe and quantify the effects of two different doses of glucose feedings compared to placebo on whole body CHO, lipid and protein oxidation. Based on Vallerand's observations (Vallerand et al., 1993), we hypothesized that CHO oxidation would increase and lipid oxidation would decrease. We found that both CHO and lipid oxidation increased with time in all conditions as metabolism increased to counter the heat loss, but the relative contribution of CHO and lipids to total heat production did not change significantly with glucose ingestion (Figure 4A and 4B and Table 5). Previous studies have shown that the initial contribution of proteins as a metabolic fuel can vary from 25% to 40% of H_{prod} at baseline depending on the individual's glycogen stores and nutritional status (F. Haman, Peronnet et al., 2004; F. Haman et al., 2002; F. Haman et al., 2005). These studies have also shown that absolute rates of protein oxidation are unaffected by cold exposure. Given that the absolute rate of protein oxidation remains stable, but metabolic rate

increases with cold exposure, the relative contribution of protein oxidation to heat production usually decreases. It has been shown that the relative contribution of proteins to H_{prod} at cold exposure proportionally decreases to 10-20% of H_{prod} , again, depending on glycogen stores, nutritional status and shivering intensity (and consequently the extent of the metabolic rate increase) (F. Haman, Peronnet et al., 2004; F. Haman et al., 2002; F. Haman et al., 2005) In this study, a small but significant increase in absolute protein oxidation was observed. The reason behind this is unknown. Yet, because this absolute increase was small, the usual decrease was seen in the relative use of protein for heat production as metabolic rate increased.

Muscle glycogen vs plasma glucose

As discussed earlier, both muscle glycogen stores and plasma glucose are important sources of CHO for shivering individuals. Low CHO availability caused by hypoglycemia (Haight & Keatinge, 1973) and/or the depletion of muscle glycogen (Martineau & Jacobs, 1989b) could eventually negatively affect CHO oxidation, reduce shivering intensity, or even inhibit shivering (Gale et al., 1981; Passias et al., 1996). This would compromise thermoregulatory thermogenesis, and accelerate the drop in core temperature (Vallerand et al., 1995). The concept of glucose ingestion during shivering is also similar to the concept of glucose ingestion during prolonged exercise, where plasma glucose has also been shown to be an important substrate (A. E. Jeukendrup, Raben et al., 1999). During exercise of a duration greater than 45 min, maintenance of plasma glucose levels sustain high rates of CHO oxidation, which has the potential to improve exercise performance (Asker E. Jeukendrup et al., 2006). Also, Tsintzas and Williams suggested that ingestion of glucose at exercise could lead to two physiological mechanisms; (i) the restoration of euglycemia and consequently increased blood glucose oxidation, or (ii) decreased rate of muscle glycogen utilization (K. Tsintzas & Williams, 1998). Based on this literature, we

hypothesized that giving exogenous glucose during shivering would increase plasma glucose oxidation and consequently possibly reduce muscle glycogen oxidation, prolonging survival in the cold.

Results from this study show that the ingestion of glucose during shivering had no effect on muscle glycogen oxidation rate. Muscle glycogen fueled approximately 30% of all heat production, regardless of condition (Figures 5 and 6). Absolute muscle glycogen oxidation, relative contribution of muscle glycogen both to CHO oxidation and to total heat production, was not altered by glucose ingestion. In addition, our results show that the ingestion of glucose during shivering decreased hepatic glucose oxidation, particularly with high doses of glucose. Absolute liver glycogen oxidation was significantly lower in the Hi condition. The relative contribution of liver glycogen to total CHO oxidation was significantly lower in the glucose conditions compared to the fasting condition, the Hi condition being significantly higher than the Lo condition as well (Figure 6B). Contribution of liver glycogen to heat production decreased to almost half at high doses of glucose ingestion compared to the fasted state. If muscle glycogen oxidation did not change between conditions, but liver glycogen contribution decreased, and total CHO oxidation increased, then exogenous glucose must have contributed in some way to heat production.

Exogenous glucose

The exogenous glucose ingested during shivering contributed to total heat production, but to a lesser extent than anticipated. Although the quantity of glucose ingested in Hi ($0.8 \text{ g}\cdot\text{min}^{-1}$) was double the quantity ingested in Lo ($0.4 \text{ g}\cdot\text{min}^{-1}$), there was no significant difference in the quantity of exogenous glucose oxidized between Lo and Hi.

Figure 8A shows the rate of exogenous CHO oxidation as a function of the rate of CHO intake. The maximal rate of exogenous CHO oxidation during shivering seems to be $\sim 0.2 \text{ g}\cdot\text{min}^{-1}$ (dotted line), and seems to be independent of the rate of glucose intake at values above $\sim 0.4 \text{ g}\cdot\text{min}^{-1}$ used in Lo. It is unclear whether the rate of oxidation of ingested CHO is limited by the rate of digestion, absorption and subsequent transport of ingested glucose into the systemic blood supply, or by the rate of glucose uptake and oxidation by the shivering muscles.

Numerous studies have investigated ingestion and oxidation rates of various types of CHO during exercise. In 1994, Hawley and colleagues (Hawley, Bosch, Weltan, Dennis, & Noakes, 1994a) suggested that the rate of oxidation of glucose by muscle during exercise is probably limited to approximately $1 \text{ g}\cdot\text{min}^{-1}$, when plasma glucose concentration is 5 mmol/L and plasma insulin concentrations are not greatly increased. In 2004, Jeukendrup (A. E. Jeukendrup, 2004) plotted peak exogenous CHO oxidation rates against rates of ingestion of a large number of studies (see Figure 8B). Results show that the maximal rate at which a single ingested CHO can be oxidized is about $1.0 \text{ g}\cdot\text{min}^{-1}$ and that the optimal ingestion rate would be 1.0 to $1.2 \text{ g}\cdot\text{min}^{-1}$. Ingesting more than $1.2 \text{ g}\cdot\text{min}^{-1}$ would not result in higher CHO oxidation rates (A. E. Jeukendrup, 2004). At shivering, we observed a maximal rate of exogenous glucose oxidation of $0.2 \text{ g}\cdot\text{min}^{-1}$. What the optimal ingestion rate should be to oxidize $0.2 \text{ g}\cdot\text{min}^{-1}$ of exogenous glucose during shivering remains to be tested, as the optimal ingestion rate is most likely below $0.4 \text{ g}\cdot\text{min}^{-1}$, the lowest dose given in our study. Future studies should examine a range of glucose ingestion rates below $0.4 \text{ g}\cdot\text{min}^{-1}$ to determine the optimal glucose ingestion rate at shivering. These studies should also try to elucidate why individuals can only oxidize $0.2 \text{ g}\cdot\text{min}^{-1}$ of glucose at shivering.

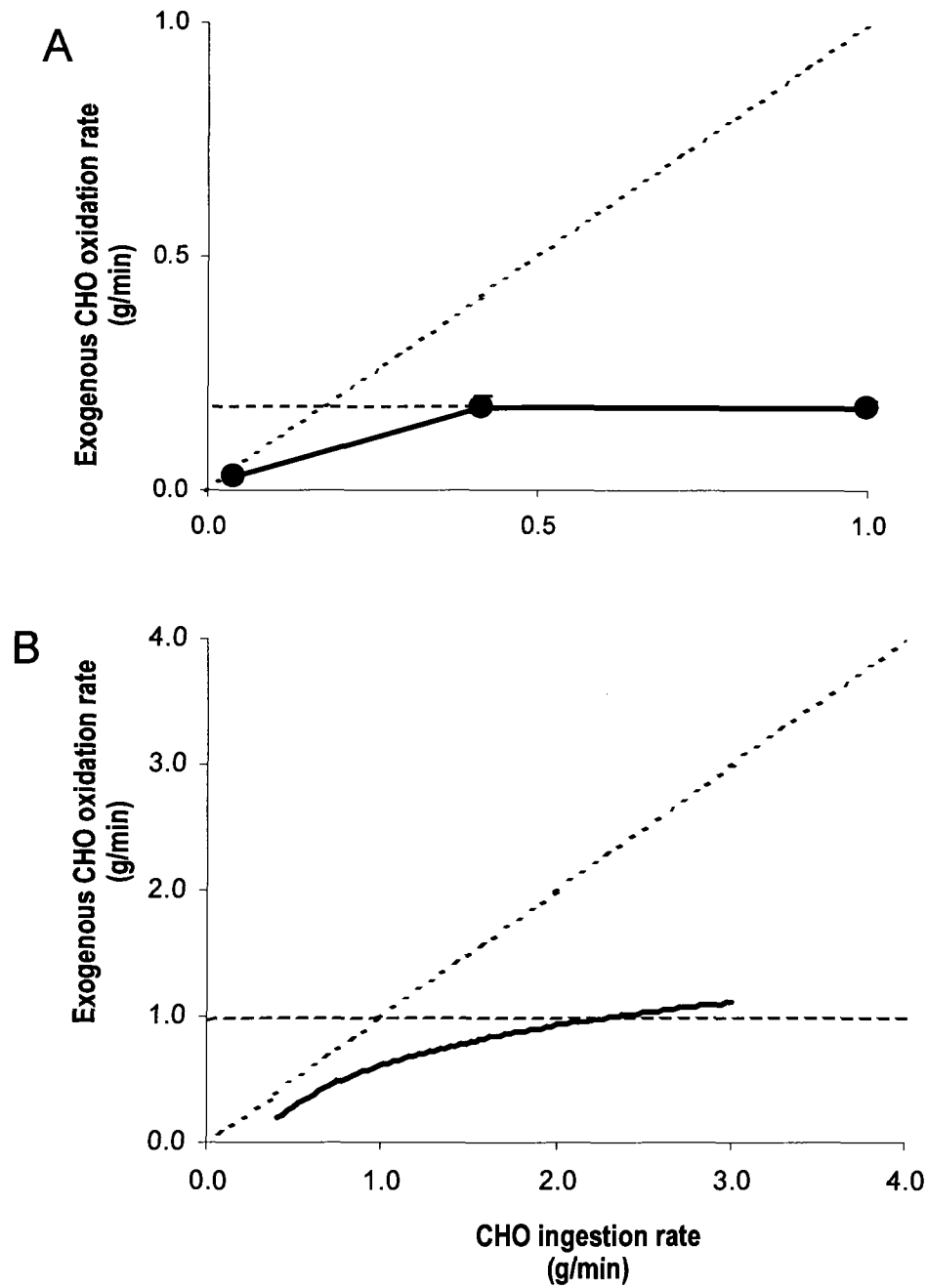


Figure 8. Exogenous carbohydrate oxidation during cold exposure (A) and at exercise (B) as a function of the rate of carbohydrate intake. The dotted line represents rate of intake at which oxidation tends to level off ($0.2 \text{ g}\cdot\text{min}^{-1}$ at cold exposure vs $1.2 \text{ g}\cdot\text{min}^{-1}$ at exercise). (A) values are means \pm SE. (B) based on Jeukendrup (2004).

The factors limiting exogenous glucose oxidation, both at exercise and during shivering, are unclear. Jeukendrup (A. E. Jeukendrup, 2004) proposed that exogenous CHO oxidation is limited by the rate of ingestion, absorption, and subsequent transport of glucose into the systemic circulation. Jeukendrup's statement was mostly based on the concept that the ingestion of a glucose and fructose mixture, or glucose and sucrose mixture, results in higher exogenous CHO oxidation rates compared with ingestion of an isoenergetic amount of glucose (Adopo, Peronnet, Massicotte, Brisson, & Hillaire-Marcel, 1994; R. L. Jentjens, Moseley, Waring, Harding, & Jeukendrup, 2004; R. L. P. G. Jentjens, Venables, & Jeukendrup, 2004). This effect is attributed to the separate transport mechanisms for glucose, fructose, and sucrose to get across the intestinal wall. However, Hawley et al. (Hawley et al., 1994a) found that intravenous glucose infusion, which bypasses digestion, absorption and subsequent transport into the systemic circulation does not result in higher rates of glucose oxidation by muscle than when glucose is ingested. This was despite similar plasma glucose concentrations, and 240 g of glucose ingested versus only 48 g infused. Hawley and colleagues' results therefore demonstrate that at exercise, the maximal rate of oxidation of exogenous glucose of approximately $1 \text{ g} \cdot \text{min}^{-1}$ is not limited by digestion, absorption and transport to the circulation, at least when plasma glucose concentrations are 5 mmol/L and plasma insulin concentrations are not greatly increased (Hawley et al., 1994a). Consequently, the maximal glucose oxidation rate of $0.2 \text{ g} \cdot \text{min}^{-1}$ in shivering individuals is also probably not limited by the rate of digestion, absorption and transport to the circulation. The interesting observation in Hawley et al.'s study (Hawley et al., 1994a) is that glucose ingestion caused plasma insulin levels to rise significantly and stay high for the length of the trial, but glucose infusion maintained baseline plasma insulin levels or even slightly decreased levels. As Hawley proposed, higher plasma insulin concentrations with CHO ingestion could have resulted from the large differences in the amounts of glucose administered to reach the same plasma

glucose levels. Consequently, in another study, Hawley and colleagues were able to increase the rate of muscle glucose oxidation to nearly $2 \text{ g}\cdot\text{min}^{-1}$ by increasing his subjects' plasma glucose concentrations to 10 mmol/L through glucose infusion (Hawley, Bosch, Weltan, Dennis, & Noakes, 1994b). This suggests that plasma glucose concentration may be the key factor regulating the rate of exogenous glucose oxidation (Hawley et al., 1994b). The fact that the body secretes insulin when glucose is ingested in order to maintain plasma glucose levels at normal levels seems to prevent the body from oxidizing more than $1 \text{ g}\cdot\text{min}^{-1}$ at exercise or $0.2 \text{ g}\cdot\text{min}^{-1}$ during shivering.

Finally, digestion, absorption and transport via circulation are not the limiting factor for CHO oxidation. Glucose oxidation can be doubled by infusing glucose, which does not cause a rise in insulin levels. It is known that insulin increases nonoxidative glucose disposal by increasing the availability of glucose transporters (GLUT-4), which enhances the uptake of glucose by tissue (Boston & Moate, 2008). Any extra glucose was thus most likely directed to non-oxidative disposal in order to maintain plasma glucose concentrations as stable as possible. It has also been shown that muscle contraction (similar to shivering) causes an increase in glucose uptake in the exercised muscles (Ivy & Holloszy, 1981; Nesher, Karl, & Kipnis, 1985). Nesher et al. (1985) observed that the increased non-oxidative disposal effects of insulin and increased glucose uptake by contractile activity were additive in rats' muscles (Nesher et al., 1985). Vallerand et al. (1987) later observed this concept in rats exposed to cold. An insulin-like effect of peripheral glucose uptake was seen in the heart, skeletal muscles, and white and brown adipose tissue (Vallerand, Perusse, & Bukowiecki, 1987). Therefore, the muscle contractions of shivering and the raised insulin levels due to glucose ingestion (Figure 7A) observed in this study support the concept that

any ingested glucose that is not oxidized during cold exposure is most likely absorbed and transported to the circulation, but sent to non-oxidative pathways.

CONCLUSIONS

Using a combination of indirect calorimetry and isotopic methods, the aims of this thesis were to determine the effects of glucose ingestion on whole body heat production and oxidative fuel selection in healthy lean young men.

Thermal response

The first objective of this thesis was to confirm that heat production and core temperature does not change with glucose feeding during shivering. We observed that ingesting exogenous glucose during shivering has no effect on total body heat production, heat loss, or tympanic temperature compared to fasting.

Whole body fuel selection

The second objective of this thesis was to quantify the effects of glucose feedings on whole body fuel selection. Both CHO and lipid oxidation increased in all conditions as metabolism increased to counter the heat loss. The relative contribution of CHO and lipids to total heat production did not change significantly with glucose ingestion.

Muscle glycogen and plasma glucose oxidation

Third, we wanted to quantify the effects of glucose feedings on whole body fuel selection during shivering. Unfortunately, our results show that the ingestion of glucose during shivering had no effect on muscle glycogen oxidation, responsible for fueling approximately 30% of heat production with or without glucose ingestion. However, ingestion of glucose during shivering

decreased hepatic glucose oxidation, particularly with high doses of glucose ingestion, where liver glycogen contribution to heat production decreased almost 50%.

Exogenous glucose oxidation

The final objective of this thesis was to quantify the oxidation of the ingested exogenous glucose. Our observations show that ingested glucose did contribute to heat production, but the maximal rate of exogenous glucose oxidation during shivering seems to be limited to $0.2 \text{ g}\cdot\text{min}^{-1}$ whether subjects ingested 0.4 or $0.8 \text{ g}\cdot\text{min}^{-1}$ of glucose. Raised plasma insulin concentrations due to glucose ingestion are thought to be the reason behind the limited exogenous glucose oxidation. By promoting non-oxidative glucose disposal to maintain normal plasma glucose levels, insulin may limit exogenous glucose oxidation at the muscle to $0.2 \text{ g}\cdot\text{min}^{-1}$.

Future perspectives

Future studies investigating oxidation of exogenous glucose oxidation during shivering could perform this protocol with lower doses of glucose ingestion. It would be interesting to see the oxidation rates at ingestion rates lower than $0.2 \text{ g}\cdot\text{min}^{-1}$. This study was performed on a limited number of healthy lean young men. Performing this study on a larger number of subjects would have required more funds and time, and would have been larger than a masters project. Consequently, future studies could also explore the substrate oxidation and exogenous glucose oxidation in women or individuals of different body compositions, such as obese individuals or glucose intolerant individuals.

APPENDIX I
GLUCOSE ASSAY PROTOCOL

Solutions Preparation:

- Start by labeling one 50 mL beaker for the Soup, one tube (10-15mL) for the Hexokinase & one microtube 1.5 mL for the glucose solution.
- Always keep all your solutions on **ice!!**

Hexokinase (HK)

- Using the balance, weigh 0.5 mg of hexokinase (HK).
- Put the Hexokinase in the tube.
- Add 2.59 mL of ddH₂O (or MilliQ H₂O).
- Vortex.

Pre-Soup (For 20 mL)

- Using the balance, weigh the following:

Name	Total Concentration (mM)	Weight (mg)	Weight (mg)
Trizma base	60	145	
Tris HCL	40	126	
MgSO ₄ , H ₂ O	1	4.9	
NaD	2	29.5	
ATP	1	12.1	

- Add these powders to the 50 mL beaker.
- In a 50 mL beaker, add 20 mL of ddH₂O (or MilliQ H₂O) using the graduated cylinder.
- Add a stir bar to the soup and mix for 5 minutes - until the powder is diluted.
- Adjust the pH @ 7.4. (Use HCl or NaOH)

Standard curves:

Glucose (2.3 mg/mL)

- Using the balance, weigh 2.3 mg of glucose
- Put the glucose in the 1.5 mL tube.
- Pipette 1 mL of ddH₂O (or MilliQ H₂O) to the tube containing the glucose.

Serial dilution

- Labeled 8 tubes (1.5 mL microtubes) with the concentration show in table 2.
- Prepare the dilution according to the following table, using the glucose solution at 2.3 mg/mL.

[Glucose] mg/mL	Glucose (uL)	ddH₂O (uL)
2.3	-	-
2.0	870 of 2.3 mg/mL	130
1.6	800 of 2.0 mg/mL	200
1.3	810 of 1.6 mg/mL	190
1.0	770 of 1.3 mg/mL	230
0.6	600 of 1.0 mg/mL	400
0.3	500 of 0.6 mg/mL	500
0	0.00	1000
Blank	0,00	1000

Assay (Microplate reader):

- Labeled the 96 wells plate. (Name, Date, Name of the experiment).
- Open the computer and the Microplate Reader.
- Load 10 μL of standards (2.3 mg/mL – 0 mg/mL)
(2 wells for each concentration).
- Load 10 μL of ddH₂O (or MilliQ H₂O) for the Blank. (2 wells)
- Load 10 μL of standard glucose solution 1 mg/mL in 4 wells.
- Load 10 μL of undiluted plasma samples in designated wells.
- Add 0.6 μL of G6PDH to the soup.
- Mix for 1 minute.
- Add 200 μL of soup (with the G6PDH) to each wells.
- Incubate 3-5 minutes.
- Read @ 340 nm for blank.
- Add 10 μL of Hexokinase (HK) to each well.
- Incubate the plate at room temperature for 30 minutes on the orbital shaker at 3000 rpm.
- Read @ 340 nm (monitoring the formation of NADH)

Quality Control

Laboratory Temperature; _____

Laboratory Humidity; _____

	<u>Lot no.</u>	<u>Réf no.</u>	<u>Exp date.</u>	<u>Compagnie</u>
<u>Hexokinase</u>	_____	_____	_____	_____
<u>Glucose</u>	_____	_____	_____	_____
<u>Trizmabase</u>	_____	_____	_____	_____
<u>Tris HCL</u>	_____	_____	_____	_____
<u>MgSO₄ . H₂O</u>	_____	_____	_____	_____
<u>NaD</u>	_____	_____	_____	_____
<u>ATP</u>	_____	_____	_____	_____
<u>G6PDH</u>	_____	_____	_____	_____
<u>Glucose stand.</u>	_____	_____	_____	_____

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EFFECTS OF GLUCOSE INGESTION ON FUEL SELECTION DURING COLD EXPOSURE

Recent studies of cold exposure have focused on the nutritional status of shivering individuals, studying the importance of carbohydrate stores for shivering muscles. No studies to date have investigated the effects glucose ingestion on fuel selection during cold exposure. Using a combination of indirect calorimetry and isotopic methods, the aims of this thesis were to determine the effects of glucose ingestion on whole body heat production and oxidative fuel selection. Six healthy lean young men were exposed to cold on three randomized single-blind occasions during which they ingested $0.04 \text{ g}\cdot\text{min}^{-1}$, $0.4 \text{ g}\cdot\text{min}^{-1}$ or $0.8 \text{ g}\cdot\text{min}^{-1}$ of glucose. Results show that whole body thermal response and total carbohydrate, muscle glycogen and lipid oxidation rates did not differ with glucose ingestion. Hepatic glucose production decreased at $0.8 \text{ g}\cdot\text{min}^{-1}$ of glucose ingestion. Finally, the maximal rate of exogenous glucose oxidation observed both at Lo and Hi was $0.2 \text{ g}\cdot\text{min}^{-1}$.

EFFETS DE L'INGESTION DE GLUCOSE SUR LA SÉLECTION DE SUBSTRATS LORS DE L'EXPOSITION AU FROID

Les récentes études sur l'exposition au froid chez l'humain discutent de l'importance des réserves de glucides disponibles lors du frissonnement. Jusqu'à maintenant, aucune étude a investigué les effets de l'ingestion de glucose au niveau des l'utilisation des substrats énergétiques lors de l'exposition au froid. À l'aide de la calorimétrie indirecte et de traceurs isotopiques, les objectifs de cette thèse étaient de déterminer les effets de l'ingestion de glucose sur la production de chaleur totale et la sélection des substrats lors du frissonnement. Six jeunes hommes furent exposés au froid à trois reprises, au cours desquelles ils ont ingéré 0.04, 0.4 et 0.8 g•min⁻¹ de glucose, de façon aléatoire. Les résultats démontrent que la réponse thermique, l'oxydation des glucides, du glycogène musculaire, et des lipides du corps entier ne changent pas lors de l'ingestion de glucose. La production de glucose hépatique diminue lors de l'ingestion de 0.8 g•min⁻¹ de glucose. Finalement, le taux maximal d'oxydation du glucose exogène observé fut de 0.2 g•min⁻¹, et ce, indépendamment de l'ingestion de glucose.