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par / by

François Haman

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OXIDATIVE FUEL SELECTION AND MUSCLE RECRUITMENT IN SHIVERING HUMANS

SUMMARY

During environmental cold exposure in adult humans, a decrease in core temperature can be prevented by increasing heat production ($\dot{H}_{prod}$) via shivering thermogenesis. The main purpose of this thesis was to determine the effects of changes in carbohydrate (CHO) availability and shivering intensity on oxidative fuel selection and muscle recruitment during cold exposure. Using a combination of metabolic and electrophysiological approaches, fuel selection and EMG activity were quantified: i) during low-intensity shivering (LOW) in individuals with normal (N), low (LO) and high CHO availability (HI), and ii) during high-intensity shivering in individuals with normal CHO availability (HIGH).

Low-intensity shivering (2.6-fold increase in $\dot{H}_{prod}$) resulted in a stimulation of plasma glucose (+138%), muscle glycogen (+109%) and lipids (+376%) oxidation rates for N (CHAPTER 2). Despite the observed increase in plasma glucose oxidation, this fuel only supplied 10% $\dot{H}_{prod}$ (or only 25% of all the glucose oxidized). Total heat production was therefore unequally shared between lipids (50%), muscle glycogen (30%), plasma glucose (10%) and proteins (10%). The same fuel selection measurements were then performed for LO and HI (CHAPTER 3). The size of CHO reserves had no effect on $\dot{H}_{prod}$, but had a major impact on fuel selection before and during shivering. In the cold, a complete shift was observed from lipid oxidation for LO (53%, 28% and 19% $\dot{H}_{prod}$ for lipids, CHO and proteins, respectively) to CHO-based metabolism for HI (23%, 65% and 12% $\dot{H}_{prod}$ for lipids, CHO and proteins, respectively). As for N, plasma

glucose oxidation was a minor fuel source (<13% $\dot{H}_{prod}$), falling to 7 % $\dot{H}_{prod}$ for LO. Therefore, plasma glucose oxidation did not compensate for changes in muscle glycogen oxidation and thus is not a strategy used for maintaining heat production. Instead, proteins and lipids compensated for the decreased in CHO availability. Most interestingly, these drastic changes in fuel metabolism were achieved without altering the electromyographic (EMG) signature of shivering muscles (CHAPTER 4). Results demonstrate that EMG shivering intensity, pattern and spectral components of eight large muscles remains unaffected by changes in fuel selection. Therefore, humans can sustain low thermogenic rates by oxidizing widely different fuel mixtures within the same muscle fibers.

Considering the low metabolic rates reached during mild shivering (LOW), oxidative fuel utilization rates were then measured at higher shivering intensity (3.5-fold rise in $\dot{H}_{prod}$) to establish whether i) the role of plasma glucose could be increased (CHAPTER 5) ii) modifications of fuel selection could be achieved via the recruitment of fuel specific muscle fibers. Plasma glucose oxidation rate was more strongly stimulated from LOW to HIGH (+122%) but this fuel still remained a minor source of heat (<15% $\dot{H}_{prod}$). In addition, during high intensity shivering, the EMG pattern was shown to provide quantitative insight into energy metabolism (CHAPTER 6). The relative use of lipids and CHO was very different between subjects, and this high variability in fuel selection was primarily explained by differences in burst shivering rate (shivering pattern associated with the recruitment of type II fibers). This finding indicates that the

recruitment of type II fibers is linked to an increase in CHO utilization and plays a key role in determining fuel selection.

RÉSUMÉ

Chez l'humain exposé au froid, le frisson constitue la source principale de production de chaleur pour prévenir une baisse de la température corporelle. Le but principal de cette thèse est de déterminer les effets d'une modification des réserves d'hydrates de carbone (HCO) et de l'intensité du frisson sur l'utilisation des carburants métaboliques et le recrutement des fibres musculaires. À l'aide de méthodes de mesures métaboliques et électrophysiologiques, le choix des carburants métaboliques et l'activité électromyographique (EMG) ont été quantifiés lors du frisson: i) de faible intensité (LOW) chez des individus avec des réserves normales (N), basses (LO) et élevées (HI) d'HCO, et ii) de haute intensité chez des individus avec des réserves normales de glycogène (HIGH).

Le frisson de faible intensité chez N (augmentation de 2.6-fois du taux de production de chaleur ($\dot{H}_{prod}$)) a eu pour effet d'augmenter l'oxydation du glucose plasmatique, du glycogène musculaire et des lipides (CHAPITRE 2). Malgré cette augmentation de l'utilisation du glucose plasmatique, ce carburant n'a fourni que 10% $\dot{H}_{prod}$ (ou seulement 25% du glucose oxydé). Conséquemment, la chaleur totale produite dans le froid a été fournie principalement par l'oxydation des lipides (50%) et du glycogène musculaire (30%) et de façon plus mineure, du glucose plasmatique (10%) et des protéines (10%). Ces mêmes mesures d'oxydation ont ensuite été effectuées chez LO et HI (CHAPTER 3). Les résultats démontrent que les changements des réserves de glycogène n'ont eu aucun effet sur le $\dot{H}_{prod}$ mais un effet majeur sur le choix des

carburants métaboliques avant et après l'exposition au froid. Dans le froid, l'oxydation des carburants a été dominée par celle des lipides pour LO (53% pour les lipides, 28% pour les HCO et 19% $\dot{H}_{prod}$ pour les protéines) alors que les HCO furent principalement oxydés lors chez HI (23% pour les lipides, 65% pour les HCO et 12% $\dot{H}_{prod}$ pour les protéines). Tel que chez N, le glucose plasmatique est demeuré une source mineure de chaleur, diminuant même à 7% $\dot{H}_{prod}$ dans le cas de LO. Les changements du taux d'oxydation du glycogène musculaire n'ont pas été compensés par une augmentation de l'utilisation du glucose plasmatique mais par celle des lipides et des protéines. De plus, ces changements importants dans le choix des carburants ont été accomplis sans modification de la signature électromyographique (EMG) des muscles actifs (CHAPTER 4). Dans l'ensemble ces résultats démontrent que l'intensité et le patron du frisson ainsi que les composantes spectrales du signal EMG de huit muscles majeurs demeurent inchangés indépendamment de l'importante modification dans le choix des carburants. Ces résultats suggèrent que l'humain puisse soutenir une thermogenèse de faible intensité en utilisant une combinaison très variable de carburants à l'intérieur de mêmes fibres musculaires.

Au vu du faible taux métabolique atteint lors du frisson de faible intensité (LOW), l'utilisation des carburants métaboliques a également été étudiée à plus haute intensité (augmentation de 3.5 fois du $\dot{H}_{prod}$) afin de déterminer i) si le rôle du glucose plasmatique pouvait être augmenté (CHAPITRE 5) et ii) si la sélection des carburants pouvait être modifiée en recrutant différentes fibres spécialisées dans l'utilisation de carburants spécifiques. Une plus grande augmentation de l'utilisation du glucose

plasmatique a été observée de LOW à HIGH (+122%) bien que ce carburant soit demeuré une source mineure utilisée dans la production de chaleur (<15% $\dot{H}_{prod}$). De plus, durant la thermogenèse à haute intensité, le patron du signal EMG fournit de l'information quantitative sur le métabolisme énergétique (CHAPITRE 6). En effet, l'utilisation relative des lipides et des HCO semble être fonction du taux de bouffée de frisson (patron de frisson associé au recrutement des fibres de types II). Dans l'ensemble, ces résultats suggèrent que le recrutement des fibres de types II est lié étroitement à une augmentation de l'utilisation des HCO et joue un rôle clé dans la détermination du choix de carburants métaboliques au cours de l'exposition au froid.

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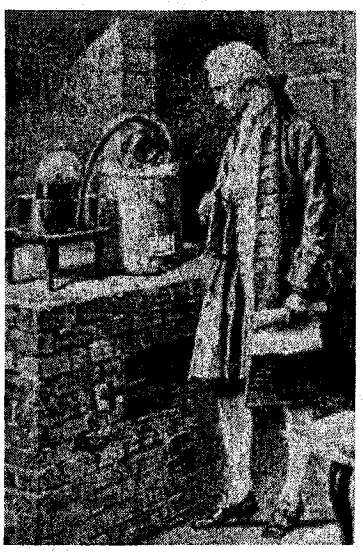


Antoine-Laurent de Lavoisier (1743-1794)

"La vie n'est qu'une combustion lente de carbone et d'hydrogène, qui est semblable en tout à celle qui s'opère dans une lampe ou dans une bougie allumée et, sous ce point de vue, les animaux qui respirent sont de véritables corps combustibles qui brûlent et se consument[1]....Comme c'est la substance même de l'animal, c'est le sang, qui fournit le combustible, si les animaux ne réparaient pas habituellement par les aliments ce qu'ils perdent par la respiration, l'huile manquerait bientôt à la lampe, et l'animal périrait, comme une lampe s'éteint lorsqu'elle manque de nourriture[2]....L'égalité de température à laquelle l'homme riche parvient avec tant de peines et de difficultés en réunissant les productions des deux hémisphères, la soie de l'Inde et la laine d'Espagne, en employant les travaux d'une multitude d'hommes employés à tisser des étoffes précieuses, la nature l'opère en faveur du pauvre d'une manière plus simple, et qui ne le met dans la dépendance de personne: elle accélère sa respiration dans la juste mesure de son besoin.[3]"

[1] Oeuvres, tome II, p. 691

[2] Oeuvres, tome II, p. 696

[3] Oeuvres, tome V, p. 388

«La vérité scientifique sera toujours plus belle que les créations de notre imagination et que les illusions de notre ignorance»

Claude Bernard (1813-1878)

CHAPTER 1. GENERAL INTRODUCTION

SURVIVAL IN THE COLD

FUELING SHIVERING THERMOGENESIS

Throughout evolution, living organisms have developed strategies to survive in ever changing environmental temperatures. These temperature adaptations provide short- and long-term solutions to defend cells against loss of function by conserving appropriate macromolecular structural parameters and by maintaining adequate metabolic fluxes and regulation (Hochachka and Somero, 1984). In the animal kingdom, the vast majority of species (*i.e.* ectotherms) have been extremely successful at assuring their survival at various temperatures by maintaining their core temperature in close relation to ambient temperature. A second group of animals, endotherms (*i.e.* birds and mammals), also managed well by regulating body temperature within a narrow range at a specific set point ranging from 36 to 38°C (Schmidt-Nielsen, 1990). Core temperature fluctuations exceeding lower or upper critical limits can have disastrous consequences that may result in permanent cell damage or even death. Even though the exact advantages of endothermy are not well understood, it is clear that maintaining a high body temperature within such a narrow range comes with considerable energy cost (Hochachka and Somero, 1984).

Endotherms regulate body temperature by adjusting rates of heat gain and heat loss using a combination of metabolic, hormonal and neural mechanisms (Himms-Hagen, 1996). Internal (*i.e.* metabolic heat production, hormone secretion, autonomous nervous system activation) or external factors (*i.e.* radiation, convection, conduction, evaporation) can shift this balance resulting in an increase or a decrease in core temperature (Brooks *et al.*, 1999, Schmidt-Nielsen, 1990). In other words, thermal responses modulate the flow of body heat to meet the need to warm or cool the body. In comparison to other

homeotherms, humans are particularly adept at dissipating heat and less efficient at conserving it (Armstrong, 2000, Vallerand *et al.*, 1988). Consequently, without proper shelter or protective clothing, human survival in the cold is limited to hours or just a few days depending on the severity of the condition.

During whole body cooling, humans like many other homeotherms prevent decreases in core temperature by increasing internal heat production. In adult humans, this process primarily takes the form of involuntary rhythmic contractions of skeletal muscle myofibrils or shivering thermogenesis (ST). However, some small (*i.e.* insectivores, hibernators and non-hibernators) and large mammals (*i.e.* newborn lambs, cattle, goats, dogs, reindeer, young monkeys) may also produce a significant amount of internal heat via nonshivering thermogenesis (NST). This process is found in brown adipose tissue (BAT), a multilocular lipid storing tissue with abundant mitochondria and uncoupling proteins (UCP). Once stimulated by the presence of fatty acids, UCP provoke heat production through the translocation of fatty acid anions into the mitochondrial matrix (Himms-Hagen, 1996, Himms-Hagen and Ricquier, 1998). BAT is also found in large quantities in various sites in newborn human infants (Himms-Hagen and Ricquier, 1998). During mild cold exposure, the metabolic rate of these newborns can be increased by close to 2-fold solely on the thermogenic action of BAT (Himms-Hagen, 1996). However, with age, BAT accumulates lipids, becomes unilocular and is practically indistinguishable from metabolically inactive white adipose tissue (WAT). In adult humans the contribution of BAT to total heat production remains controversial. Measurements of UCP concentration in adult adipose tissue suggests that the maximal

contribution of BAT to total heat production is minimal (1 or 2%) (Himms-Hagen, 1996). Consequently, ST is always assumed to be the largest (if not the only) source of heat in non-exercising adult humans exposed to the cold.

FUELING SHIVERING THERMOGENESIS

Involuntary muscle contractions during shivering are fueled by a combination of carbohydrates (CHO), lipids and proteins. To produce ATP within working muscles, these fuels may be provided from two possible sources: i) uptake from the circulation and ii) *in situ* utilization of intra-muscular reserves. During cold exposure, little is known about the relative importance of these two sources because oxidation rates have never been quantified directly.

Carbohydrates. Even though CHO represent only a minor fraction of total energy availability in mammals (~1% of total energy reserves), they remain an essential fuel source for energy metabolism. CHO are also the only fuel source that can be used to produce adenosine triphosphate (ATP) at high rates under aerobic (30 μmol $ATP \cdot g^{-1} \cdot min^{-1}$) and anaerobic conditions (60 μmol $ATP \cdot g^{-1} \cdot min^{-1}$). In contracting skeletal muscles, CHO oxidation is fueled by two possible glucose sources i) uptake of plasma glucose provided by hepatic output (via glycogenolysis and gluconeogenesis) and/or ii) in situ utilization of muscle glycogen reserves.

During cold exposure, plasma glucose and muscle glycogen have both been shown to play significant roles in heat production (Jacobs et al., 1994). Vallerand *et al.*

(1995, 1999a) calculated that plasma glucose and muscle glycogen would contribute equally to total CHO oxidation assuming that 100% of hepatic glucose production (R_aGLU) is oxidized. However, as pointed out by these authors, at such low rates of oxygen consumption this assumption is probably not met because non-oxidative glucose disposal could be important. Studies where measurements of R_aGLU and plasma glucose oxidation were carried out simultaneously indicate that non-oxidative disposal ranges between 25% R_aGLU during submaximal exercise (45% $\dot{V}O_{2max}$) and 70% R_aGLU at rest (Friedlander et al., 1997). Under these conditions, neglecting to subtract nonoxidative glucose disposal from R_aGLU may cause a significant overestimation of glucose oxidation rates.

A series of studies have also shown that 90 min of cold-water immersion causes a reduction of glycogen concentration in biopsies from the *vastus lateralis* (Jacobs *et al.*, 1985, Martineau and Jacobs, 1988, Martineau and Jacobs, 1989a, Martineau and Jacobs, 1989b, Martineau and Jacobs, 1991). Unfortunately, estimating total use of muscle glycogen, at the whole-organism level, from small biopsy samples is inaccurate at best, because: i) glycogen content varies greatly between and within individual muscles and, ii) the specific muscles involved in heat generation and their level of recruitment are unknown. Therefore, the exact contributions of plasma glucose and muscle glycogen are presently unclear because their rates of oxidation have never been measured directly during shivering.

Lipids. Lipid oxidation generally accounts for a large fraction of energy metabolism in most tissues. Circulatory lipids are transported from the gut, and from hepatic or adipose tissue stores to tissue mitochondria. To allow significant circulatory transport, these lipids must be bound to a plasma protein (Peters Jr and Davidson, 1991), thereby increasing their solubility in plasma. Similarly, lipid transport in the aqueous cytosol is mediated by smaller cell-specific fatty acid binding proteins (FABP) (Clarke and Armstrong, 1989, Sweetser *et al.*, 1987).

During cold exposure, the dual role of lipids as a heat insulation layer and as a large, energy-dense, metabolic fuel (>95% of total energy stored) has been recognized for a long time (Schmidt-Nielsen, 1990). In terrestrial and marine mammals, very large quantities of lipids can be stored in subcutaneous and abdominal adipocytes. This thick layer of blubber protects very efficiently against rapid changes in body temperature by reducing heat conductance. Consequently, increasing the subcutaneous fat layer is an important strategy for improving survival time in the cold. Moreover, a large increase in the relative use of lipids can maintain heat production for a longer period of time, and, therefore, also improve chances of survival.

Little is known about the relative importance of circulatory non-esterified fatty acids (NEFA) and intra-muscular triacylglycerol (TAG) reserves for thermogenesis. However, indirect evidence suggests that both sources may play a significant role in heat production. Recently, Vallerand *et al.* (1999a) reported that NEFA turnover rate increases by more than 3 fold in the cold even exceeding total fat oxidation by 2-fold. This observation suggests that non-oxidative disposal is extremely high but the exact fate

of this fuel source is still unclear. Direct measurements of NEFA oxidation rates would be needed to clarify this issue. In another study, the importance of intra-muscular TAG for sustaining heat production was shown to be as equally important (Martineau and Jacobs, 1991). Plasma NEFA availability was reduced by the administration of nicotinic acid (inhibitor of lypolysis) in subjects with low glycogen reserves. Independently of these important decreases in metabolic fuel availability, total heat production remained unaffected and lipid oxidation still represented 52% of all the heat produced. Whether this response is compensatory remains unclear, but it indicates that humans are capable of sustaining their thermogenic rate using a variety of fuel mixture.

Proteins. Proteins are not only required to form body structures and enzymes but specific amino acids such as alanine, leucine and glutamine also play an essential role for energy production (i.e. prolonged exercise, fasting) as substrate for gluconeogenesis (Brooks et al., 1999). Complete oxidation of amino acids has an energy potential slightly higher than that of glucose (~20 $kJ \cdot g^{-1}$ for proteins vs. 16.3 $kJ \cdot g^{-1}$ for glucose). The contribution of protein oxidation to shivering thermogenesis is generally assumed to be negligible, and, therefore, is rarely measured directly (Haman *et al.*, 2002, Vallerand *et al.*, 1995, Vallerand *et al.*, 1999a).

FUEL SELECTION IN THE COLD

As for exercise, CHO and lipids are the main sources of substrate for sustaining shivering thermogenesis whereas the contribution of proteins remains minor (<10% $\dot{H}_{prod}$) (Jacobs et al., 1994, Vallerand et al., 1995, Vallerand and Jacobs, 1989). Unfortunately, the respective importance of CHO and lipid oxidation in the cold has never been clearly established (Weller *et al.*, 1998). For example, some researchers imply that CHO is the preferred fuel source in the cold (~60% of total heat production) (Glickman-Weiss *et al.*, 1993, Glickman-Weiss *et al.*, 1994, Jacobs, 1997, MacNaughton *et al.*, 1990, Vallerand *et al.*, 1995, Vallerand and Jacobs, 1989, Vallerand and Jacobs, 1990, Vallerand *et al.*, 1989, Vallerand *et al.*, 1993, Vallerand *et al.*, 1999a), while others show a greater reliance on lipids (~60% of total heat production) (Jacobs, 1997, Martineau and Jacobs, 1988, Martineau and Jacobs, 1989a, Martineau and Jacobs, 1989b, Tikuisis et al., 2000b, Weller et al., 1998). Possible reasons for such discrepancies between studies are differences in shivering intensity, cooling protocol and/or nutritional state (i.e. CHO availability).

A comparison of CHO and lipid oxidation rates measured in these 16 studies were recalculated from non-protein respiratory exchange ratios measured between 60 and 90 min of cold exposure and plotted in Figure 1.1. Initially, this comparison was done independently of differences in shivering intensity, cooling protocol or nutritional state. This analysis suggests that both CHO and lipids provide equally to total heat production.

Far more interesting patterns of fuel selection emerge when shivering intensity and cooling protocols are considered.

Shivering intensity. The effect of shivering intensity on the respective roles of CHO, lipids and proteins is still unknown (Weller et al., 1998). When fuel selection measurements from previously mentioned studies are plotted as a function of shivering intensity (Fig. 1.2), it is very interesting to note that the reported dominance of either CHO or lipids has no clear link with differences in metabolic rate. This finding is difficult to reconcile with the well-established fuel selection pattern found during exercise where the ratio of CHO to lipid utilization rates is well known to increase with work intensity (Brooks et al., 1999). In addition, during low intensity shivering, results are conflicting with most studies reporting a greater reliance on CHO (~60 $\%H_{prod}$) (Glickman-Weiss *et al.*, 1993, Glickman-Weiss *et al.*, 1994, Jacobs, 1997, MacNaughton *et al.*, 1990, Vallerand *et al.*, 1995, Vallerand and Jacobs, 1989, Vallerand and Jacobs, 1990, Vallerand *et al.*, 1989, Vallerand *et al.*, 1993, Vallerand *et al.*, 1999a) and another on lipids (~60 $\% \dot{H}_{prod}$) (Weller *et al.*, 1998). In contrast, during high intensity shivering, results consistently report that lipids are the dominant fuel source (~60% of $\dot{H}_{prod}$, (Jacobs, 1997, Martineau and Jacobs, 1988, Martineau and Jacobs, 1989a, Martineau and Jacobs, 1989b, Tikuisis *et al.*, 2000b).

If one considers the relatively low metabolic rates reached during shivering (15-42% $\dot{V}O_{2max}$), a high reliance on lipid oxidation, even during high intensity shivering, would not be that surprising. Exercise studies reveal that lipid oxidation predominates

for prolonged work at all intensities below 50% $\dot{V}O_{2max}$ (Bergman and Brooks, 1999, Brooks *et al.*, 1999, Roberts, 1996). Therefore, even at the highest possible metabolic rates reached during maximum shivering (about 5 times RMR or ~40% $VO_{2\,max}$; (Eyolfson et al., 2001)), lipids may still play a significant role in heat generation, if fuel selection patterns are identical between exercise and shivering (Fig. 1.3). However, exercise and shivering are not necessarily analogous (Tipton *et al.*, 1997) and additional work would be needed to establish more clearly the pattern of fuel selection during cold exposure.

Cooling protocol. Cold exposure studies have employed a variety of procedures to study metabolic responses. These include cold water immersion, cold air exposure and the use of a liquid conditioned suit (or water perfusion suit). In a final analysis, the relative contributions of CHO and lipids to total heat production were plotted as a function of cooling method (Fig. 1.4). Interestingly, a close correlation between cooling protocol and the dominance of either CHO or lipids is clearly observed. While subjects exposed to cool air used CHO preferentially (~60% of $\dot{H}_{prod}$, (Glickman-Weiss *et al.*, 1993, Glickman-Weiss *et al.*, 1994, Jacobs, 1997, MacNaughton *et al.*, 1990, Vallerand *et al.*, 1995, Vallerand and Jacobs, 1989, Vallerand and Jacobs, 1990, Vallerand *et al.*, 1989, Vallerand *et al.*, 1993, Vallerand *et al.*, 1999a)), those cooled by water immersion or by liquid conditioned suit (LCS) favored lipid utilization (~60% of $\dot{H}_{prod}$, (Jacobs, 1997, Martineau and Jacobs, 1988, Martineau and Jacobs, 1989a, Martineau and Jacobs, 1989b,

Tikuisis et al., 2000b, Weller et al., 1998)). Physiological reasons for such a difference are unclear and further research would be needed to explain it.

CHO AVAILABILITY AND FUEL SELECTION

CHO oxidation can account for up to 60% of total heat production during cold exposure (Jacobs *et al.*, 1994). Modifying glycogen availability has a profound effect on fuel selection during shivering, to the extent that a complete shift from CHO dominance to lipid dominance can be elicited (Martineau and Jacobs, 1989b, Young *et al.*, 1989). Several studies show that shivering humans (prolonged immersion in 18°C-water) produce ~80% of total heat from CHO oxidation when glycogen reserves are high, and the same percentage - *but from lipid oxidation* - when glycogen reserves are depleted (Martineau and Jacobs, 1989b, Young *et al.*, 1989). Usually, such drastic changes in fuel selection have no effect on cold tolerance because total heat production appears to be independent of glycogen availability (Martineau and Jacobs, 1989b, Young *et al.*, 1989) (Figure 1.5). This important observation indicates that a compensatory shift to a greater use of lipids (and possibly proteins even though their oxidation rate was never measured in these studies) occurs when glycogen reserves are depleted. In addition, this observation suggests that CHO availability may not be essential for sustaining thermogenesis.

Two separate sources of CHO are available for heat production: hepatic glucose provided to shivering muscles by the circulation, and muscle glycogen. Only two papers have addressed the problem of oxidative fuel selection in relation to altered glycogen

reserves (Martineau and Jacobs, 1989b, Young *et al.*, 1989). During high intensity shivering, Young *et al,* (1989) reported no change in muscle glycogen levels in glycogen-depleted and glycogen-loaded individuals. In the other study, Martineau *et al,* (1989b) found a significant increase in glycogen utilization rate (1 $mmol \cdot kg^{-1}$ dry muscle $mass \cdot min^{-1}$) when CHO reserves were high but no significant change when they were low. The exact reasons for these discrepancies are unclear, but methodological limitations need to be considered. As mentioned previously, it is well known that estimating whole-body glycogen utilization from muscle biopsies is extremely difficult because: 1) glycogen concentration is variable within and among muscles and, 2) the relative contribution of *vastus lateralis* to total shivering activity is not known. Clearly, more work would be needed to provide information on the effect of glycogen availability on the relative importance of plasma glucose and muscle glycogen to total CHO oxidation.

MUSCLE FIBER RECRUITMENT AND FUEL SELECTION

To sustain shivering thermogenesis over prolonged periods of cold exposure, muscle recruitment and fuel metabolism must be tightly coordinated. Modifying fuel selection in contracting muscles can be acheived in 2 ways: i) by mobilizing different metabolic pathways within the same fibers, or ii) by recruiting distinct fiber populations specialized for different fuels. Over the last several decades, mechanisms of motor unit (MU) recruitment during voluntary contractions have received a lot of attention (see Gardiner, 2001, Linnamo *et al.*, 2003, Wakeling *et al.*, 2002 for review), but very few

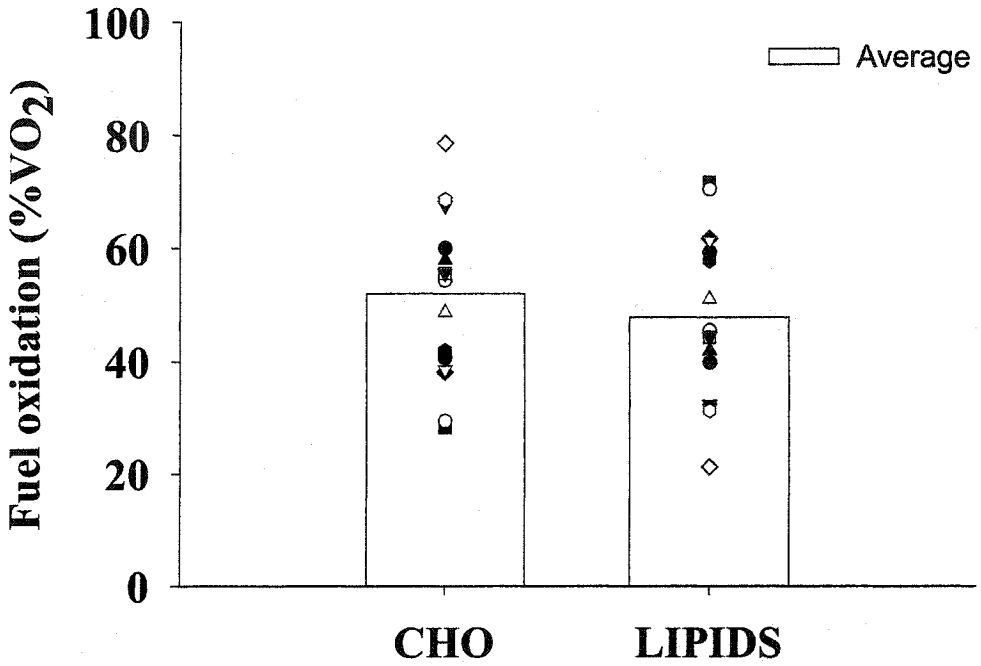
studies have investigated this process during shivering (Meigal, 2002, Meigal *et al.*, 1993, Meigal *et al.*, 1995, Petajian and Williams, 1972).

During exercise, fiber recruitment occurs according to the "size principle" described for the first time almost four decades ago (Henneman et al., 1965). This principle stipulates that muscle fiber recruitment is determined by force requirements, with smaller and slow muscle fibers (Type I) being activated for low-force contractions and increasingly larger and fast muscle fibers (Type II) being progressively activated to supply greater force (Brooks *et al.*, 1999, De Luca *et al.*, 1982, Freund *et al.*, 1975, Linnamo *et al.*, 2003, Milner-Brown *et al.*, 1973, Moritani and Muro, 1987, Wakeling *et al.*, 2002). This pattern of fiber recruitement is closely related to the pattern of metabolic fuel selection (Roberts et al., 1996). At low exercise intensities (e.g. walking), recruitment of slow-oxidative fibers (type I) is related to an increase in lipid utilization whereas, at higher intensities (e.g. sprinting), CHO utilization plays a gradually increasing role with the progressive recruitment of more fast-glycolytic muscle fibers (type II) (Brooks *et al.*, 1999, Roberts *et al.*, 1996). However, mechanisms of fuel selection depend on numerous factors and may not be identical between exercise and shivering.

Far less is known on the principles that govern fiber recruitment during shivering and on the possible effect of recruitment of specific MU has on heat production or fuel selection. Previous work has suggested that all fiber types are involved in shivering (Jacobs *et al.*, 1994, Meigal, 2002). Jacobs *et al.* (1994) reported that glycogen concentration decreased in all fiber types of the *vastus lateralis* after cold exposure.

Electromyography (EMG) recordings also reveal two distinct patterns of shivering: i) thermoregulatory muscle tone, or continuous, low-intensity shivering (at 4-8 Hz), and ii) bursts of high-intensity shivering occurring at much lower frequencies (0.1-0.2 Hz) (Israel and Pozos, 1989, Meigal, 2002). These two patterns are associated with the recruitment of specific MU (Meigal, 2002, Meigal *et al.*, 1993, Petajian and Williams, 1972). While continuous, low-intensity shivering is linked to low-threshold MU (type I, slow-oxidative, fatigue-resistant fibers), shivering bursts are associated with high-threshold MU (type II, fast-glycolytic, more fatigable fibers). Human type II fibers (IIA and IIX) show lower activities for oxidative enzymes than type I fibers (-60%) and much higher activities for glycolytic enzymes and creatine kinase (+300-400%) (Gardiner, 2001). Because of these large biochemical differences, the two shivering patterns observed may reflect the use of distinct metabolic substrates, type I being mostly geared towards lipid use and type II towards CHO use. Very little is known about the relative importance of continuous, low-intensity shivering and of burst shivering to total heat generation. How they are partitioned may have important implications on survival in the cold, and additional research is needed to determine the physiological significance of this dual pattern.

Figure 1.1. Literature review - relative contribution of carbohydrates (CHO) and lipids to total heat production measured between 60 and 90 min of cold exposure (i.e. water, air, liquid conditioned suit). Values are presented as means of the 16 studies (*t*-test assuming equal variance, P=0.59)



Air

- ● Vallerand *et al.*, 1989 (10°C)
- ○ Glickman-Weiss *et al.*, 1994 (12°C)
- ▼ MacNaughton *et al., 1990* (5°C)
- ▽ Glickman-Weiss et al., 1994 (8°C)
- □ Glickman-Weiss *et al.*, 1993 (8°C)
- ▼ Vallerand *et al.*, 1993 (10°C)
- ◇ Vallerand *et al.*, 1995 (10°C)
- ▲ Vallerand *et al.*, 1990 (10°C)
- △ Vallerand *et al.*, 1989 (10°C)
- ○ Vallerand *et al.*, 1999 (5°C)

Water

- ● Jacobs, 1997 (18°C)
- ○ Martineau and Jacobs, 1988 (18°C)
- ◆ Tikuisis *et al.*, 2000 (18°C)
- ▽ Martineau *et al.*, 1989 (18°C)

Liquid conditioned suit

- ■ Weller *et al.*, 1998 (7°C)
- ● Haman *et al.*, 2002 (10°C)

Figure 1.2. Literature review - relative contribution of carbohydrates (CHO) and lipids to total oxygen consumption ($\%VO_2$) as a function of shivering intensity (values taken from Fig. 1.1). Values are presented as means ± S.E.

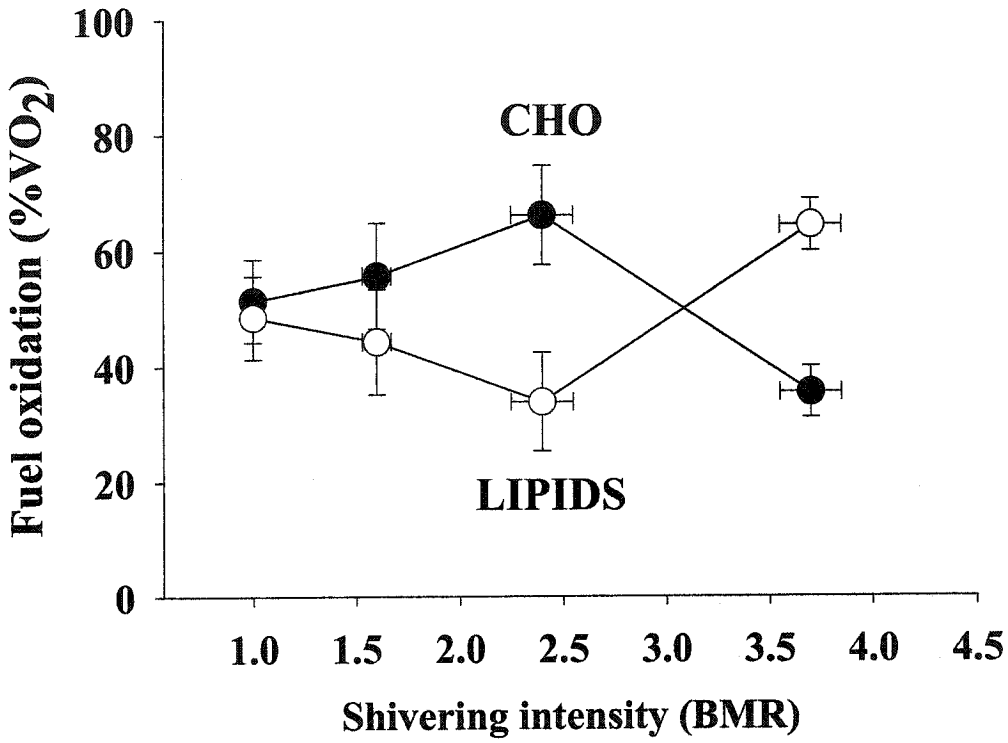
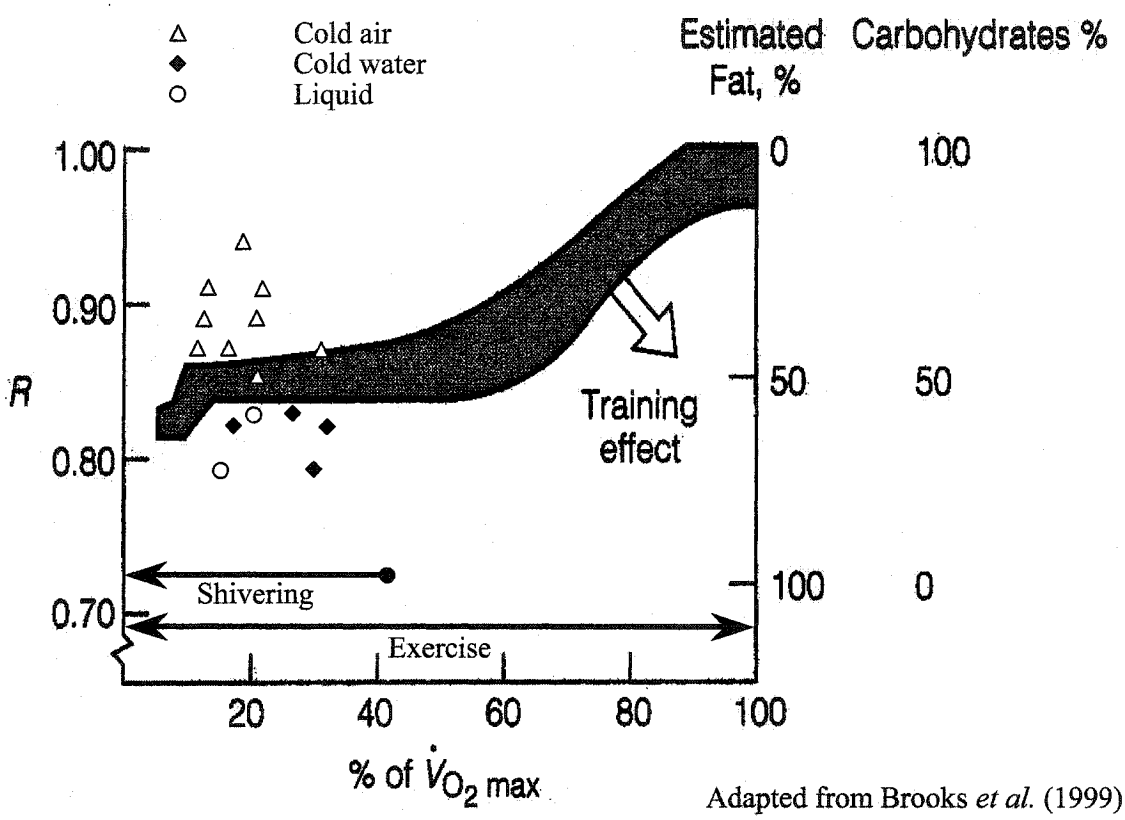

100
80
60
40
20
0
Fuel oxidation (%VO2)
CHO
LIPIDS
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Shivering intensity (BMR)

Figure 1.3. Literature review – changes in respiratory exchange ratio (R) and the relative contribution of carbohydrates (%) and lipids (Fat,%) to total energy expenditure as a function of exercise and shivering intensity. Maximal shivering intensity is equivalent to ~42%VO_2max or 5 times resting metabolic rate (Eyolfson et al., 2001). Values are presented separately for studies where individuals were cooled with water (open triangle), air (closed diamond) or a liquid conditioned suit (open circle).



Adapted from Brooks *et al.* (1999)

Figure 1.4. Literature review - relative contribution of carbohydrates (CHO) and lipids (FAT) to total heat production (values taken from Fig. 1.1) as a function of cooling protocol (i.e. water, air, liquid conditioned suit; LCS). Values are presented as means ± S.D.

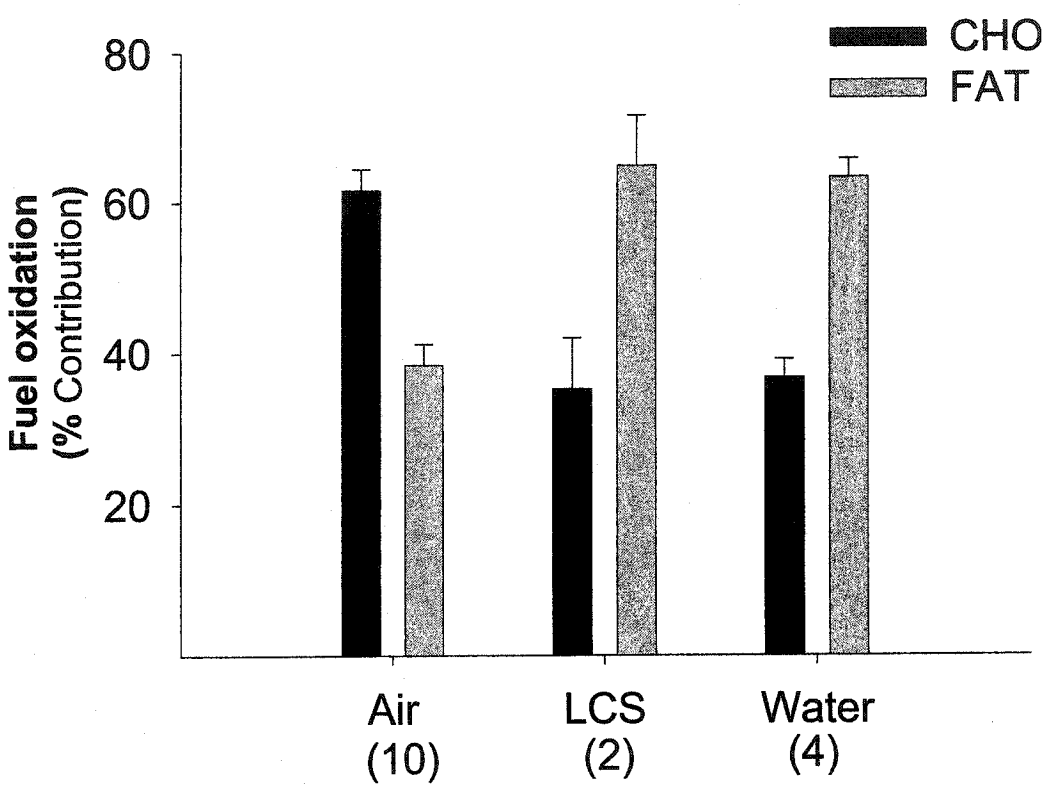
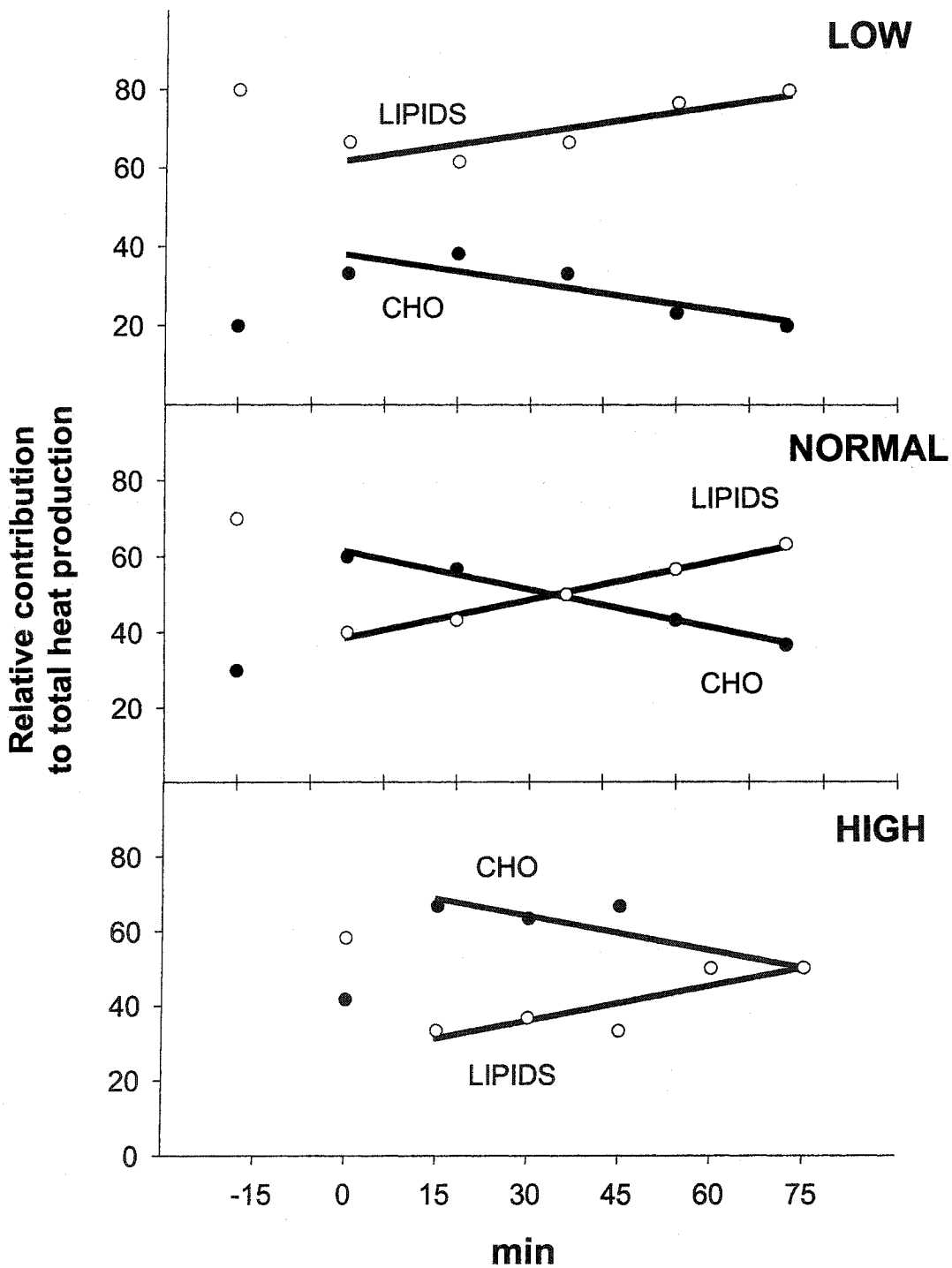

CHO
FAT
Fuel oxidation
(% Contribution)
80
60
40
20
Air
(10)
LCS
(2)
Water
(4)

Figure 1.5. Changes in the relative contribution of carbohydrates (CHO) and lipids to total heat production during high intensity shivering (~3.5 times RMR) in individuals with low- (LOW), normal and high-glycogen reserves. Values were calculated from average nonprotein respiratory exchange ratios reported in Martineau *et al.* (1989).


LOW
NORMAL
HIGH
LIPIDS
CHO
LIPIDS
CHO
CHO
LIPIDS
Relative contribution to total heat production
80
60
40
20
0
-15
0
15
30
45
60
75
min

GOALS OF THE INVESTIGATION

The purpose of this thesis was to characterize some of the effects of CHO availability and shivering intensity on oxidative fuel selection (specifically, plasma glucose, muscle glycogen, lipids and proteins) and EMG pattern during cold exposure. All experiments were conducted on humans, a homeotherm that depends essentially on shivering for heat production and, whose furless body makes it ideal to study cold exposure. Previous research in this field falls into two broad categories dealing either with muscle metabolism (Jacobs et al., 1994) or with electrophysiological aspects of muscle recruitment (Bell *et al.*, 1992, Meigal, 2002, Tikuisis *et al.*, 2000a). These two complementary perspectives on the same problem have not been traditionally integrated and this thesis is a first attempt at doing so.

To achieve this objective, a series of studies were designed to study the effects of CHO availability and shivering intensity on the preferential use of CHO sources (plasma glucose and muscle glycogen) and on muscle fiber recruitment. The following questions were addressed:

1. How do changes in CHO availability and shivering intensity modify the relative contributions of plasma glucose, muscle glycogen, lipids and proteins to total heat production? (CHAPTERS 2, 3, 5)
2. How do changes in CHO availability and shivering intensity modify muscle recruitment and the EMG signature of working muscles? (CHAPTER 4 and 6)
3. Does the EMG shivering pattern provide quantitative information on energy metabolism? (CHAPTER 6)

In more detail, CHAPTER 2 quantifies the respective contributions of plasma glucose, muscle glycogen and lipid oxidation to total heat production during prolonged, low-intensity shivering using a combination of stable isotope and indirect calorimetry methods. It is hypothesized that plasma glucose oxidation will play a lesser role than previously suggested (Vallerand *et al.*, 1995, Vallerand *et al.*, 1999a) and that lipid oxidation will be a major pathway for heat generation based on the small change in metabolic rate observed in the cold. Under the same cold conditions, the goal of CHAPTER 3 is to quantify the effects of changes in CHO availability on the oxidation rates of circulating glucose, proteins and lipids, and to determine their role in compensating for low / high glycogen availability. In view of the minor role played by circulating glucose in normal subjects (CHARTER 2), it is hypothesized that changes in plasma glucose oxidation will not be used to offset changes in glycogen oxidation. Therefore, it is predicted that lipids and proteins will maintain heat production by compensating for the variable contribution from glycogen. In CHAPTER 4, the first objective is to quantify the effects of changes in glycogen stores (CHAPTER 3) on the shivering activity of 8 large muscles representing >90% of total shivering muscle mass. The second is to characterize the detailed shivering pattern and spectral parameters of individual muscles in an attempt to uncover more subtle effects of CHO availability on the shivering response. The chosen experimental design also allows us to investigate whether changes in fuel selection are achieved by recruiting "fuel-specific fibers" as previously suggested (Roberts et al., 1996). It is hypothesized that total shivering activity would not be affected by changes in CHO availability but that the relative contribution of

specific muscles would be altered to maintain heat production. Within each muscle, it is anticipated that the shivering pattern and EMG spectral parameters would change with CHO availability because distinct fuel-specific fiber populations would be recruited. Experiments in CHAPTERS 5 and 6 were conducted at a high thermogenic rate. The aim of CHAPTER 5 is 2-fold: i) to determine whether the role of plasma glucose will be increased during high-intensity shivering and, ii) to investigate the effect of shivering intensity on fuel selection pattern. It is hypothesized that i) while plasma glucose oxidation rate will increase with shivering intensity, the relative contribution of this fuel to total heat production will be minor and, ii) lipid oxidation will remain the major pathway for heat generation even at a higher shivering intensity, if the fuel selection pattern is the same for shivering and exercise. In CHAPTER 6, an experiment was designed to determine whether muscle recruitment pattern (EMG) could provide metabolic information on fuel selection during high-intensity shivering. Because burst shivering is associated with the recruitment of fast-glycolytic MU (Meigal, 2002, Meigal *et al.*, 1993, Petajian and Williams, 1972), it is hypothesized that changes in CHO utilization would be correlated with burst shivering rate. Finally, general conclusions are presented in CHAPTER 7 where the relative importance of oxidative fuels and the importance of shivering pattern on the pattern of fuel selection during cold exposure are discussed.

CHAPTER 2. EFFECT OF COLD EXPOSURE ON FUEL UTILIZATION IN HUMANS: PLASMA GLUCOSE, MUSCLE GLYCOGEN AND LIPIDS

Based in part on

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INTRODUCTION

During environmental cold exposure in adult humans, a decrease in core temperature is prevented by increasing heat production via shivering thermogenesis. Involuntary muscle contractions during shivering are mainly fueled by CHO and lipids, while the contribution of protein oxidation remains minor (~10%) (Jacobs *et al.*, 1994, Vallerand *et al.*, 1995, Vallerand and Jacobs, 1989). However, the relative importance of CHO and lipid oxidation has not been clearly established. For example, some researchers imply that CHO is the preferred fuel in the cold (~60% of total heat production) (Glickman-Weiss *et al.*, 1993, Glickman-Weiss *et al.*, 1994, Jacobs, 1997, MacNaughton *et al.*, 1990, Vallerand *et al.*, 1995, Vallerand and Jacobs, 1989, Vallerand and Jacobs, 1990, Vallerand *et al.*, 1989, Vallerand *et al.*, 1993, Vallerand *et al.*, 1999a), while others show a greater reliance on lipids (~60% of total heat production) (Jacobs, 1997, Martineau and Jacobs, 1988, Martineau and Jacobs, 1989a, Martineau and Jacobs, 1989b, Tikuisis *et al.*, 2000b, Weller *et al.*, 1998). Possible reasons for such discrepancies between studies are differences in shivering intensity (but SEE DISCUSSION), cooling protocol and/or nutritional state. Over the last decade, most studies of fuel selection during shivering have focused on CHO metabolism as a probable limiting factor for heat production. However, two important issues remain unresolved: 1) the relative contributions of hepatic glucose and muscle glycogen to total CHO oxidation have not been quantified, and 2) the potentially important role of triacylglycerol stores (adipose tissue, liver and muscle) has often been underrated, particularly during prolonged, low-intensity shivering.

Plasma glucose and muscle glycogen have both been shown to play significant roles in heat production during cold exposure (Jacobs et al., 1994). Vallerand *et al.* (1995, 1999a) calculated that plasma glucose and muscle glycogen would contribute equally to total CHO oxidation, assuming that 100% of hepatic glucose production (R_aGLU) is oxidized. However, as pointed out by these authors, at such low rates of oxygen consumption, this assumption is probably not met because non-oxidative glucose disposal could be important. Studies where measurements of R_aGLU and plasma glucose oxidation were carried out simultaneously indicate that non-oxidative disposal ranges between 25% R_aGLU during submaximal exercise (45% $\dot{V}O_{2max}$) and 70% R_aGLU at rest (Friedlander et al., 1997).

A series of studies have shown that 90 min of cold-water immersion causes a reduction of glycogen concentration in biopsies from the *vastus lateralis* (Jacobs *et al.*, 1985, Martineau and Jacobs, 1988, Martineau and Jacobs, 1989a, Martineau and Jacobs, 1989b, Martineau and Jacobs, 1991). Unfortunately, estimating total use of muscle glycogen, at the whole-organism level, from small biopsy samples is inaccurate at best, because: 1) glycogen content varies greatly between and within individual muscles and, 2) the specific muscles involved in heat generation and their level of recruitment are unknown. Therefore, the exact contributions of plasma glucose and muscle glycogen are presently unclear because their rates of oxidation have never been measured directly during shivering.

The purpose of this study was to quantify the respective contributions of plasma glucose, muscle glycogen and lipid oxidation to total heat production during prolonged,

low-intensity shivering, using a combination of stable isotope and indirect calorimetry methods. In human subjects exposed to low-intensity shivering (10°C for 2 h using a liquid conditioned suit), we hypothesize that plasma glucose oxidation will play a lesser role than previously suggested (Vallerand *et al.*, 1995, Vallerand *et al.*, 1999a) and that lipid oxidation will be a major pathway for heat generation due to the small change in metabolic rate observed in the cold.

METHODS

Subjects

Six healthy and trained men volunteered for this study approved by the Health Sciences Ethical Committee of the University of Ottawa, and written consent was obtained from the participants. Percent body fat (underwater weighing; Brosek *et al.* (1963) and maximal oxygen consumption using a progressive treadmill protocol were measured 5-7 days prior to the experiments. Physical characteristics of the subjects are presented in Table 2.1.

Experimental protocol

Experiments were conducted between 9:00 and 13:00, following 36-h without heavy physical activity. The last evening meal was standardized (~988 kcal, ~52% CHO, ~18% lipids, ~30% proteins) and subjects were asked to report to the laboratory the next morning (9:00 AM) after a 12-14h fast. Ingestion of carbohydrates from plants naturally rich in ^{13}C (C_4 photosynthetic cycle) was avoided for 24h to maintain low ^{13}C background enrichment in plasma glucose and expired CO_2. Care was taken to minimize thermal stimuli between awakening and the start of the experiment (i.e. avoid exposure to hot or cold temperatures, very low intensity exercise during transit from home to the laboratory). Upon their arrival in the laboratory, subjects were instrumented with thermal probes and an indwelling catheter (18G, 32 mm, Medical Inc., Arlington, TX) placed in

Table 2.1. Physical characteristics of subjects (n=6).
Values are presented as means ± SE.

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

an antecubital vein (left arm) for blood sampling, and were fitted with a liquid conditioned suit (LCS; Three piece Delta Temax, Pembroke, ON, Canada). Subjects were then asked to empty their bladder (t = 0 min) and sit quietly for two hours at 28.1±0.3°C (758±4 mmHg, 20-30% RH). Following this habituation period, they were transferred to an environmental chamber (11.1±0.1°C, 760±4 mmHg, 40-57% RH) and a 10°C water perfusion was started through the LCS using a temperature controlled circulation bath (Endocal, NESLAB and Model 200-00, Micropump, Vancouver, WA). Thermal response, metabolic rate and fuel utilization were measured at 28°C and during the subsequent 2-h cold exposure.

Thermal response

Central body temperature (T_{es}) was monitored continuously using a pediatric esophageal temperature probe (Mon-a-therm general purpose, Mallinckrodt Medical Inc, St-Louis, MO) which was inserted through the nose to a depth placing the tip of the thermocouple at the level of the left atrium, or one quarter of the standing height of the subject (Mekjavic and Rempel, 1990). Heat flux transducers (Concept Engineering, Old Saybrook, CT) were used to estimate skin temperature and non-evaporative heat flux from the forehead, chest, biceps, forearm, abdomen, lower and upper back, front and back calf, quadriceps, hamstrings and finger. Mean skin temperature ($\overline{T}_{skin}$) and mean heat flux were calculated using an area-weighted equation (Dubois and Dubois, 1916). Heat flux measurements were used to calculate whole body radiative ($\dot{R}$) and convective ($\dot{C}$) heat exchange. Respiratory evaporative ($\dot{E}_{resp}$) and convective ($\dot{C}_{resp}$) heat

exchanges were determined from ventilation measurements by estimating water loss via the respiratory tract (2,411.3 J of heat per gram of evaporated water) (Livesey and Elia, 1988). It was assumed that evaporative heat loss from the skin was negligible under the LCS. Whole-body heat loss ($\dot{H}_{loss}$ in watts) was calculated as follows:

$$\dot{H}_{loss} = (\dot{R} + \dot{C}) + (\dot{E}_{resp} + \dot{C}_{resp}) \quad (2.1)$$

Metabolic rate and fuel utilization

Ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were determined by open-circuit spirometry (250 l, chain-compensated gasometer, Warren Collins inc., Braintree, MA). All expired gas collections were made at ambient temperature outside the experimental chamber. A mouthpiece, an unidirectional valve (2700 Series, Hans Rudolph, Kansas City, MO) and a 44 mm plastic tube were used to direct all expired gases to the collection tank. Expired gases were collected for 5 min every 30 min at 28°C and during cold exposure. Ventilation ($\dot{V}_E$, $l \cdot min^{-1}$, BTPS) was calculated from the displacement of the cylinder and corrected for temperature and pressure. Oxygen and carbon dioxide concentrations in dry expired gases ($CaSO_4$, Drierite, 8 Mesh, Fisher Scientific inc., Ottawa, Canada) were determined directly from the spirometer using calibrated electrochemical gas analyzers (AMETEK Model S-3A/1 and CD 3A, Applied Electrochemistry, Pittsburg, PA).

Total carbohydrate (RG_{ox}), lipid (RF_{ox}) and protein (RP_{ox}) oxidation rates were calculated using the following equations (Livesey and Elia, 1988):

$$RG_{ox}\ (g \cdot min^{-1}) = 4.59 \dot{V}CO_2 (l \cdot min^{-1}) - 3.23\ \dot{V}O_2 (l \cdot min^{-1}) \quad (2.2)$$

$$RF_{ox}\ (g \cdot min^{-1}) = -1.70\ \dot{V}CO_2 (l \cdot min^{-1}) + 1.70\ \dot{V}O_2 (l \cdot min^{-1}) \quad (2.3)$$

$$RP_{ox} (g \cdot min^{-1}) = 2.9 \times\ UREA_{urine} (g \cdot min^{-1}) \quad (2.4)$$

where $\dot{V}CO_2$ and $\dot{V}O_2$ (Eq. 2.2 and 2.3) were corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1.010 and 0.843 $l \cdot g^{-1}$, respectively).

Estimates of RP_{ox} (*Eq.* 2.4) were made by measuring urinary urea excretion ($UREA_{urine}$) from urine samples collected for a period of 120 min at 28°C and 10°C. A correction for urea accumulation in plasma was not required because plasma levels did not change during cold exposure (P=0.28; Paired t-test) (Jéquier et al., 1987). Urinary and plasma urea concentrations were determined on a Synchron Clinical System (CX7, Beckman, Anaheim, CA). Respective contributions of glucose, lipid and protein oxidation to total heat production ($\%\dot{H}_{prod}$) were calculated using energy potential of 16.3 $kJ \cdot g^{-1}$, 40.8 $kJ \cdot g^{-1}$ and 19.7 $kJ \cdot g^{-1}$, respectively (Elia, 1991, Péronnet and Massicotte, 1991).

Plasma glucose oxidation

For the measurement of plasma glucose oxidation, the subjects ingested 10 g of glucose (7 x 1.4 g in 100 mL of water; corn sugar, with a $^{13}C/C = 0.01098$) artificially enriched with ^{13}C (U-^{13}C-glucose, $^{13}C/C > 99\%$, Isotec, Miamisburg, OH) to obtain a final $^{13}C/C$ ratio of 0.0476 (R_{exo}).

After measuring baseline $^{13}C/^{12}C$ in plasma and expired CO_2 (t = 30 min), subjects ingested the first dose of ^{13}C-glucose. Subsequent doses were then taken every 30 min until the end of the experiment. Isotopic composition of plasma glucose (R_{glu}) and expired CO_2 (R_{exp}) were determined in blood and expired gas samples every 30 min prior to the ingestion of the next dose. Upon collection, blood samples were put on ice, spun in a refrigerated centrifuge, separated, and the plasma was kept frozen at –20°C until analyzed.

The isotopic composition of plasma glucose was determined as previously described (Péronnet *et al.*, 1998). Briefly, plasma samples (1 ml) were deproteinized (BaOH; 1.5 ml, 0.3N and $ZnSO_4$ 1.5 ml, 0.3N) and centrifuged to precipitate the proteins. Double-bed ion exchange chromatography with superimposed columns (resins: AG 50W-X8 H^+, 200-400 mesh, and AG 1-X8 chloride, 200-400 mesh) was used to isolate plasma glucose. Following evaporation, glucose was combusted (60 min at 400°C) in the presence of CuO, and CO_2 was recovered. Measurements of $^{13}C/^{12}C$ in expired CO_2 and in CO_2 obtained from glucose combustion were determined in a Prism mass spectrometer (VG, Manchester, UK). Isotopic composition was expressed as ‰ difference in comparison to PDB-1 Chicago standard using the equation of Craig (1957):

$$\delta^{13}C‰ = \{[(^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{standard})]-1\}x10^3 \qquad (2.5)$$

The rate of exogenous glucose oxidation ($RG_{ox\text{-}exo}$, $g \cdot min^{-1}$) was estimated from R_{exp} and R_{exo} as follows (Mosora et al., 1976):

$$RG_{ox\text{-}exo}\ (g \cdot min^{-1}) = \dot{V}CO_2\ (l \cdot min^{-1}, STPD)(R_{exp}\text{-}R_{ref}/R_{exo}\text{-}R_{ref})(1/k1 \cdot k2) \qquad (2.6)$$

where $\dot{V}CO_2$ is in $l \cdot min^{-1}$ (STPD), R_{ref} is the isotopic composition of expired CO_2 at 28°C before ingestion of the first ^{13}C-glucose dose, k1 ($0.7426\ l \cdot g^{-1}$) is the volume of CO_2 produced from the complete oxidation of glucose and k2 is the fractional recovery at the mouth of CO_2 produced in tissues (Péronnet and Massicotte, 1991). A fractional recovery of $^{13}CO_2$ at the mouth (k2) of 0.8 and 1 was used at 28°C and during cold exposure, respectively (Wolfe, 1992). Due to the large size of the bicarbonate pool, only values in the last 30 min at 28°C and at 10°C were used in the calculation of plasma glucose oxidation rate ($RG_{ox\text{-}plasma}$). This delay allows sufficient time for equilibrium of the $^{13}C/^{12}C$ ratio to be attained in the bicarbonate pool (Pallikarakis et al., 1991).

$RG_{ox\text{-}plasma}$ was calculated from $^{13}CO_2$ excretion and the isotopic enrichment of plasma glucose using the following equation (Derman *et al.*, 1996, Wolfe, 1992):

$$RG_{ox\text{-}plasma} = \dot{V}CO_2\ (R_{exp}\text{-}R_{ref}/R_{glu}\text{-}R_{ref})(1/k1 \cdot k2) \qquad (2.7)$$

Oxidation of glucose released from the liver ($RG_{ox\text{-}liver}$) was estimated by subtracting the low rate of exogenous glucose oxidation ($RG_{ox\text{-}exo}$) from $RG_{ox\text{-}plasma}$. Calculation of glucose oxidation derived from glycogen stores ($RG_{ox\text{-}mus}$; $g \cdot min^{-1}$) in the tissues, either directly or through the lactate shuttle (Brooks, 1986) was calculated by subtracting $RG_{ox\text{-}plasma}$ (*Eq.* 2.7) from RG_{ox} (*Eq.* 2.2).

$$RG_{ox\text{-}mus} = RG_{ox} - RG_{ox\text{-}plasma} \qquad (2.8)$$

Blood analysis

Plasma glucose and lactate concentrations were measured spectrophotometrically at 340 nm on a Beckman DU 640 (Bergmeyer, 1985) while total plasma nonesterified fatty acid (NEFA) concentration was determined using an analytical assay kit (NEFA C, Wako Chemicals, Osaka, Japan). Insulin concentration was measured using a radioimmunoassay (#KTSP-11001, Medicorp Inc, Montréal, Qc, Canada).

Statistical analyses

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

RESULTS

Thermal response

Changes in T_{es} and $\overline{T}_{skin}$ are presented in Fig. 2.1. While T_{es} remained constant at 36.4±0.1°C throughout the experiment, $\overline{T}_{skin}$ decreased from 34.0±0.02°C to 27.2±0.02°C in the initial 90 min of cold exposure and did not change for the last 30 min. Absolute $\dot{H}_{loss}$ and $\dot{H}_{prod}$ increased by a maximum of 3.3 (77.7±0.6 W to 258.4±10.6 W) and 2.6 fold (95.3±2.2 W to 243.8±4.2 W), respectively (Fig. 2.2). After reaching a maximum 20 min after the onset of cold exposure (t=140 min), $\dot{H}_{loss}$ decreased by 16% over the next 100 min (238.3±0.6 W). Maximal $\dot{H}_{prod}$ was reached after 90 min of cold exposure and stayed constant for the remainder of the experiment. Observed shivering activity appeared minimal over the first 60 min of cold exposure, but increased progressively in the last hour.

Metabolic response and fuel utilization

Changes in ventilation, oxygen consumption and respiratory exchange ratio (RER) are shown in Fig. 2.3. $\dot{V}_E$ and $\dot{V}O_2$ increased by 2.4- and 2.6-fold, respectively (Fig. 2.3A and B). A small decrease in RER was observed during cold exposure, but it did not reach overall statistical significance (one-way ANOVA with replication; P=0.075) averaging 0.84 ±0.01 throughout the experiment (Fig. 2.3C; 0.86 ±0.01 at 28°C and 0.83 ±0.02 between 150 and 180 min at 10°C).

Rates of lipid, CHO and protein oxidation and their respective contributions to total heat production throughout cold exposure are plotted in Fig. 2.4. Lipid and CHO utilization increased 3.8-fold (Fig. 2.4A; 39 ± 2 at 28°C to 177 ± 17 mg fatty acids·min^{-1} at 10°C) and 2.2-fold (Fig. 2.4B; 165 ± 9 at 28°C to 358 ± 41 mg glucose·min^{-1} at 10°C), respectively. Protein utilization was not affected significantly by the change in temperature and averaged 62.1±3.1 at 28°C and 77.7±5.0 mg·min^{-1} at 10°C. A trend towards an increase in the relative contribution of lipids and a decrease in the relative contribution of CHO during cold exposure was noticed in the cold. However, these observed changes failed to reach statistical significance (Fig. 2.4B; P=0.10). In contrast, the relative contribution of protein oxidation to total heat production decreased significantly throughout cold exposure, from 21.2±0.8 at 28°C to 10.7±0.8% at 10°C.

Plasma concentrations

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

CHO oxidation: plasma glucose vs. muscle glycogen

The amount of ^{13}C-glucose administered provided a sufficient signal in CO_2 and plasma glucose to quantify circulatory glucose oxidation (Figs. 2.6A and B). The change in isotopic enrichment of expired CO_2 and plasma glucose (δ ^{13}C PDB-1), as well as the calculated values of $RG_{ox\text{-}plasma}$ and $RG_{ox\text{-}exo}$ obtained throughout the experiment are plotted in Fig. 2.6. At 28°C, 60 min after glucose ingestion, $RG_{ox\text{-}plasma}$ averaged 39.4±2.4 mg·min^{-1} ($RG_{ox\text{-}liver}$ was 37.7±2.3 mg·min^{-1} and $RG_{ox\text{-}exo}$ only 2.1±0.4 mg·min^{-1}) and increased progressively throughout cold exposure to reach a maximal value of 107.3±6.1 mg·min^{-1} ($RG_{ox\text{-}liver}$ was 84.0±6.0 mg·min^{-1} and $RG_{ox\text{-}exo}$ only 9.6±5.5 mg·min^{-1}; Fig. 6C).

Table 2.2 summarizes average values measured at 28°C (90-120 min) and 10°C (210-240 min), for all the parameters of fuel utilization estimated in this study ($RG_{ox\text{-}plasma}$, $RG_{ox\text{-}mus}$, RF_{ox} and RP_{ox}). Expired CO_2 and plasma glucose isotopic composition did not change significantly over the last 30 min at 28°C (t = 90 and 120 min) and over the last 30 min at 10°C (t = 210 and 240 min)(ANOVA, $p>0.05$). However, Fig. 2.6A and B suggest that priming of the bicarbonate pool with $NaH^{13}CO_3$ would have produced a better isotopic steady-state. It is therefore possible that $RG_{ox\text{-}plasma}$ rate was slightly underestimated. As a result of cold exposure, $RG_{ox\text{-}plasma}$ and $RG_{ox\text{-}mus}$ increased by 2.1- and 2.4-fold, respectively. While RF_{ox} increased by as much as 3.8 fold at 10°C, RP_{ox} remained constant throughout the experiment. The relative contributions of $RG_{ox\text{-}plasma}$ and $RG_{ox\text{-}mus}$ to total $\dot{H}_{prod}$ did not change when subjects were exposed to the cold whereas that of RF_{ox} increased 1.5-fold and that of RP_{ox} decreased 2-fold.

Table 2.2. Absolute oxidation ($mg \cdot min^{-1}$) and relative ($\%\dot{H}_{prod}$) contributions of plasma glucose ($RG_{ox\text{-}plasma}$; Ref *Eq.* 2.7), muscle glycogen ($RG_{ox\text{-}mus}$, Ref *Eq.* 2.8), lipid (RF_{ox}, Ref *Eq.* 2.3) and protein oxidation (RP_{ox}, Ref *Eq.*2.4) to total heat production at 28°C (90-120 min) and steady-state at 10°C (210-240 min). *Significantly different from values at 28°C, (paired *t*-test, $P \leq 0.05$).

	28°C	10°C
Plasma glucose ($RG_{ox\text{-}plasma}$)		
$mg \cdot min^{-1}$	39.4 ± 2.4	*93.9 ± 5.5
$\%\dot{H}_{prod}$	10.7 ± 0.5	10.5 ± 0.9
Muscle glycogen ($RG_{ox\text{-}mus}$)		
$mg \cdot min^{-1}$	126.6 ± 7.8	*264.2 ± 36.9
$\%\dot{H}_{prod}$	35.0 ± 1.9	29.2 ± 3.8
Lipids (RF_{ox})		
$mg \cdot min^{-1}$	46.9 ± 3.2	*176.5 ± 17.3
$\%\dot{H}_{prod}$	33.2 ± 2.0	*49.6 ± 4.4
Proteins (RP_{ox})		
$mg \cdot min^{-1}$	62.1 ± 3.1	77.7 ± 5.0
$\%\dot{H}_{prod}$	21.0 ± 0.8	*10.6 ± 0.8

Figure 2.1. Esophageal (T_{es}) and mean skin ($\overline{T}_{skin}$) temperature at 28°C and during whole body 10°C cold exposure. Arrows indicate the times at which ^{13}C-glucose solutions were ingested.

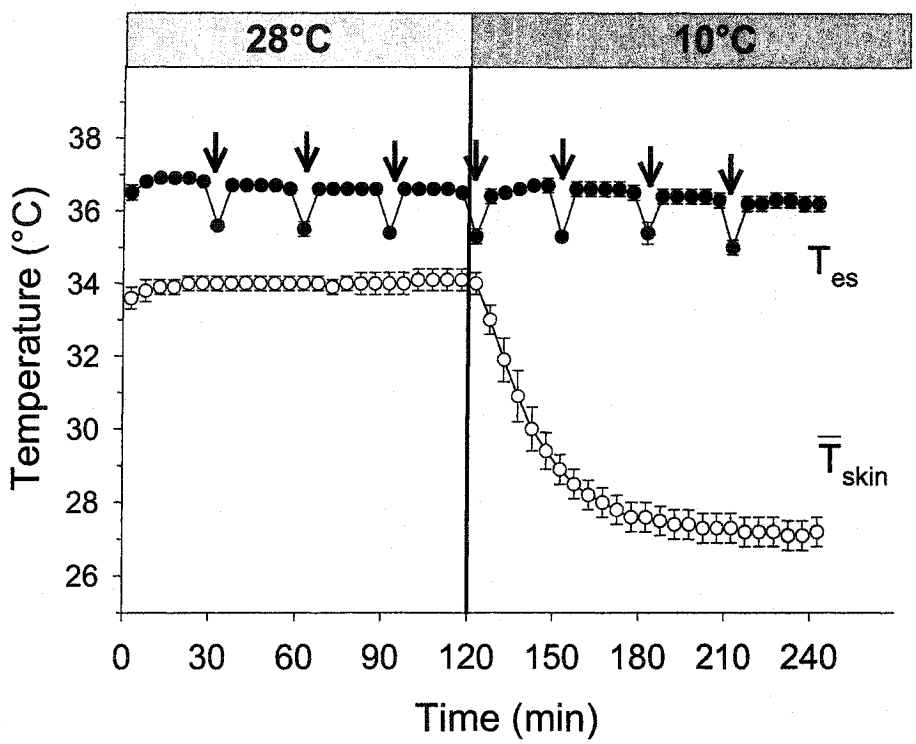

28°C
10°C
38
36
34
32
30
28
26
Temperature (°C)
T_{es}
$\overline{T}_{skin}$
0
30
60
90
120
150
180
210
240
Time (min)

Figure 2.2. Absolute rates of heat loss (A, $\dot{H}_{loss}$, W, *Eq.* 2.1) and heat production (B, $\dot{H}_{prod}$, W) at 28°C and during whole body 10°C cold exposure. * Significantly different from values at 28°C (One-way ANOVA with replication, P≤0.05) † Significantly different from maximal value reached during cold exposure (One-way ANOVA with replication and Bonferroni *post-hoc t*-test, P≤0.05, n=6).

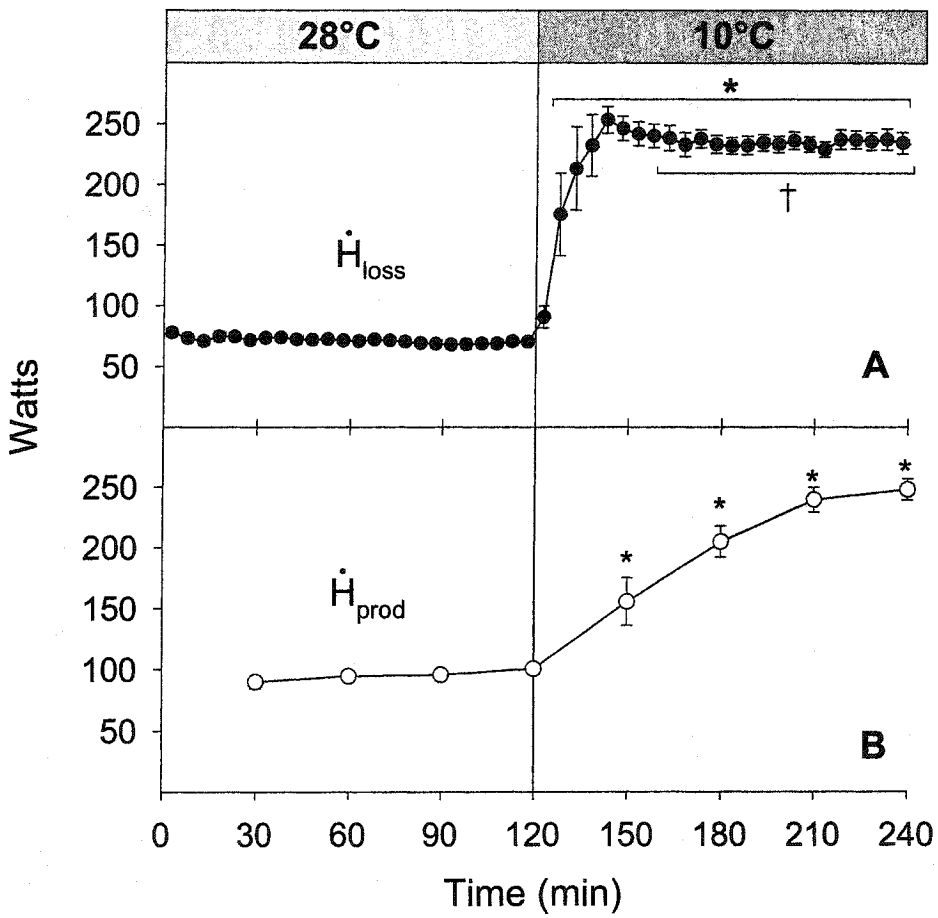

28°C
10°C
*
†
250
200
150
100
50
$\dot{H}_{loss}$
A
250
200
150
100
50
$\dot{H}_{prod}$
B
*
*
*
*
Watts
0
30
60
90
120
150
180
210
240
Time (min)

Figure 2.3. Ventilation (A, $\dot{V}_E$, $l \cdot min^{-1}$, BTPS), absolute oxygen consumption (B, $\dot{V}O_2$, l $O_2 \cdot min^{-1}$, STPD) and respiratory exchange ratio (C, RER) at 28°C and during whole body 10°C cold exposure. * Significantly different from values at 28°C (One-way ANOVA with replication and Bonferroni *post-hoc* *t*-test, $P \leq 0.05$, n=6)

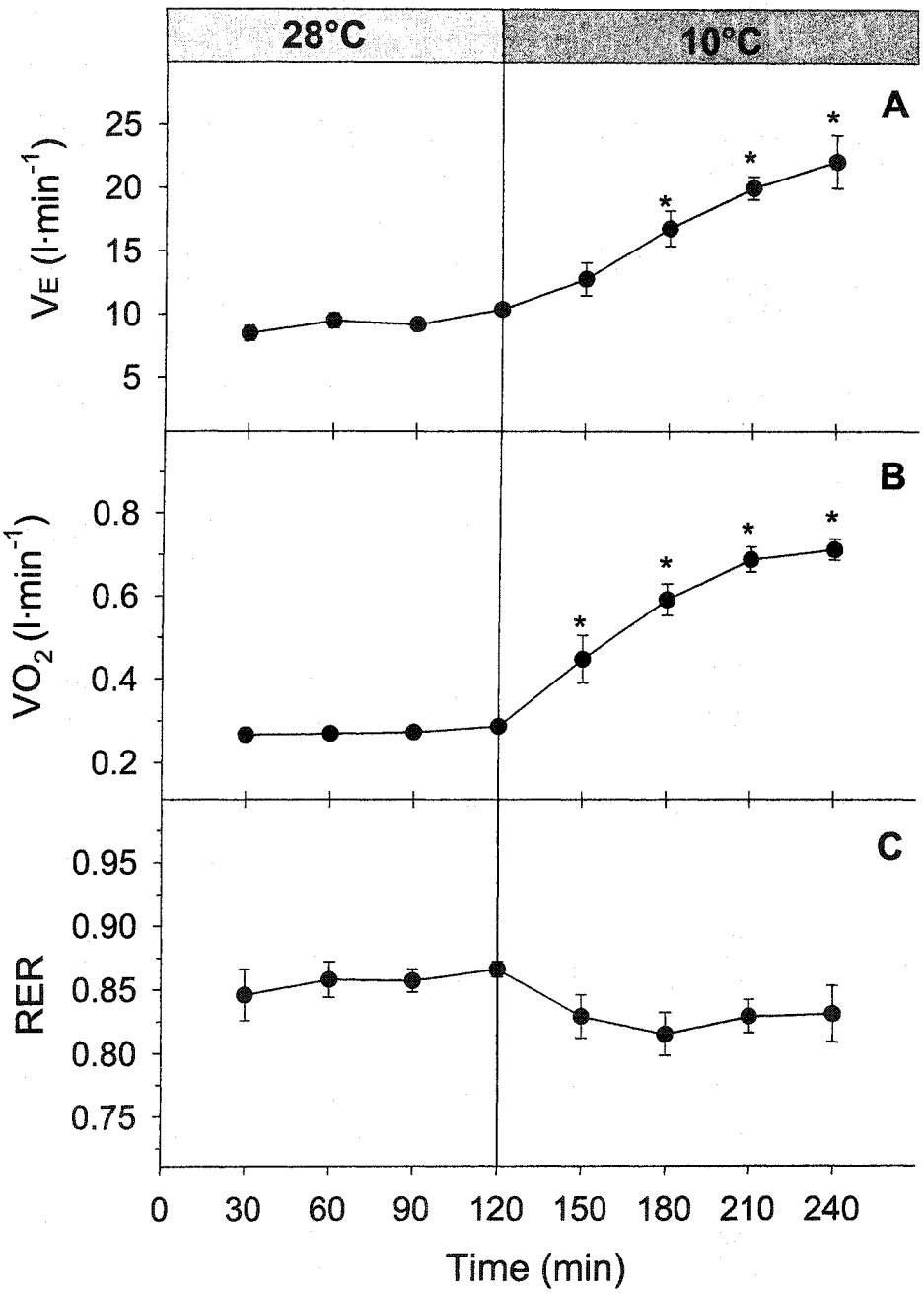

28°C
10°C
A
B
C
V_E (l·min^{-1})
VO_2 (l·min^{-1})
RER
25
20
15
10
5
0.8
0.6
0.4
0.2
0.95
0.90
0.85
0.80
0.75
0
30
60
90
120
150
180
210
240
Time (min)

Figure 2.4. Glucose (RG_{ox}, closed circles, *Eq.* 2.2) and lipid (RF_{ox}, open circles, *Eq.* 2.3) utilization rates (A, $mg \cdot min^{-1}$; protein oxidation was constant at 62.1 ± 3.1 $mg \cdot min^{-1}$ at 28°C and 77.7 ± 5.0 $mg \cdot min^{-1}$ at 10°C) as well as their relative contribution to total heat production (B, $\% \dot{H}_{prod}$; proteins, open squares) at 28°C and during whole body 10°C cold exposure. * Significantly different from values at 28°C (One-way ANOVA with replication and Bonferroni *post-hoc t*-test, $P \leq 0.05$, n=6)

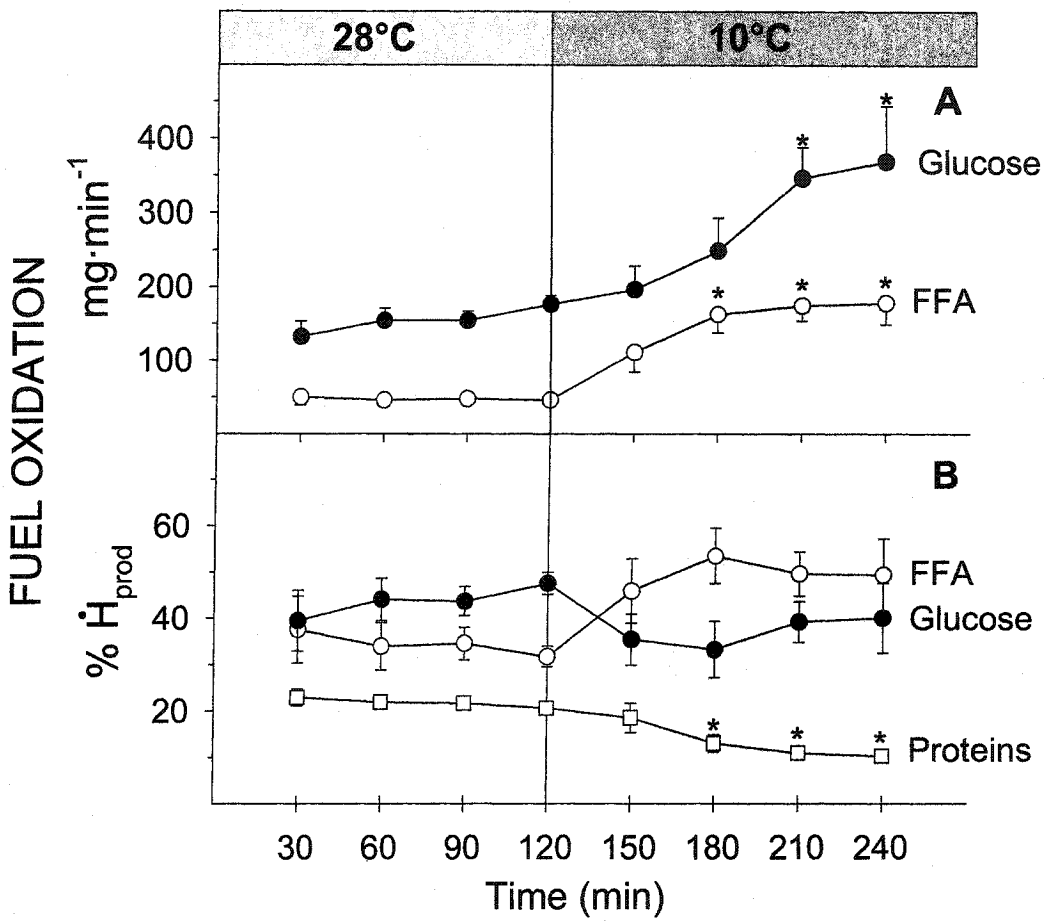

28°C
10°C
A
Glucose
FFA
400
300
200
100
mg·min-1
B
FFA
Glucose
Proteins
60
40
20
% Ḣprod
30
60
90
120
150
180
210
240
Time (min)
FUEL OXIDATION

Figure 2.5. Plasma insulin, glucose, lactate and nonesterified fatty acid (NEFA) concentrations at 28°C and during whole body 10°C cold exposure. * Significantly different from values at 28°C (One-way ANOVA with replication and Bonferroni *post-hoc t*-test, $P \leq 0.05$, n=6)

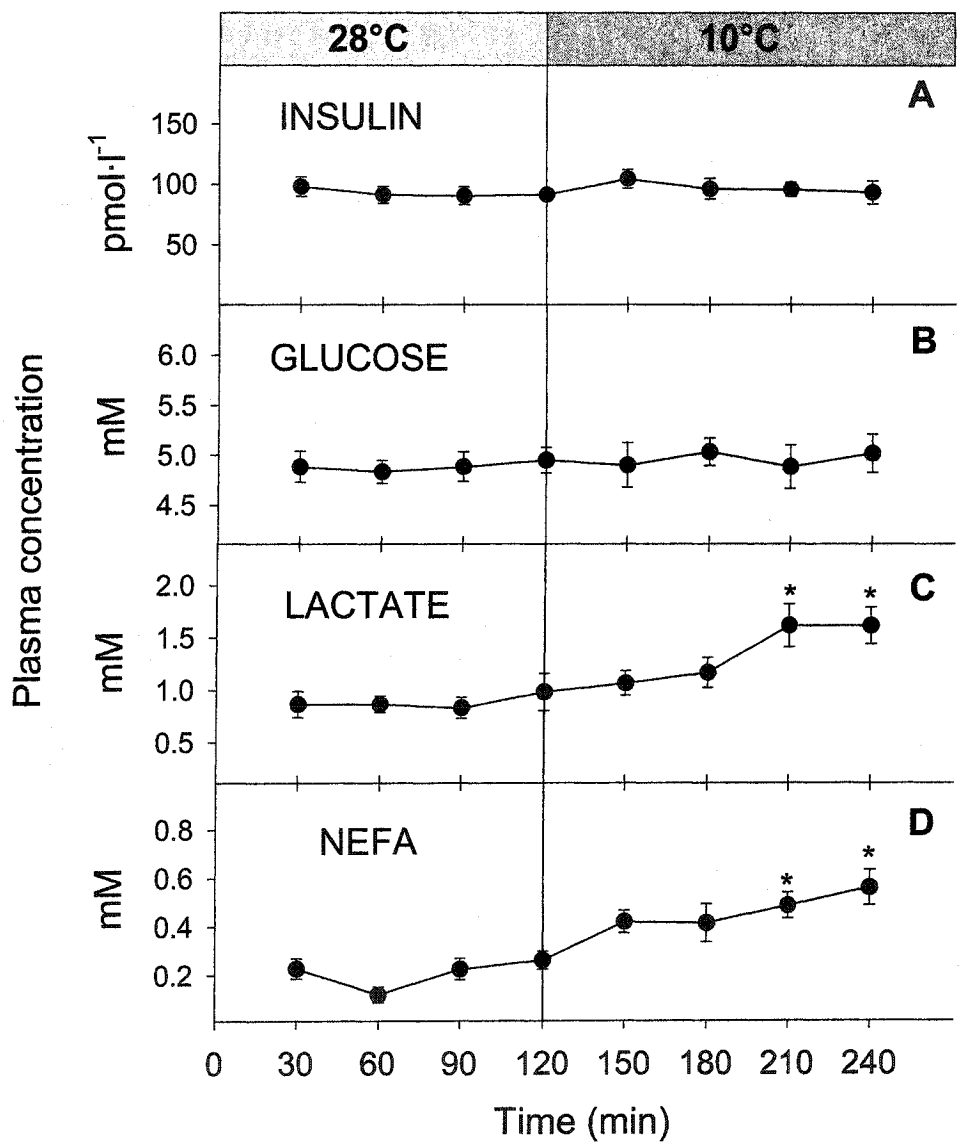
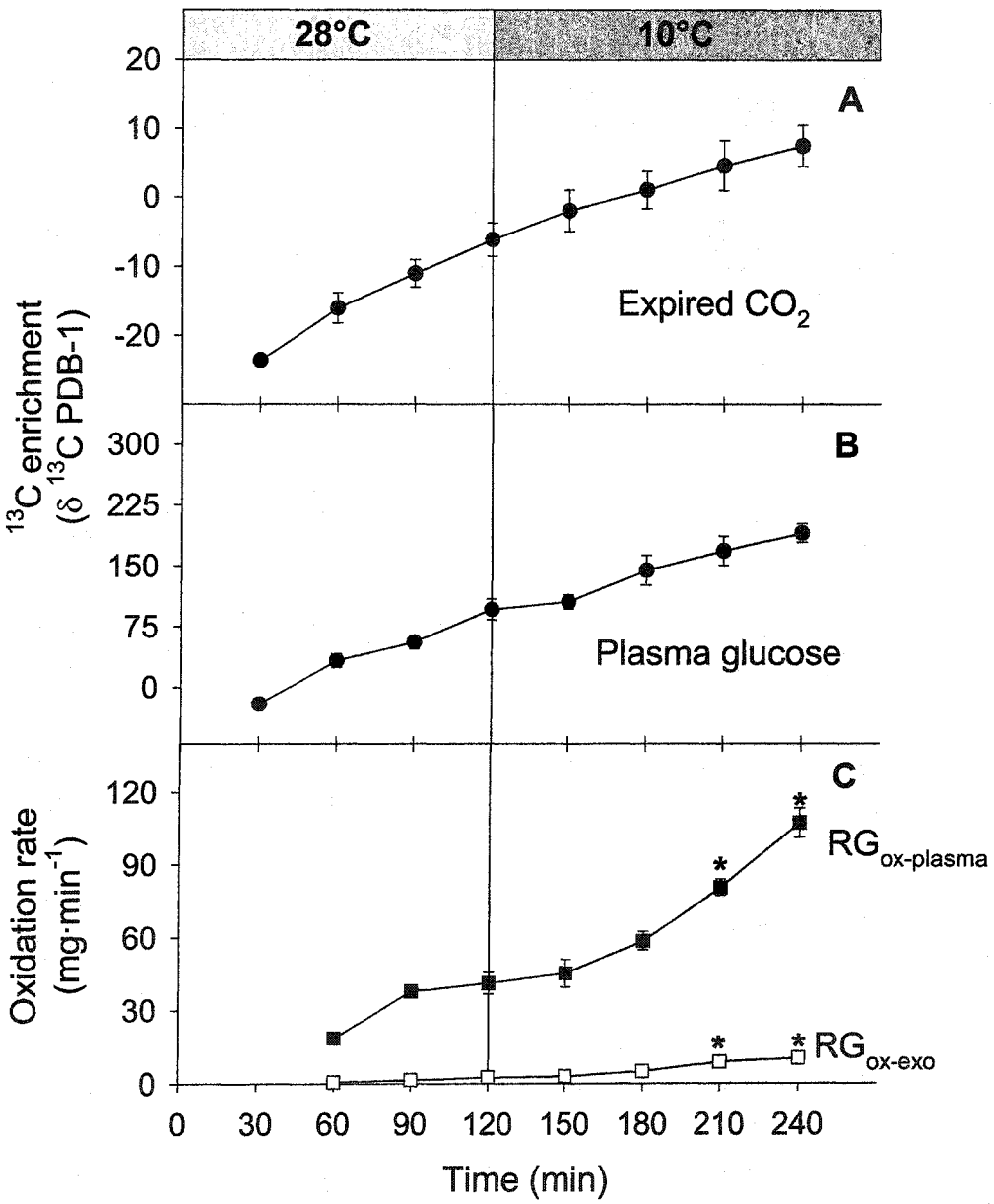

28°C
10°C
INSULIN
A
pmol·l-1
150
100
50
GLUCOSE
B
mM
6.0
5.5
5.0
4.5
LACTATE
C
mM
2.0
1.5
1.0
0.5
NEFA
D
mM
0.8
0.6
0.4
0.2
*
0
30
60
90
120
150
180
210
240
Time (min)
Plasma concentration

Figure 2.6. Expired CO_2 (A) and plasma glucose (B) ^{13}C enrichment (δ ^{13}C PDB-1) and calculated plasma glucose ($RG_{ox\text{-}plasma}$, $mg \cdot min^{-1}$) and exogenous glucose ($RG_{ox\text{-}exo}$, $mg \cdot min^{-1}$) oxidation rates measured at 28°C and during whole body 10°C cold exposure. * Significantly different from values at 28°C (One-way ANOVA with replication and Bonferroni *post-hoc t*-test, $P \leq 0.05$, n=6).


28°C
10°C
A
Expired CO_2
B
Plasma glucose
C
$RG_{ox\text{-}plasma}$
$RG_{ox\text{-}exo}$
^{13}C enrichment (δ ^{13}C PDB-1)
Oxidation rate (mg·min^{-1})
Time (min)
20
10
0
-10
-20
300
225
150
75
0
120
90
60
30
0
0
30
60
90
120
150
180
210
240

DISCUSSION

Even though plasma glucose oxidation is strongly stimulated during low-intensity shivering (+138%), we show that this fuel only plays a minor role in total heat production (10 % $\dot{H}_{prod}$). Also, muscle glycogen oxidation doubled during mild cold exposure, providing 75% of total CHO oxidized. Interestingly, lipids are the most important fuel, showing close to a 4-fold increase in oxidation rate and accounting for the production of as much heat as all other metabolic substrates combined.

This study quantifies the oxidation rate of plasma glucose during cold exposure. It shows that $RG_{ox\text{-}plasma}$ is stimulated in direct proportion to metabolic rate (Table 2.2, Figs 2.3B), and that its relative contribution to total heat production remains constant and low. During low-intensity shivering, the contribution of plasma glucose is as minor as that of proteins (Table 2.2). In contrast, muscle glycogen stores play a more prominent role, providing 3 times more glucose units for oxidation than the circulation (Table 2.2).

The ^{13}C-glucose ingestion technique selected for this study allowed us to quantify the role of circulatory glucose as an oxidative fuel to support shivering. Results show that, during mild cold exposure, circulatory glucose plays a more minor role than previously suggested when oxidation was estimated from measurements of R_aGLU (Vallerand *et al.*, 1995, Vallerand *et al.*, 1999a). As anticipated, neglecting to subtract non-oxidative glucose disposal from R_aGLU caused a significant overestimation of glucose oxidation rates. Under conditions of steady state (i.e. when plasma glucose concentration remains constant over time), R_aGLU and glucose disposal were matched.

However, the disposal of glucose can take place through two distinct metabolic pathways: oxidation ($RG_{ox\text{-}plasma}$) and storage (or non-oxidative disposal). At low metabolic rates (rest, mild exercise or low-intensity shivering), non-oxidative disposal represents a significant fraction of R_aGLU and, therefore, a direct measurement of oxidation is necessary to quantify the role of plasma glucose as an oxidative fuel.

The stimulation of circulatory glucose utilization in the cold was not accompanied by changes in plasma glucose or insulin concentrations (Fig. 2.5A and B), as previously observed in several other studies (Martineau and Jacobs, 1989a, Tipton *et al.*, 1997, Vallerand *et al.*, 1995, Vallerand and Jacobs, 1992, Vallerand *et al.*, 1999a). Constant glycemia shows that rates of hepatic glucose production and glucose disposal were both increased in parallel. The increase in glucose uptake taking place during cold exposure is therefore not dependent on changes in plasma insulin. However, it may be the consequence of a cold-induced increase in insulin sensitivity and/or GLUT translocation as previously proposed for humans (Vallerand *et al.*, 1988, Vallerand *et al.*, 1987) as well as animals (Smith and Davidson, 1982, Weekes *et al.*, 1983). An insulin-independent control of glucose uptake may allow an increase in glucose delivery specifically to shivering muscles rather than indiscriminately to all insulin-sensitive tissues.

The major source of CHO oxidation was muscle glycogen, providing three quarters of all the glucose oxidized in the cold (Table 2.2). Even though whole-body glycogen stores only represent 1% of total energy stores, shivering studies in humans have shown that glycogen availability - modified through diet or exercise - affects fuel selection (Martineau and Jacobs, 1989b, Tikuisis *et al.*, 2000b, Young *et al.*, 1989), and

possibly body cooling rate (Martineau and Jacobs, 1989b). In these studies, while $\dot{H}_{prod}$ was the same between glycogen depleted, glycogen loaded and normal glycogen controls immersed in 18°C water, RER values were significantly lower for glycogen depleted than for glycogen loaded and normal glycogen, indicating a compensatory shift to a greater relative use of lipids when glycogen reserves are depleted (Martineau and Jacobs, 1989b, Tikuisis *et al.*, 2000b, Young *et al.*, 1989). However, these studies do not provide information on the effect of glycogen availability on the relative importance of $RG_{ox\text{-}plasma}$ and $RG_{ox\text{-}mus}$ to total CHO oxidation.

Oxidizing lipids to generate heat

The dual role of lipids as a heat insulation layer and as a large, energy-dense, metabolic fuel (>95% of total energy stored) has been recognized for a long time (Schmidt-Nielsen, 1990). However, the quantitative importance of lipids as a substrate to support prolonged low-intensity shivering has been somewhat neglected because, over the last decade, most studies have focused on CHO-dependent heat production (Jacobs et al., 1994). Our results show that RF_{ox} provides 50% of all the heat produced (Table 2.2). The relative importance of lipid oxidation measured here is consistent with several studies (Jacobs, 1997, Martineau and Jacobs, 1988, Martineau and Jacobs, 1989a, Martineau and Jacobs, 1989b, Tikuisis et al., 2000b, Weller et al., 1998), whereas many others found that CHO oxidation is dominant (Glickman-Weiss *et al.*, 1993, Glickman-Weiss *et al.*, 1994, MacNaughton *et al.*, 1990, Vallerand *et al.*, 1995, Vallerand and Jacobs, 1989, Vallerand and Jacobs, 1990, Vallerand *et al.*, 1989, Vallerand *et al.*, 1993,

Vallerand *et al.*, 1999a). In all these studies, it is very interesting to note that the reported dominance of either CHO or lipids has no clear link with differences in shivering intensity, but might be correlated with the cooling protocol. While subjects exposed to cool air used CHO preferentially (~60% of $\dot{H}_{prod}$, (Glickman-Weiss *et al.*, 1993, Glickman-Weiss *et al.*, 1994, Jacobs, 1997, MacNaughton *et al.*, 1990, Vallerand *et al.*, 1995, Vallerand and Jacobs, 1989, Vallerand and Jacobs, 1990, Vallerand *et al.*, 1989, Vallerand *et al.*, 1993, Vallerand *et al.*, 1999a)), those cooled by water immersion or by LCS favored lipid utilization (~60% of $\dot{H}_{prod}$, (Jacobs, 1997, Martineau and Jacobs, 1988, Martineau and Jacobs, 1989a, Martineau and Jacobs, 1989b, Tikuisis et al., 2000b, Weller et al., 1998)). Physiological reasons for such a difference are unclear and further research will be needed to explain it.

If one considers the relatively low metabolic rates reached here during mild shivering (15-20% $\dot{V}O_{2max}$), the observed utilization of lipids is not that surprising. Exercise studies reveal that lipid oxidation predominates for prolonged work at all intensities below 50% $\dot{V}O_{2max}$ (Bergman and Brooks, 1999, Brooks *et al.*, 1999, Roberts *et al.*, 1996). Therefore, even at the highest possible metabolic rates reached during maximum shivering (about 5 times RMR or ~40% $\dot{V}O_{2max}$; (Eyolfson et al., 2001)), lipids may still play a significant role in heat generation, if fuel selection patterns are identical between exercise and shivering.

The large increase in lipid utilization observed here during low-intensity shivering (Table 2.2) is a strategy to spare limited CHO reserves. Any increase in the relative use

of lipids allows the maintenance of $\dot{H}_{prod}$ for a longer period of time, and, therefore, improves chances of survival in the cold. We can estimate theoretical values for maximum cold endurance under the conditions of our experiments, assuming that the relative use of the different fuels remains the same as measured after 2 h of mild shivering. An average adult male would be able to shiver at 245 W (Fig. 2.2) for ~20h under the specific conditions of the present study before depleting muscle glycogen reserves (assuming that 80% of muscle glycogen is available for oxidation, mean muscle glycogen concentration = 100 mmol glucosyl units·kg^{-1} wet mass; actively shivering muscle mass = 70% of 36 kg; $RG_{ox\text{-}mus}$ = 18.4 μmol·kg^{-1} body mass·min^{-1}, Table 2.2).

The stimulation of RF_{ox} (Table 2.2) found here was accompanied by a 2-fold increase in circulatory NEFA levels (Fig. 2.5D) as observed in other studies (Tipton *et al.*, 1997, Vallerand *et al.*, 1995, Vallerand and Jacobs, 1990, Vallerand and Jacobs, 1992, Vallerand *et al.*, 1999a, Weller *et al.*, 1998). Vallerand *et al.* (1999a) found that men exposed to 5°C for 3 h showed a parallel increase in NEFA concentration and NEFA disappearance rate. Together these observations suggest that the oxidation of circulatory NEFA increased throughout cold exposure. However, the relative contributions of NEFA from the circulation (adipose and liver) and from muscle triacylglycerol to the 4-fold increase in total fat oxidation remain to be established.

In conclusion, this study shows that total $\dot{H}_{prod}$ during prolonged low-intensity shivering is unequally shared between lipids (50%), muscle glycogen (30%), circulatory glucose (10%) and proteins (10%). Therefore, the importance of plasma glucose

oxidation is only minor and future research should focus on lipid and muscle glycogen stores that provide most of the energy for $\dot{H}_{prod}$.

CHAPTER 3. EFFECTS OF CARBOHYDRATE AVAILABILITY ON SUSTAINED SHIVERING: I. OXIDATION OF PLASMA GLUCOSE, MUSCLE GLYCOGEN AND PROTEINS

Based in part on

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INTRODUCTION

Even though CHO only represents ~1% of energy stores in normal subjects, their oxidation can account for up to 60% of total heat production during cold exposure (Haman *et al.*, 2002, Jacobs *et al.*, 1994). Modifying glycogen availability has a profound effect on fuel selection during shivering, to the extent that a complete shift from CHO dominance to lipid dominance can be elicited (Martineau and Jacobs, 1989b, Young *et al.*, 1989). Several studies show that shivering humans (prolonged immersion in 18°C-water) produce ~80% of total heat from CHO oxidation when glycogen reserves are artificially elevated, and the same percentage - *but from lipid oxidation* - when glycogen reserves are depleted (Martineau and Jacobs, 1989b, Young *et al.*, 1989). Usually, such drastic changes in fuel selection have no effect on cold tolerance because total heat production appears to be independent of glycogen availability (Martineau and Jacobs, 1989b, Weller *et al.*, 1998, Young *et al.*, 1989). However, when glycogen reserves are low, conflicting results have been reported for body cooling rate that shows a significant increase in one study (Martineau and Jacobs, 1989b), but no change in another (Young et al., 1989).

Two separate sources of CHO are available for heat production: hepatic glucose provided to shivering muscles by the circulation, and muscle glycogen. The relative importance of these two CHO sources in humans with normal glycogen stores was recently determined during sustained, low-intensity shivering (Haman *et al.*, 2002). In these subjects, most of the CHO oxidized came from muscle glycogen (~75%) whereas the contribution of circulating glucose was minor (~25% of total CHO oxidized or ~10%

of total heat produced). Several earlier studies investigated the effect of changes in the size of glycogen reserves on fuel metabolism during shivering. Using biopsies from the *vastus lateralis*, Martineau *et al.* (1989b) showed higher glycogen use in glycogen-loaded than in glycogen-depleted subjects, whereas Young *et al.* (1989) did not find a significant difference between the two conditions.

The roles played by the oxidation of blood glucose and body proteins have never been characterized in relation to the size of glycogen stores. Consequently, the purpose of this study was to measure the effects of changes in CHO availability on oxidative fuel selection during sustained shivering. More specifically, the oxidation rates of circulating glucose, proteins and lipids were quantified to determine their role in compensating for low / high glycogen availability. Using a combination of indirect calorimetry and stable isotope tracer methods, fuel oxidation was monitored in adult human male subjects with low and high glycogen levels while they were exposed to cold for 2-h. In view of the minor role played by circulating glucose in normal subjects (Haman *et al.*, 2002), we hypothesized that changes in plasma glucose oxidation would not be used to offset changes in glycogen oxidation. Therefore, we predicted that lipids and proteins would maintain heat production by compensating for the variable contribution from glycogen.

In the second part of this study, reported in the next chapter, we investigated a potential mechanism for changing fuel selection. It has been suggested that switching oxidative fuels could simply be achieved by recruiting different populations of muscle fibers specialized for lipid or CHO oxidation (Roberts et al., 1996). However, this concept of "fuel-specific fibers" has never been tested directly, probably because it is

very difficult to make simultaneous measurements of substrate metabolism and muscle fiber recruitment, particularly during exercise. It occurred to us that shivering could be an ideal model to test this hypothesis because electrical noise caused by limb movements is considerably lower than in exercise. In Chapter 4, we report electromyography data (EMG) recorded simultaneously with the substrate metabolism results presented here and correlate fuel selection with muscle fiber recruitment.

METHODS

Subjects

Six healthy male subjects volunteered to participate in this study approved by the Health Sciences Ethical Committee of the University of Ottawa. Physical characteristics of the subjects are presented in Table 3.1. Five to seven days prior to the experimental trials, maximal oxygen consumption was determined separately for upper and lower body using progressive cycloergometer protocols (90 W and 180 W, 2-min ramps for upper and lower body, respectively). Body composition was estimated by underwater weighing (Brosek et al., 1963).

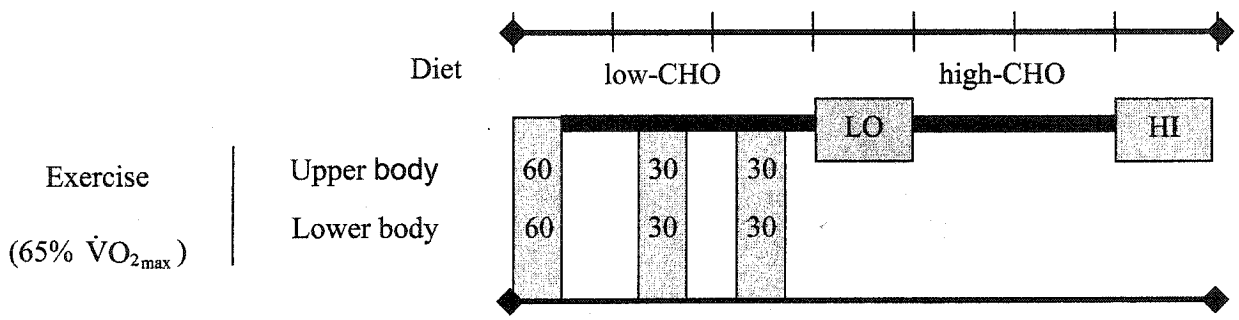
Experimental protocol

Five to six days prior to the experiments, a 1-h session was held to familiarize the subjects with the equipment and the level of cold exposure faced in the experiments. For the actual experiments, subjects were exposed to the cold on two separate occasions following: i) a diet low in CHO and heavy exercise bouts (LO) and, ii) a diet high in CHO without exercise bouts (HI). A detailed description of the diet and exercise regimen is given below. On the day of these experimental sessions, care was taken to minimize exercise or other thermal stresses between awakening and the start of the experiment (i.e. avoid exposure to hot or cold temperatures, very low intensity exercise during transit from home to the laboratory). Upon their arrival in the laboratory (8:00 AM; 12h post-absorptive), subjects were instrumented with thermal probes, an indwelling catheter (18G, 32 mm, Medical Inc., Arlington, TX) placed in an antecubital vein for blood

Table 3.1. Physical characteristics of subjects (n=6).
Values are presented as means ± SE.

Age, yr	22.2 ± 0.3
Body mass, kg	70.6 ± 3.2
Height, cm	174.1 ± 1.5
Body surface area, m^2	1.8 ± 0.04
Percent body fat, %	12.6 ± 1.0
$\dot{V}O_{2max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	
Upper body	37.1 ± 3.9
Lower body	51.7 ± 3.0

Figure 3.1. Seven day diet and exercise protocol to decrease (LO) and increase (HI) glycogen reserves. Thick black lines represent duration of low- and high-CHO diets. Experiments for LO and HI were performed on the morning of day 4 and day 7. Numbers in the grey boxes (in exercise section) indicate the duration (min) of exercise bouts for upper- and lower-body.


Diet
low-CHO
high-CHO
LO
HI
Exercise
(65% $\dot{V}O_{2max}$)
Upper body
Lower body
60
60
30
30
30
30

sampling, and they were fitted with a LCS (Three piece Delta Temax, Pembroke, ON, Canada). After voiding their bladder (T= 0 min), subjects remained seated comfortably for the next 2-h at 23.2 ± 0.01°C (758 ± 2 mmHg, 39.8 ± 3.6% RH). Following this period, they were transferred to an environmental chamber (10.5 ± 0.01°C, 755 ± 3 mmHg, 61 ± 2 % RH) and a 10°C water perfusion was started through the LCS using a temperature controlled circulation bath (Endocal, NESLAB and Model 200-00, Micropump, Vancouver, WA). Average environmental conditions in the laboratory and thermal chamber were the same for LO and HI experiments. Thermal response was monitored continuously at 23°C and during the subsequent 2-h cold exposure using a Hewlett Packard data acquisition and control unit (model 3497A). Metabolic and thermal comfort data were collected periodically throughout the experimental session.

Also, disposable surface electrodes (Blue Sensor, Medicotest Inc., USA) were positioned over the bellies of eight large and centrally located muscles: *trapezius* (TR), *latissimus dorsi* (LA), *pectoralis major* (PE), *rectus abdominis* (RA), *vastus lateralis* (VL), *rectus femoris* (RF), *vastus medialis* (VM) and *gastrocnemius* (GA). Shivering EMG was collected periodically prior and during cold exposure. Full description of EMG methodology and analysis is given in Chapter 4.

LO and HI diet and exercise regimen

In an attempt to modify glycogen availability, a combination of exercise and dietary manipulations was used within a seven-day period (Fig. 3.1). LO and HI diet composition are presented in Table 3.2. Total caloric intake was identical for both diets

and was calculated based on the weight of each subject (~170 $kJ \cdot kg^{-1} \cdot day^{-1}$). Care was taken to avoid ingestion of CHO from plants naturally rich in ^{13}C (C_4 photosynthetic cycle) to maintain low ^{13}C background enrichment in plasma glucose and expired CO_2. Three days prior to the LO experiment, glycogen depletion phase began with subjects performing upper and lower body exercises for 2 h at 65% $\dot{V}O_{2max}$ on a cycloergometer and a hand crank ergometer (alternating every 30 min between legs and arms). Following these exercises, subjects were prescribed a LO diet for the next three days until the evening of LO experiment. During this phase, additional 1h exercises were performed each day (Day 2 and 3) with upper and lower body (30 min each at 65% $\dot{V}O_{2max}$). Immediately following LO experiment (Day 4), subjects began a HI diet (Table 3.2) for the next 2.5 days and were asked to refrain from exercising during this period. HI experiment was performed on the morning of Day 7. This experimental design was selected to minimize inter-individual variability in the shivering response (i.e. same subjects for LO and HI), and to take advantage of the initial depletion in glycogen reserves to maximize glycogen loading on days 4 to 7. Using this non-randomized design could potentially result in an order effect associated with muscle fatigue, leading to a reduction in shivering intensity. However, results from this and the next study (Haman *et al.*, 2004a) show that thermal responses, heat production (indirect calorimetry) and shivering intensity (EMG) were not different between LO and HI. This indicates that muscle fatigue was most likely minimal because glycogen depletion exercise was performed at least 20 h before shivering measurements, and muscle work for thermogenesis was always very low (only up to 25% $\dot{V}O_{2max}$).

Thermal response

Whole body $\dot{H}_{loss}$ (in watts) was estimated using the following equation:

$$\dot{H}_{loss} = (\dot{R} + \dot{C}) + (\dot{E}_{resp} + \dot{C}_{resp}) \quad (3.1)$$

where, $\dot{R}$ and $\dot{C}$ represent rates of radiative and convective heat loss and, $\dot{E}_{resp}$ and $\dot{C}_{resp}$ are rates of evaporative and convective heat loss by ventilation (2,411.3 J of heat per gram of evaporated water). $\dot{R}$ and $\dot{C}$ were estimated using heat flux transducers (Concept Engineering, Old Saybrook, CT) placed on the surface of the skin at 11 sites (i.e. forehead, chest, biceps, forearm, abdomen, lower and upper back, front and back calf, quadriceps, hamstrings) and calculated using an area-weighed equation (Dubois and Dubois, 1916). Evaporative heat loss from the skin was assumed to be negligible at 23°C and 10°C (Nishi, 1981). Total $\dot{H}_{prod}$ was calculated by indirect respiratory calorimetry corrected for protein oxidation (see below). Percent of shivering peak was determined by dividing $\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$) values measured in the cold by the calculated shivering peak ($Shiv_{peak}$ in $ml \cdot kg^{-1} \cdot min^{-1}$) estimated for each subject using the following equation (Eyolfson et al., 2001):

$$Shiv_{peak} = 30.5 + (0.348 \times \dot{V}O_{2_{max}}) - (0.909 \times BMI) - (0.233 \times age) \quad (3.2)$$

where, $\dot{V}O_{2_{max}}$ is the maximal oxygen consumption ($ml \cdot kg^{-1} \cdot min^{-1}$), BMI is the body mass index ($kg \cdot m^{-2}$) and the age is in years.

T_{es} was monitored continuously using a pediatric esophageal probe (Mon-a-therm general purpose, Mallinckrodt Medical Inc, St-Louis, MO) and $\overline{T}_{skin}$ was averaged from 12 sites (i.e. finger tip plus 11 heat transducer sites mentioned above for heat flux measurements) using an area-weighed equation (Dubois and Dubois, 1916). Subjective thermal perception was determined every 30 min by asking the subjects to identify their comfort level based on an 11-point Likert scale (Gregory, 1992) (–5 and +5 being the coldest and warmest ever experienced, with 0 feeling neither cold nor warm). This scale was modified from the original version described by Thanasis *et al.* (1996).

Metabolic rate and fuel utilization

Pulmonary $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$ were determined by open-circuit spirometry (250 l, chain-compensated gasometer, Warren Collins inc., Braintree, MA). All expired gas collections were made at ambient temperature outside the experimental chamber. A mouthpiece, a unidirectional valve (2700 series, Hans Rudolph, Kansas City, MO), and a 44-mm plastic tube were used to direct all expired gases to the collection tank. Expired gases were collected for 5 min every 30 min before and during cold exposure and the mouthpiece was only removed for ~2 min after collecting expired gases to allow ingestion of the ^{13}C-glucose solution. Oxygen and carbon dioxide concentrations in dry expired gases were determined using calibrated electrochemical gas analyzers (AMETEK Model S-3A/1 and CD 3A, Applied Electrochemistry, Pittsburg, PA).

Total RP_{ox}, RG_{ox} and RF_{ox} oxidation rates (in $g \cdot min^{-1}$) were calculated using the following equations (Burelle *et al.*, 1999, Livesey and Elia, 1988):

$$RP_{ox}(g \cdot min^{-1}) = 2.9 \times UREA_{urine}(g \cdot min^{-1}) \quad (3.3)$$

$$RG_{ox}(g \cdot min^{-1}) = 4.59 \dot{V}CO_2(l \cdot min^{-1}) - 3.23 \dot{V}O_2(l \cdot min^{-1}) \quad (3.4)$$

$$RF_{ox}(g \cdot min^{-1}) = -1.70 \dot{V}CO_2(l \cdot min^{-1}) + 1.70 \dot{V}O_2(l \cdot min^{-1}) \quad (3.5)$$

where $\dot{V}CO_2$ ($l \cdot min^{-1}$) and $\dot{V}O_2$ ($l \cdot min^{-1}$) were corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1.010 and 0.843 $l \cdot g^{-1}$, respectively).

RP_{ox} was estimated from $UREA_{urine}$ in urine samples collected for a period of 120 min at 23°C and 10°C. Urinary urea concentrations were determined on a Synchron Clinical System (CX7, Beckman, Anaheim, CA). Energy potentials of 16.3 $kJ \cdot g^{-1}$, 40.8 $kJ \cdot g^{-1}$ and 19.7 $kJ \cdot g^{-1}$ were used to calculate the amount of heat produced from glucose, lipid and protein oxidation, respectively (Elia, 1991, Péronnet and Massicotte, 1991).

Plasma glucose oxidation

On the evening of the experiment, 3 g of glucose ($^{13}C/C = 0.01098$) artificially enriched with ^{13}C (U-^{13}C-glucose, $^{13}C/C > 99\%$, Isotec, Miamisburg, OH) to obtain a final $^{13}C/C$ ratio of 6.32 (R_{exo}) were diluted into 700 ml of water and split into seven equal doses (100 ml). Following the measurement of baseline $^{13}C/C$ in plasma and expired CO_2 (t = 30 min), subjects ingested the first dose of ^{13}C-glucose. Subsequent doses were then taken every 30 min until the end of the experiment. Isotopic composition of plasma glucose and expired CO_2 were determined in blood and expired gas samples every 30 min prior to the ingestion of the next dose. Upon collection, blood samples were put on ice,

spun in a refrigerated centrifuge, separated, and the plasma was kept frozen at –20°C until analyzed.

Plasma glucose was isolated by double-bed ion exchange chromatography with superimposed columns (resins: AG 50W-X8 H^+, 200-400 mesh, and AG 1-X8 chloride, 200-400 mesh). Following evaporation, glucose was combusted (60 min at 400°C) in the presence of CuO, and CO_2 was recovered. Measurements of $^{13}C/^{12}C$ in expired CO_2 (R_{exp}) and in CO_2 obtained from glucose combustion (R_{glu}) were determined in a Prism mass spectrometer (VG, Manchester, UK). Isotopic enrichment was expressed as $\%^{13}C/C$.

The $RG_{ox\text{-}plasma}$ was calculated from $^{13}CO_2$ production at the mouth and plasma glucose isotopic enrichment (Fig. 2A and B) using the following equation (Derman *et al.*, 1996, Wolfe, 1992):

$$RG_{ox\text{-}plasma} = \dot{V}CO_2\ (R_{exp}\text{-}R_{ref}/R_{glu}\text{-}R_{ref})(1/k1 \cdot k2) \qquad (3.6)$$

where $\dot{V}CO_2$ is in $l \cdot min^{-1}$ (STPD), R_{ref} is the isotopic composition of expired CO_2 prior to the ingestion of the first ^{13}C-glucose dose, k1 (0.7426 $l \cdot g^{-1}$) is the volume of CO_2 produced from the complete oxidation of glucose and k2 is the fractional recovery at the mouth of CO_2 produced in tissues (Pallikarakis et al., 1991). A fractional recovery of $^{13}CO_2$ at the mouth (k2) of 0.8 and 1 was used before and during cold exposure, respectively (Wolfe, 1992). Due to the large size of the bicarbonate pool, only values in the last 30 min before and during cold exposure were used in the calculation of $RG_{ox\text{-}plasma}$. In addition, $RG_{ox\text{-}plasma}$ was corrected to account for the fraction of plasma glucose

oxidized from exogenous sources (Haman *et al.*, 2002 and Chapter 2). Oxidation of glucose derived from muscle glycogen stores ($RG_{ox\text{-}mus}$; $g \cdot min^{-1}$), directly or through the lactate shuttle (Brooks et al., 1999), was calculated by subtracting $RG_{ox\text{-}plasma}$ from RG_{ox}.

$$RG_{ox\text{-}mus} = RG_{ox} - RG_{ox\text{-}plasma} \quad (3.7)$$

Blood analysis

Plasma glucose and lactate concentrations were measured spectrophotometrically at 340 nm on a Beckman DU 640 (Bergmeyer, 1985) while total plasma nonesterified fatty acid (NEFA) and β-hydroxybutyrate (β-HB) concentration were determined using an analytical assay kit (NEFA C, Wako Chemicals, Osaka, Japan and Sigma Kit #310, Sigma-Aldrich Canada, Oakville, ON, Canada). Insulin concentration was measured using a radioimmunoassay (#KTSP-11001, Medicorp Inc, Montréal, Qc, Canada).

Statistical analyses

Changes in T_{es}, $\overline{T}_{skin}$, $\dot{H}_{prod}$, $\dot{H}_{loss}$, expired CO_2 and plasma glucose isotopic enrichments, and gas exchange were assessed by two-way analysis of variance for repeated measures (ANOVA). For each sampling time, a Boferroni *t*-test was used to detect potential differences with control values observed at baseline. Differences in $\dot{H}_{prod}$, fuel utilization for CHO (RG_{ox}, $RG_{ox\text{-}exo}$, $RG_{ox\text{-}plasma}$, $RG_{ox\text{-}mus}$), lipids (RF_{ox}) and proteins (RP_{ox}) as well as plasma metabolite concentrations over the last 30 min before and during cold exposure were determined using a two-way analysis of variance to verify the main effect of diet (LO vs. HI) and cold exposure (23°C vs. 10°C). Statistical

differences were considered significant when $p \leq 0.05$. The statistical power of our tests was calculated for key parameters (oxidation rates, and relative contributions of plasma glucose, muscle glycogen, total CHO and lipids to total heat production) and it ranged from 0.945 to 1.000. All values presented are means ± SE ($n = 6$) unless indicated otherwise.

RESULTS

Thermal response

Changes in absolute $\dot{H}_{loss}$ and $\dot{H}_{prod}$ before and during cold exposure for LO and HI are presented in Fig. 3.3. Absolute $\dot{H}_{loss}$ and $\dot{H}_{prod}$ increased as a result of cold exposure but no significant difference was found between LO and HI. Maximal $\dot{H}_{loss}$ was reached after 10 min of cold exposure (76.7±3.6 W at 23°C to 220.5 ± 4.4 W at 10°C and 75.9±4.7 W to 210.8±4.4 W for LO and HI, respectively) and decreased gradually by 11%, stabilizing in the last 60 min at 10°C (192.2±6.6W for LO and 191.3±10.6W for HI). $\dot{H}_{prod}$ averaged 94.5±5.3 and 86.1±5.9 W at 23°C and reached a maximum of 224.3±17.4 and 209.1±26.4 W after 120 min in the cold for LO and HI, respectively. Metabolic rate reached by the end of cold exposure was not different between LO (34.4 ± 3.8 $\%Shiv_{peak}$) and HI (30.4 ± 2.0 $\%Shiv_{peak}$). As shown in Fig. 3.4, changes in T_{es}, $\overline{T}_{skin}$ and subjective thermal comfort were not different between LO and HI. While T_{es} did not change as a result of cold exposure (36.5±0.3°C), $\overline{T}_{skin}$ decreased from 33.6±0.4°C at 23°C to 27.4±0.5°C in the first 90 min of cold exposure and remained constant until the end of the experiment. Thermal comfort averaged +2 at 23°C and decreased continuously reaching –3 at the end of the cold exposure.

Plasma concentrations

Average plasma concentrations of insulin, glucose, lactate, total NEFA and β-HB calculated in the last 30 min before and during cold exposure are presented in Table 3.3.

Before cold exposure, while insulin, glucose and lactate concentrations were significantly lower for LO than HI (-22, -4 and -46%, respectively), NEFA and β-HB were 4.3- and 10.3-fold higher for LO than for HI. Insulin and β-HB concentrations were not affected by the change in temperature whereas glucose concentration increased 4% and 7.5% by the end of cold exposure for LO and HI, respectively. Cold exposure caused a 1.8-fold for LO and 1.5-fold for HI increase in lactate concentration over values at 23°C and total NEFA concentration increased 1.5- and 2.5-fold over values prior to cold exposure for LO and HI, respectively.

Metabolic fuel utilization

RG_{ox} and RF_{ox} for LO and HI are shown in Fig. 3.5. RG_{ox} was significantly higher for HI before (58.1±20.0 $mg \cdot min^{-1}$ and 240.6±27.4 $mg \cdot min^{-1}$ for LO and HI, respectively) as well as during cold exposure (Fig. 3.5A; maximum of 221.5±45.0 $mg \cdot min^{-1}$ and 469.9±61.7 $mg \cdot min^{-1}$ for LO and HI, respectively). Prior to cold exposure, RF_{ox} was not significantly different from zero for HI (non-protein respiratory exchange ratio was equal or larger than 1 indicating a stimulation of lipogenesis due to the high CHO uptake (Poppitt et al., 1988)) and averaged 58.2±6.0 $mg \cdot min^{-1}$ for LO. In the cold, RF_{ox} increased continuously reaching maximal values of 163.2±21.6 for LO and 68.4±22.0 $mg \cdot min^{-1}$ for HI after 90 min in the cold (Fig. 3.5B). As shown in Table 3.4, a close to 2-fold difference in protein oxidation rate (RP_{ox}) was observed between LO and HI before cold exposure (111.5±23.2 $mg \cdot min^{-1}$ and 63.6±6.0 $mg \cdot min^{-1}$ for LO and HI, respectively) and in the cold (118.3±12.9 $mg \cdot min^{-1}$ and 65.5±5.9 $mg \cdot min^{-1}$ for LO and HI,

respectively). Changes in the respective contributions of CHO (%RG_{ox}), lipid (%RF_{ox}) and protein (%RP_{ox}) oxidation to total $\dot{H}_{prod}$ under LO and HI conditions are presented in Fig. 3.6. Prior to cold exposure, %RG_{ox} was found to be 4.3-fold lower for LO (17.5±5.7% $\dot{H}_{prod}$) when compared to HI (75.7±5.0% $\dot{H}_{prod}$) (Fig. 3.6A). The subsequent decrease in environmental temperature had not effect on %RG_{ox} with values averaging 24.1±6.1% $\dot{H}_{prod}$ for LO and 67.3±6.0% $\dot{H}_{prod}$ for HI. A continuous increase in %RF_{ox} was observed for HI averaging a value not different from zero at 23°C and increasing to a maximum of 21.7±5.3% $\dot{H}_{prod}$ in the last hour of cold exposure (Fig. 3.6B). For LO, %RF_{ox} in the cold (58.2±6.0% $\dot{H}_{prod}$) was not different from control values at 23°C (52.5±4.5% $\dot{H}_{prod}$; Fig. 3.6B). As a result of cold exposure, a 2.2-fold decrease %RP_{ox} was found for LO (40.2±7.8 at 23°C to 18.7±1.5% $\dot{H}_{prod}$ at 10°C) and HI (25.3±3.6 at 23°C to 11.7±1.5% $\dot{H}_{prod}$ at 10°C; Fig. 3.6C).

Table 3.5 summarizes average values measured before cold exposure (90-120 min) and in the last 30 min at 10°C (210-240 min), for all parameters of fuel utilization ($\dot{H}_{prod}$, RF_{ox}, RG_{ox}, $RG_{ox\text{-}plasma}$, $RG_{ox\text{-}mus}$, and RP_{ox}) estimated in this study under LO and HI. The dietary and exercise regimen used to modify glycogen availability had a significant impact on the absolute and relative contributions of CHO, lipids and proteins to total heat production prior and during cold exposure. Prior to cold exposure, RF_{ox} was not different from zero for HI and averaged 58.2 ± 6.0 $mg \cdot min^{-1}$ for LO, whereas RG_{ox} and RP_{ox} showed a respective 4.1- and 1.8-fold difference between LO and HI. In

addition, $RG_{ox\text{-}plasma}$ was only 1.3-fold higher for HI than for LO while $RG_{ox\text{-}mus}$ showed a 6.8-fold difference between LO and HI. The relative contribution of proteins to total heat production was 1.6-fold higher for LO than for HI whereas that of CHO was 4.3-fold higher for HI than for LO and that of lipids was not different from 0 for HI and averaged 41.6±3.6% $\dot{H}_{prod}$ for LO. Furthermore, $RG_{ox\text{-}plasma}$ contributed relatively less for LO than for HI (+138%) and $RG_{ox\text{-}mus}$ was 697% higher for HI than for LO. As a result of cold exposure, RG_{ox} increased respectively 4.7- and 2.0-fold for LO and HI whereas RP_{ox} did not change. RF_{ox} increased 2.6-fold for LO and from a value not different from zero at 23°C to 68.4 ± 22.0 $mg \cdot min^{-1}$ by the end of cold exposure. Increases 5.5- and 1.8-fold for $RG_{ox\text{-}mus}$ as well as 1.9- and 1.4-fold $RG_{ox\text{-}plasma}$ were observed as a result of cold exposure for LO and HI, respectively. While proteins contributed 1.6-fold more to total $\dot{H}_{prod}$ under LO than HI, $\%RG_{ox}$ was 2.4-fold higher for HI than for LO. In addition, the relative contribution of $RG_{ox\text{-}plasma}$ and $RG_{ox\text{-}mus}$ to total heat production in the cold was 179% and 256% higher for HI than for LO, respectively.

Table 3.2. Macronutritional composition of low- and high-CHO diets. Values are means ± SE; (n=5).

	low-CHO	high-CHO
CHO, g/day	67.9 ± 10.4	494.0 ± 54.8
Lipids, g/day	204.4 ± 19.7	38.4 ± 4.8
Proteins, g/day	170.4 ± 20.5	111.5 ± 10.9
CHO, %	9.7 ± 0.5	71.5 ± 0.5
Lipids, %	65.8 ± 1.2	12.5 ± 0.5
Proteins, %	24.3 ± 0.8	16.2 ± 0.4

Table 3.3. Plasma insulin, glucose, lactate, nonesterified fatty acid (NEFA) and β-hydroxybutyrate (β-HB) concentrations in men with low (LO) / high (HI) CHO reserves before (23°C) and during cold exposure (10°C; T=210-240 min).

	LO		HI	
	23°C	10°C	23°C	10°C
Insulin, pM	77.0±1.7	82.0±2.4	99.2±4.2[b]	95.1±2.9[c]
Glucose, mM	4.7±0.1	4.9±0.1[a]	4.9±0.1[b]	5.3±0.2[ac]
Lactate, mM	0.7±0.1	1.3±0.3[a]	1.3±0.1[b]	1.9±0.2[c]
NEFA, μM	431±34	651±33[a]	100±21[b]	253±53[ac]
β-HB, μM	322±50	482±64	31±9[b]	24±6[c]

[a]Significantly different from control values at 23°C; [b]Significantly different from control values at 23°C in LO; [c]Significantly different from values at 10°C in LO. Differences were assessed by a two-way ANOVA, $P \leq 0.05$, n=6.

Table 3.4. Urinary urea excretion rate and absolute oxidation rate of protein in men with low (LO) / high (HI) CHO availability before (23°C) and during cold exposure (10°C).

	LO		HI	
	23°C	10°C	23°C	10°C
Urinary urea excretion, $g \cdot 120min^{-1}$	4.8 ± 0.6	5.0 ± 0.9	2.6 ± 0.3[b]	2.7 ± 0.2[c]
Protein oxidation rate, $g \cdot 120min^{-1}$	13.4 ± 2.8	14.2 ± 1.6	7.6 ± 0.7	7.8 ± 0.7

[a]Significantly different from control values at 23°C; [b]Significantly different from control values at 23°C in LO; [c]Significantly different from values at 10°C in LO. Differences were assessed by a two-way ANOVA, $P \leq 0.05$, n=6.

Table 3.5. Absolute oxidation rate ($mg \cdot min^{-1}$) and relative ($\% \dot{H}_{prod}$) contributions of lipids (RF_{ox}), total CHO (RG_{ox}), plasma glucose ($RG_{ox\text{-}plasma}$), muscle glycogen ($RG_{ox\text{-}mus}$) and proteins (RP_{ox}) to total heat production in men with low (LO) and high (HI) CHO availability before (23°C; 90-120 min) and during cold exposure (10°C; 210-240 min).

	LO		HI	
	23°C	10°C	23°C	10°C
$\dot{H}_{prod}$, $kJ \cdot min^{-1}$	5.5 ± 0.2	12.6 ± 0.8[a]	5.0 ± 0.4	11.7 ± 1.3[a]
Lipids (RF_{ox}), $mg \cdot min^{-1}$	58.2 ± 6.0	163.2 ± 21.6[a]	0*	68.4 ± 22.0[ac]
$\% \dot{H}_{prod}$	41.6 ± 3.6	53.4 ± 4.5[a]	0*	23.0 ± 5.2[ac]
CHO (RG_{ox}), $mg \cdot min^{-1}$	60.1 ± 18.7	221.5 ± 45.0[a]	248.6 ± 27.9[b]	469.9 ± 61.7[ac]
$\% \dot{H}_{prod}$	18.1 ± 5.4	27.7 ± 5.2[a]	77.1 ± 3.8[b]	65.3 ± 5.4[ac]
Liver ($RG_{ox\text{-}plasma}$), $mg \cdot min^{-1}$	29.3 ± 2.4	53.8 ± 6.1[a]	38.5 ± 5.0	89.6 ± 9.7[ac]
$\% \dot{H}_{prod}$	8.9 ± 0.8	7.3 ± 0.9	12.3 ± 1.2	13.1 ± 1.4
Muscle ($RG_{ox\text{-}mus}$), $mg \cdot min^{-1}$	30.7 ± 21.0	167.8 ± 43.1[a]	210.1 ± 24.0[b]	380.3 ± 58.3[ac]
$\% \dot{H}_{prod}$	9.3 ± 5.9	20.4 ± 4.6[a]	64.8 ± 3.3[b]	52.3 ± 4.9[ac]
Proteins (RP_{ox}), $mg \cdot min^{-1}$	111.5 ± 23.2	118.3 ± 12.9	63.6 ± 6.0	65.5 ± 5.9
$\% \dot{H}_{prod}$	40.2 ± 7.8	18.7 ± 1.5[a]	25.3 ± 3.6[b]	11.7 ± 1.5[ac]

[a]Significantly different from control values at 23°C, [b]Significantly different from control values at 23°C in LO, [c]Significantly different from values at 10°C in LO. Differences were assessed by a two-way ANOVA, $P \leq 0.05$, n=6.
*see RESULTS

Figure 3.2. Expired CO_2 (A) and plasma glucose (B) isotopic enrichment (% $^{13}C/C$) before and during cold exposure for LO (closed circles) and HI (open circles). * Significantly different from control values before cold exposure (Two-way ANOVA with replication and Bonferroni *post-hoc t*-test, $P \leq 0.05$, n=6).

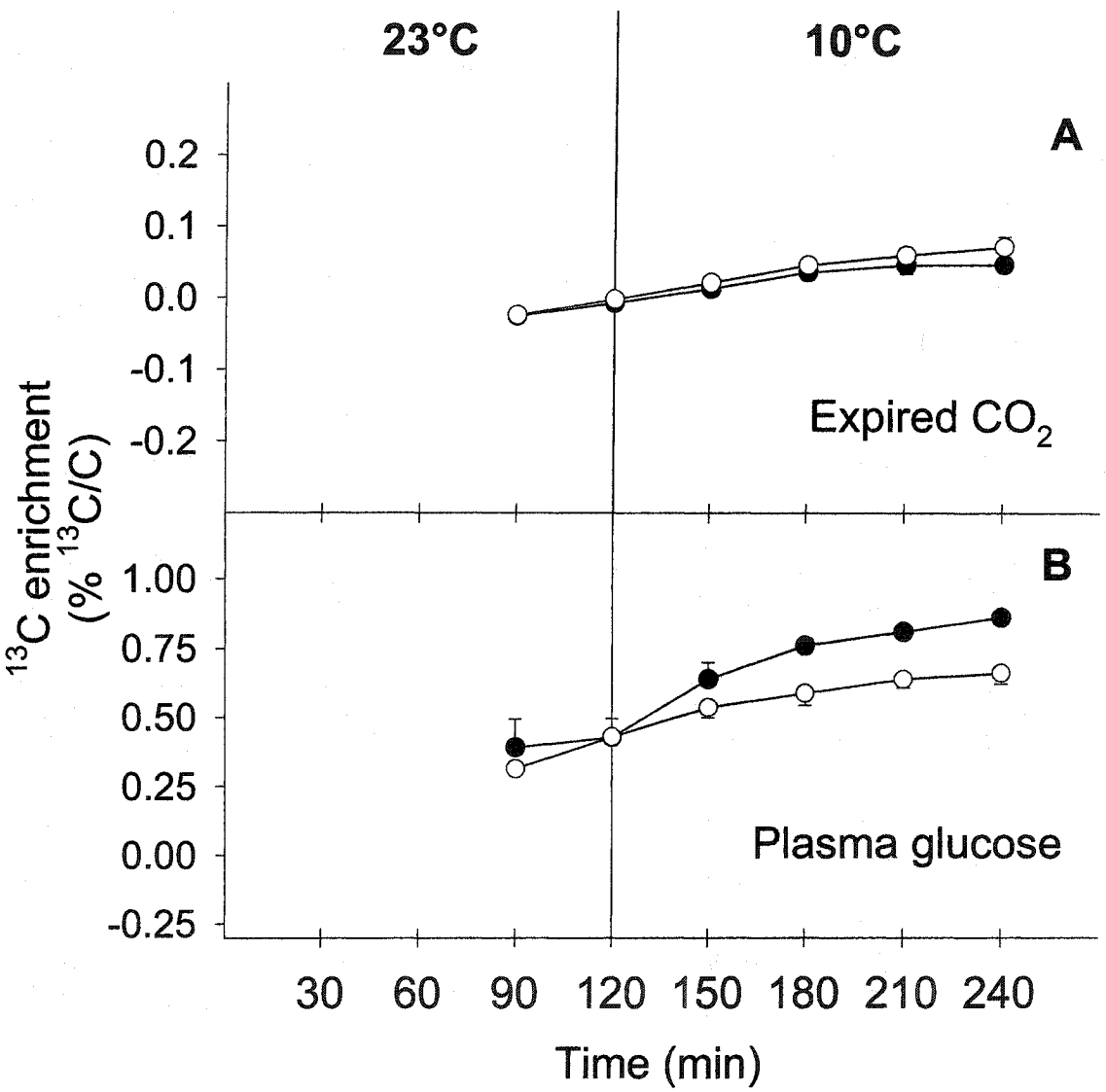

23°C
10°C
A
0.2
0.1
0.0
-0.1
-0.2
Expired CO_2
B
1.00
0.75
0.50
0.25
0.00
-0.25
Plasma glucose
^{13}C enrichment (% $^{13}C/C$)
30
60
90
120
150
180
210
240
Time (min)

Figure 3.3. Changes in heat loss (A: $\dot{H}_{loss}$) and heat production (B: $\dot{H}_{prod}$) before and during cold exposure for LO (closed circles) and HI (open circles).

* Significantly different from control values before cold exposure (Two-way ANOVA with replication and Bonferroni *post-hoc t*-test, $P \leq 0.05$, n=6).

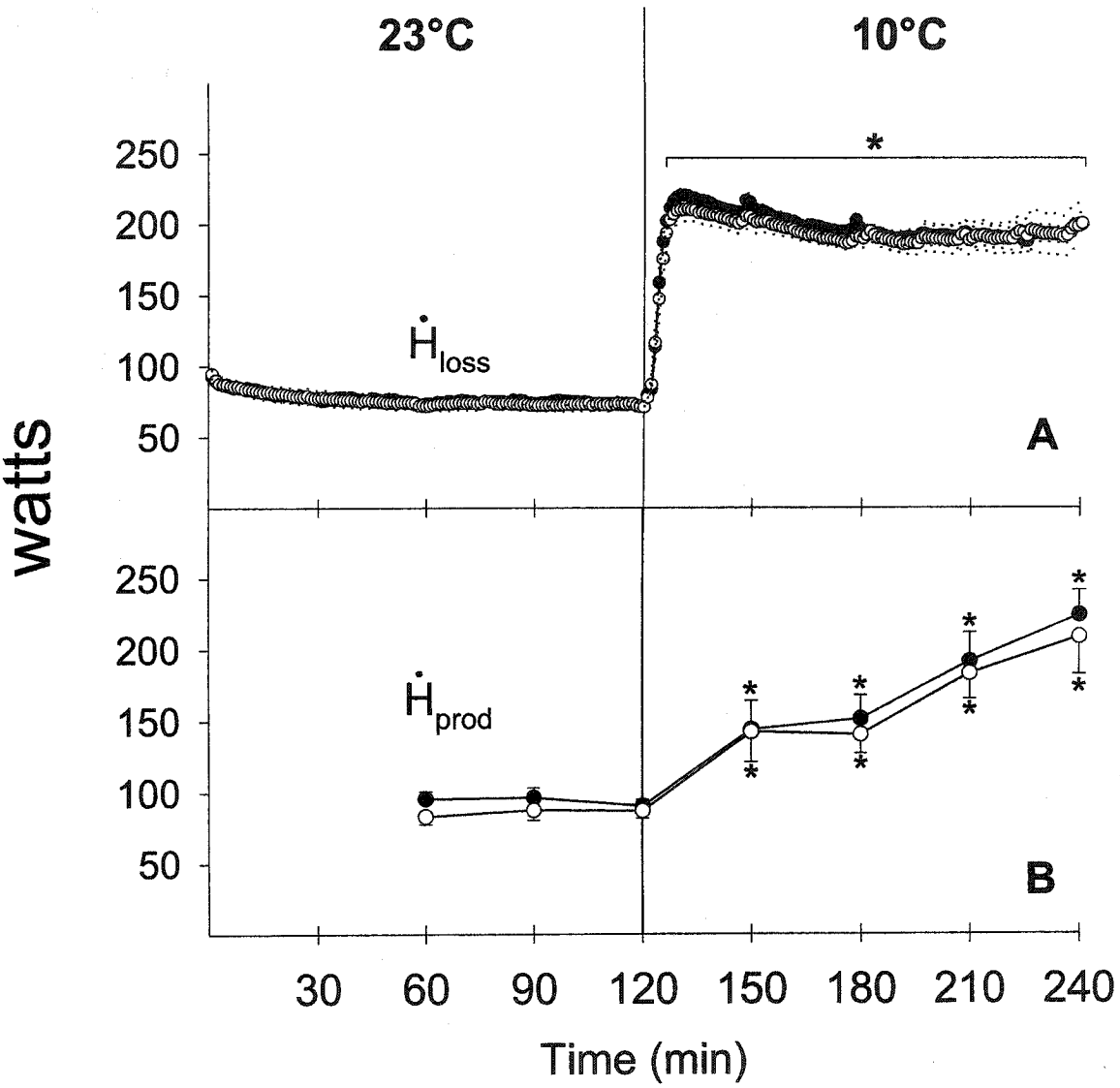

23°C
10°C
watts
250
200
150
100
50
$\dot{H}_{loss}$
A
$\dot{H}_{prod}$
B
30
60
90
120
150
180
210
240
Time (min)

Figure 3.4. Changes in esophageal (T_{es}) and mean skin ($\overline{T}_{skin}$) temperature as well as subjective thermal comfort before and during cold exposure for LO (closed circles) and HI (open circles). Arrows indicate times when the ^{13}C-glucose solutions were ingested. * Significantly different from control values before cold exposure (Two-way ANOVA with replication and Bonferroni *post-hoc* *t*-test, $P \leq 0.05$, n=6).

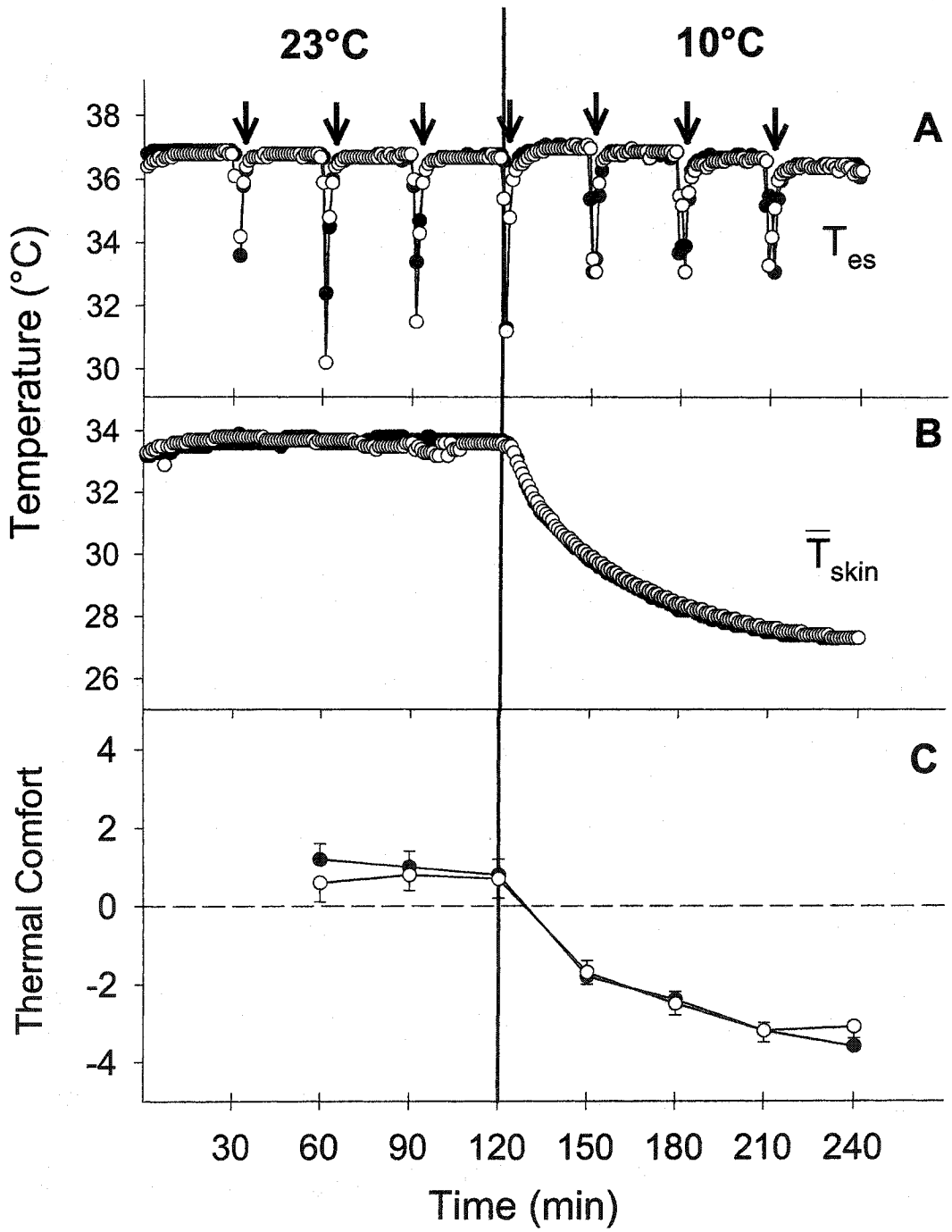

23°C
10°C
A
B
C
T_{es}
$\overline{T}_{skin}$
Temperature (°C)
Thermal Comfort
Time (min)
38
36
34
32
30
28
26
4
2
0
-2
-4
30
60
90
120
150
180
210
240

Figure 3.5. Changes in absolute CHO (RG_{ox}) and lipid (RF_{ox}) oxidation rates before and during cold exposure for LO (closed circles) and HI (open circles). Gray circles represent previously published values in men with normal CHO reserves (Haman *et al.*, 2002). * Significantly different from control values before cold exposure (Two-way ANOVA with replication and Bonferroni *post-hoc* *t*-test, $P \leq 0.05$, n=6).

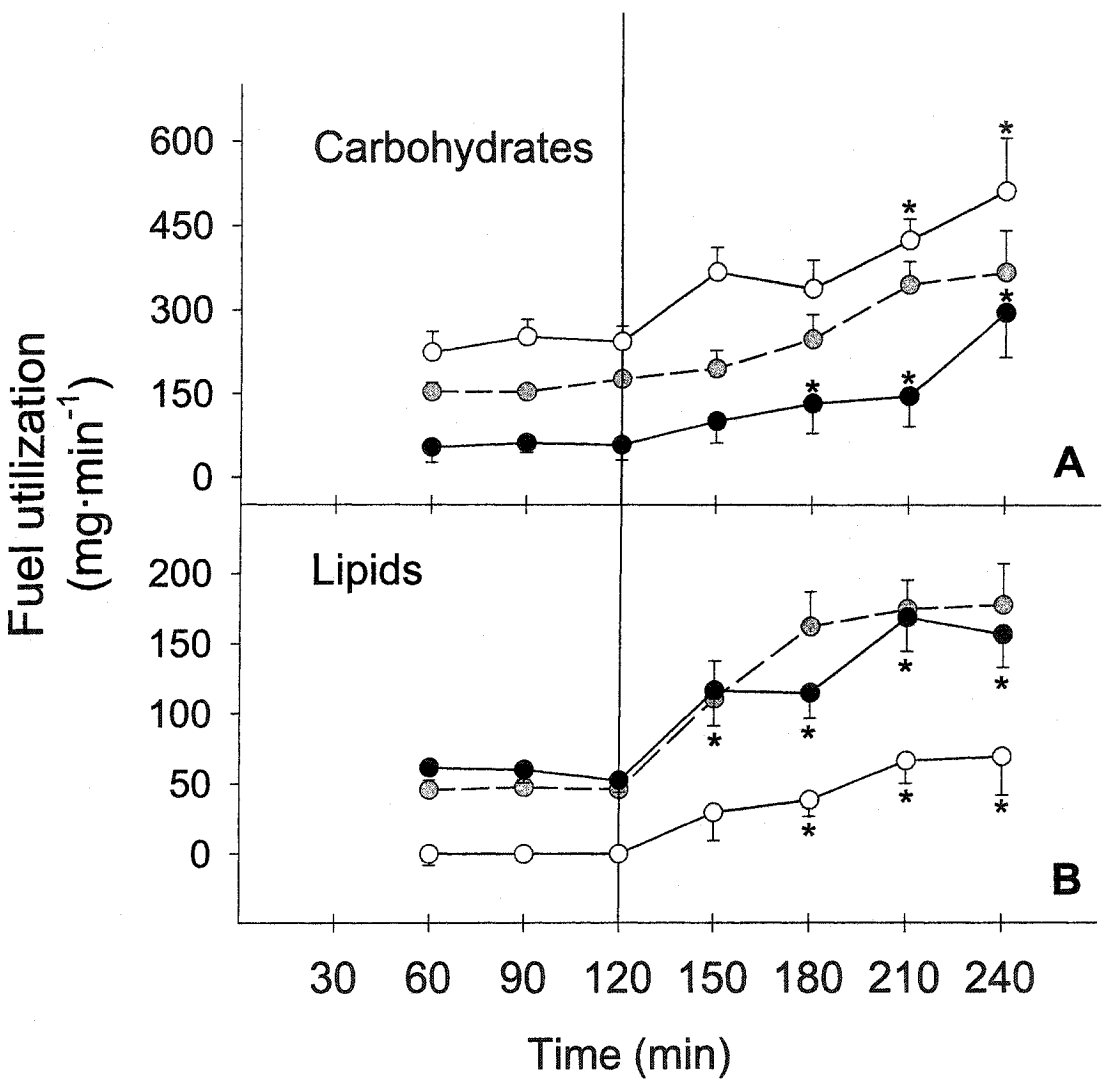

Carbohydrates
Lipids
A
B
Fuel utilization (mg·min-1)
600
450
300
150
0
200
150
100
50
0
30
60
90
120
150
180
210
240
Time (min)

Figure 3.6. Changes in the relative contribution of CHO (RG_{ox}), lipid (RF_{ox}) and protein (RP_{ox}) to total heat production before and during cold exposure for LO (closed circles) and HI (open circles). Gray circles represent previously published values for men with normal CHO reserves (Haman *et al.*, 2002). * Significantly different from control values before cold exposure (Two-way ANOVA with replication and Bonferroni *post-hoc t*-test, $P \leq 0.05$, n=6).

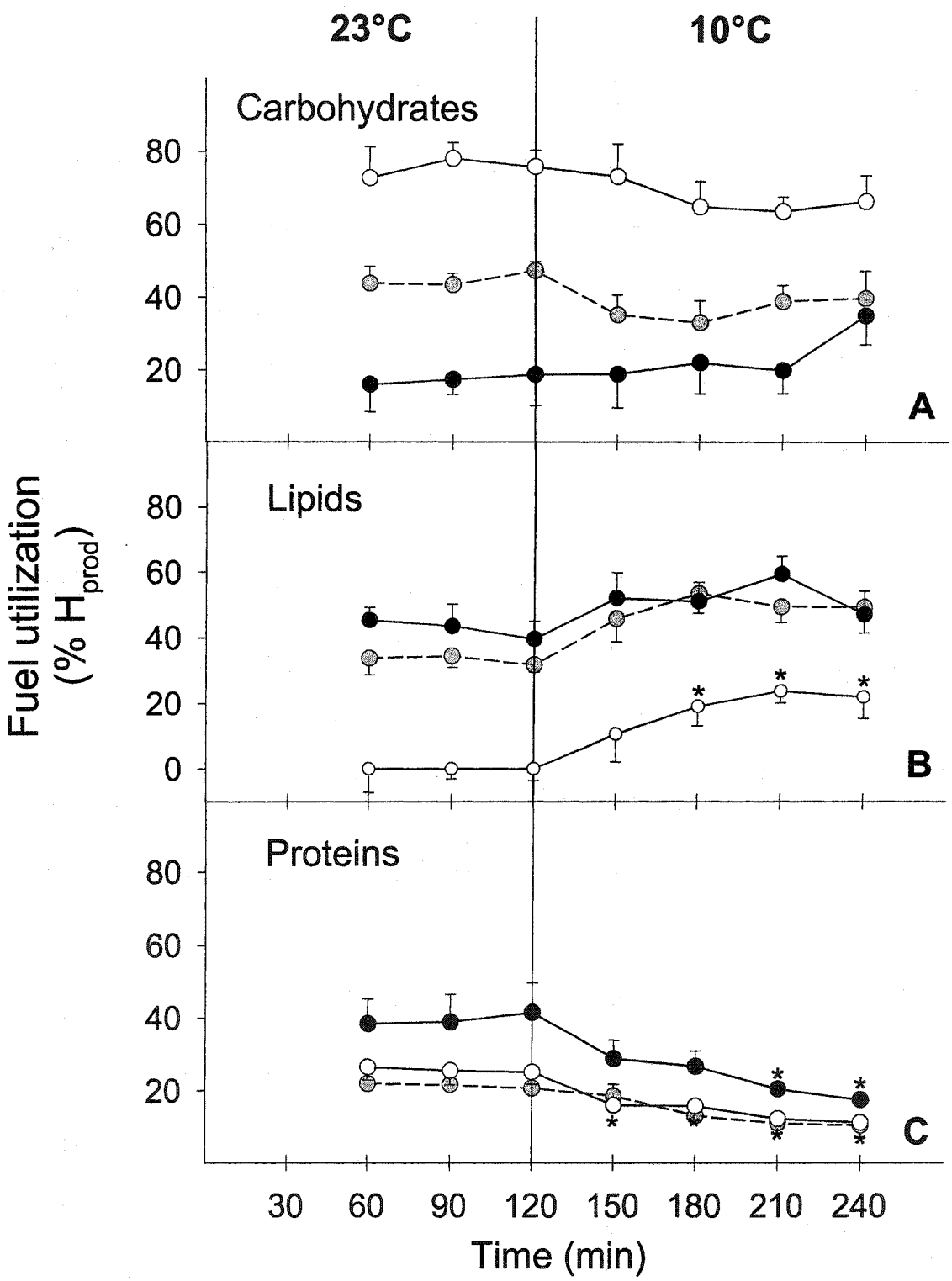
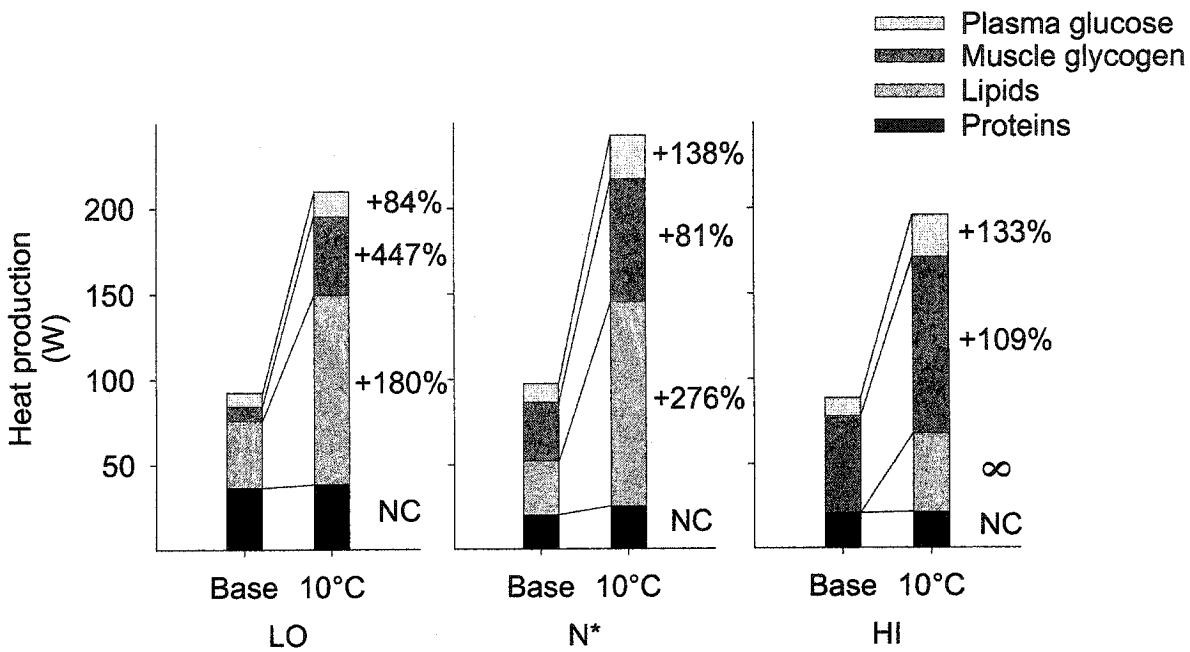

23°C
10°C
Carbohydrates
Lipids
Proteins
A
B
C
Fuel utilization
(% H_{prod})
80
60
40
20
0
30
60
90
120
150
180
210
240
Time (min)

Figure 3.7. Relative effects of prolonged low-intensity cold exposure on the contribution of protein, lipid, plasma glucose and muscle glycogen to total heat production in men with low, normal and high CHO reserves. Relative changes from baseline were calculated from oxidation rates measured before and during the last 30 min of cold exposure. NC indicates no significant change. (Two-way ANOVA, $P \leq 0.05$, $n=6$)


Plasma glucose
Muscle glycogen
Lipids
Proteins
Heat production (W)
200
150
100
50
+84%
+447%
+180%
NC
+138%
+81%
+276%
NC
+133%
+109%
∞
NC
Base 10°C
LO
Base 10°C
N*
Base 10°C
HI

DISCUSSION

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

Blood glucose oxidation

Previous work in subjects with normal (N) CHO availability (Haman *et al.*, 2002), and this study in glycogen-loaded and –depleted subjects (Table 3.5), show that the relative role of plasma glucose is always minor during sustained, low-intensity shivering (< 13% $\dot{H}_{prod}$). Cold exposure stimulates $RG_{ox\text{-}plasma}$ in direct proportion to metabolic rate in HI (2.3-fold; as previously observed in normal subjects, (Haman *et al.*, 2002)),

whereas a smaller increase is observed in LO (1.8-fold). These results suggest that hepatic glucose production is lower in glycogen-depleted subjects, because gluconeogenesis does not make up for decreased liver glycogenolysis in this group. Plasma glucose oxidation does not compensate for the large decrease in muscle glycogen oxidation of LO, and other fuels must be used to sustain heat production. Clearly, however, plasma glucose oxidation rates of our subjects were not limited by maximal hepatic glucose production, because much higher $RG_{ox\text{-}plasma}$ have been reported during exercise (6 to 8 $mg \cdot kg^{-1} \cdot min^{-1}$ vs 0.7 to 1.3 $mg \cdot kg^{-1} \cdot min^{-1}$ in the cold) (Bosch, 1993, Bosch *et al.*, 1994, Bosch *et al.*, 1996, Coggan *et al.*, 1990, Friedlander *et al.*, 1997, Jeukendrup *et al.*, 1999, Péronnet *et al.*, 1998). Friedlander *et al.* (1997) showed that $RG_{ox\text{-}plasma}$ is stimulated by as much as 5-fold during exercise at 45% $\dot{V}O_{2max}$, the highest metabolic rate observed during maximal shivering (Eyolfson et al., 2001). Considering the low metabolic rates reached in this study (2.3 times resting metabolic rate or 20% $\dot{V}O_{2max}$), further research is needed to establish whether plasma glucose plays a more important role at higher shivering intensities.

Muscle glycogen oxidation

This study provides the first *whole-body* measurements of glycogen utilization during sustained, low-intensity shivering. Muscle glycogen supplied 75 to 80% of total CHO oxidized (Table 3.5) and played an important role in total heat production (20% and 50% $\dot{H}_{prod}$ in LO and HI, respectively). The absolute rate of muscle glycogen utilization was two times higher in HI (380.3±58.3 $mg \cdot min^{-1}$) than in LO (167.8±43.1 $mg \cdot min^{-1}$), but

the greatest relative change caused by cold exposure was observed in LO (+445% for LO vs +80% for HI). These results show that muscle glycogen reserves are always strongly mobilized for thermogenesis, even when they have been reduced before cold exposure.

Only two other papers have addressed the problem of oxidative fuel selection in relation to altered glycogen reserves (Martineau and Jacobs, 1989b, Young *et al.*, 1989). They have reported much lower or higher rates of muscle glycogen utilization than observed here. The conclusions drawn from these studies were based on the direct measurement of glycogen concentration in *vastus lateralis* biopsies, and this experimental approach may be responsible for the large variability. Even though their measurements were made at higher shivering intensities, Young *et al.* (1989) reported no change in muscle glycogen levels in LO or HI. In the other study, Martineau *et al.* (1989b) found an average glycogen utilization rate approximately four times higher (~20 mg·kg body weight^{-1}·min^{-1}) than values reported here for HI, but no significant change for LO. The exact reasons for these discrepancies are unclear, but methodological limitations may be responsible. For example, it is well known that estimating whole-body glycogen utilization from muscle biopsies is extremely difficult because: 1) glycogen concentration is variable within and among muscles and, 2) the relative contribution of *vastus lateralis* to total shivering activity is not known.

Protein oxidation

Altering glycogen reserves through dietary and exercise manipulations had a major effect on the relative use of proteins before cold exposure. At 23°C, protein

oxidation was responsible for 25% $\dot{H}_{prod}$ in HI, and this value increased to 40% $\dot{H}_{prod}$ in LO (Table 3.4). Absolute rates of protein oxidation were not affected by cold exposure, and, therefore, the 2.3-fold increase in metabolic rate observed in the cold was simply translated as a proportional decrease in the relative role of proteins for both groups (12% $\dot{H}_{prod}$ in HI and 19% $\dot{H}_{prod}$ in LO; Table 3.5). The contribution of protein oxidation to shivering thermogenesis has generally been assumed to be minor (~10% $\dot{H}_{prod}$), and, therefore, rarely measured directly (Haman *et al.*, 2002, Vallerand *et al.*, 1995, Vallerand *et al.*, 1999a). Our results show that this assumption may still be true when glycogen reserves are artificially elevated, but that the relative importance of proteins increases substantially when CHO reserves are reduced. In fact, the increased contribution of proteins plays a key role in compensating for the decrease in CHO use after glycogen depletion. Failing to take protein oxidation into account in fuel oxidation budgets during low-intensity shivering leads to a significant overestimation of CHO and lipid oxidation rates, particularly when glycogen reserves are depleted.

Fuel metabolism before cold exposure

It is clear that our dietary and exercise manipulations had the desired impact on glycogen reserves, but muscle glycogen concentrations were not directly measured in our study. Previous experiments where muscle biopsies were taken provide strong evidence that our glycogen-loading and depleting protocols affected muscle glycogen levels significantly (Bergström *et al.*, 1967, Conlee, 1987, Martineau and Jacobs, 1989b, Martineau and Jacobs, 1991, Young *et al.*, 1989). Using the same experimental protocol,

Young *et al.* (1989) found a 4-fold difference in glycogen levels of the *vastus lateralis* between LO and HI (144±14 vs. 543±53 mmol glucose·kg^{-1} dry muscle, respectively). Even when intense exercise was not used (in addition to diet) to deplete glycogen (Martineau and Jacobs, 1989b, 1991), a 2-fold difference in muscle glycogen levels was still reported between LO (247±15 mmol glucose·kg^{-1} dry muscle) and HI (548±42 mmol glucose·kg^{-1} dry muscle). The various effects on fuel selection and plasma metabolite concentrations found in this study are also consistent with large changes in glycogen levels (Fig. 3.5 and 3.6, Tables 3.4 and 3.5). Before cold exposure, we show a complete shift from lipid and protein oxidation for LO (40% $\dot{H}_{prod}$ each, for lipids and proteins) to CHO-based metabolism for HI (80% $\dot{H}_{prod}$ accounting for by CHO). This large difference in total CHO oxidation (20% to 80% $\dot{H}_{prod}$) was almost entirely caused by changes in $RG_{ox\text{-}mus}$ (30.7 ± 21.0 mg·min^{-1} for LO and 210.13±324.0 mg·min^{-1} for HI) because $RG_{ox\text{-}plasma}$ remained minor in both groups. Together, these results show that the protocol selected for our study had the anticipated effects on glycogen reserves.

Same heat production, very different fuel mixtures

The contributions of all oxidative fuels to total heat production before and after cold exposure, as well as the % changes caused by shivering, are summarized in Fig. 3.7 for LO, N (Haman *et al.*, 2002), and HI. Altering glycogen reserves had a major effect on fuel selection before, but also during shivering (Fig. 3.5, Fig. 3.6, Table 3.5). In the cold, total heat production was unequally shared between lipids (53% $\dot{H}_{prod}$), CHO

(28% $\dot{H}_{prod}$), and proteins (19% $\dot{H}_{prod}$) in LO, but the pattern of fuel selection was widely different for HI: CHO (65% $\dot{H}_{prod}$), lipids (23% $\dot{H}_{prod}$), and proteins (12% $\dot{H}_{prod}$). These drastic differences in oxidative fuel selection had no impact on total heat production because $\dot{H}_{prod}$ was the same in both groups. Similar results have been observed at higher shivering intensities (~3.5 times RMR for men immersed in 18°C for up to 90 min) when large changes in substrate utilization only had a minor (Martineau and Jacobs, 1989b) or no effect (Young et al., 1989) on total heat production.

However, some discrepancies in the relative contributions of CHO and lipids between these studies and ours still remain. Because oxidation rates reported in these previous papers were not corrected for protein oxidation, we have recalculated our values using non-protein respiratory exchange ratios for comparison. After this adjustment, CHO and lipid oxidation rates were found to be consistent in all studies for LO, but not for HI. A greater reliance on CHO was observed in Young *et al.* (1989) and our study (65% $\dot{H}_{prod}$) than in Martineau *et al.* (1989b) (50% $\dot{H}_{prod}$). This difference may be related to the glycogen loading/depletion protocols or to the experimental design (longitudinal design here and in Young *et al.* (1989) vs transversal design in Martineau *et al.* (1989b)). Such small discrepancies have no impact on the general observation that lipids and proteins can adequately compensate for important changes in CHO oxidation to maintain heat production for at least 2 h of cold exposure.

Lipid oxidation

In both groups, circulating NEFA concentration showed a large increase during shivering (1.5- and 2.5-fold in LO and HI, respectively; Table 3.3). Such changes are consistent with several other shivering studies where RF_{ox} was stimulated in normal subjects (Haman *et al.*, 2002, Tipton *et al.*, 1997, Vallerand *et al.*, 1995, Vallerand and Jacobs, 1990, Vallerand and Jacobs, 1992, Vallerand *et al.*, 1999a, Weller *et al.*, 1998) and they reflect the well known correlation between plasma NEFA concentration and NEFA flux.

The importance of lipids as substrate for low-intensity shivering has been demonstrated recently for individuals with normal CHO reserves (Haman *et al.*, 2002, Weller *et al.*, 1998). In these studies, lipid oxidation provided a dominant fraction of the heat produced in the cold (50-70% $\dot{H}_{prod}$). In glycogen-depleted and glycogen–loaded individuals (this study), we also found that lipids play a significant role for maintaining shivering (23% $\dot{H}_{prod}$ in HI and 53% $\dot{H}_{prod}$ in LO). A strong stimulation of lipid oxidation was observed in HI where cold exposure caused RF_{ox} to increase from 0 to 68 $mg \cdot min^{-1}$. For LO, a 2.8-fold increase was found and, as previously observed in N (Haman *et al.*, 2002), lipids provided as much heat as all other metabolic fuels combined. Strong stimulation of lipid use represents an important strategy to spare small CHO reserves (~1% of total energy reserves in normal subjects) and to prolong survival in the cold. We have estimated theoretical values of maximal cold endurance for LO and HI, assuming that the relative use of the different fuels would remain the same as after 2 h of shivering. Under our conditions of cold exposure, an average subject could shiver at 200

W (Fig. 3.3) for ~20 h before depleting muscle glycogen, and, surprisingly, this maximal duration would be the same for LO and HI (assuming that: a) 80% of total muscle glycogen is available for oxidation, b) active muscle mass during shivering is 70% of 36 kg, c) mean muscle glycogen concentrations are 62 and 137 mmol glucosyl units/ kg wet mass as observed in Young *et al.* (1989), and d) $RG_{ox\text{-}mus}$ is 14 and 29 μmol·kg body $mass^{-1}\cdot min^{-1}$ for LO and HI, respectively; Table 3.5). This calculation suggests that glycogen loading does not improve cold endurance.

Wissler (1985) has proposed a more elaborate model to predict shivering endurance based on empirical observations from Beckman and Reeves (1966). In his model, shivering fatigue was determined from the onset of muscle cramping observed in a group of men exposed to 24°C water. Under our conditions, the model of Wissler predicts that shivering at 200 W could be sustained for 33 to 42 h. In addition, Tikuisis *et al.* (2002) recently suggested that the Wissler model may underestimate true values. Our calculated shivering endurance of 20 h based on whole-body oxidation of glycogen is clearly shorter than predicted by Wissler (1985) or Tikuisis (2002). This observation suggests two possible shortcomings for our simple approach: a) glycogen may not be essential, and low-intensity shivering is sustainable solely on lipids and proteins and/or, b) a significant shift in fuel selection to spare glycogen takes place after 2 h of shivering (in fact, a progressive increase in fat oxidation was observed by Tikuisis *et al.* (2002) during prolonged shivering lasting for up to 4 h). No information is available on fuel selection for shivering in excess of 4 h, and it is still unclear whether glycogen depletion coincides with muscle fatigue. Future studies should address this interesting problem.

This study only included male subjects and was not designed to measure gender-differences in shivering physiology. However, in view of the significant sex differences reported previously during exercise and cold exposure, future research may uncover interesting metabolic differences between shivering males and females. For example, during submaximal exercise at same %VO_2max, women were shown to use more lipids and, consequently, less muscle glycogen and less plasma glucose than men (see Tarnopolsky and Ruby, 2001 for review). Similarly, during low-intensity shivering (~1.8 times RMR), lipid oxidation was reported to be significantly higher in women than men (64 vs 53 % $\dot{H}_{prod}$) (Pettit *et al.*, 1999), suggesting that women may show higher glycogen sparing and, possibly, longer survival in the cold. However, no such gender difference in fuel use was found during high-intensity shivering (Tikuisis *et al.*, 2000b). Future work should address whether plasma glucose and muscle glycogen oxidation are different in shivering males and females.

Conclusions

This study shows that large changes in glycogen reserves have no effect on thermogenesis during sustained shivering in men. Total CHO oxidation provides 65% of the heat in glycogen-loaded individuals (50% for muscle glycogen and 15% for plasma glucose), but only 27% in glycogen-depleted subjects (20% for glycogen and 7% for plasma glucose). We show that heat production of glycogen-depleted individuals is not compromised, because protein and lipid oxidation are both stimulated to compensate for the reduced contribution from CHO. Depletion of CHO reserves reduces the relative use

of plasma glucose by shivering muscles even below that of subjects with normal CHO reserves. This study provides clear evidence that proteins can play a significant thermogenic role when CHO reserves are depleted (19% $\dot{H}_{prod}$). Finally, electromyography (EMG) signals from shivering muscles were also collected in the present experiments, and the following paper investigates the patterns of muscle fiber recruitment in relation to the large changes in fuel selection reported here.

CHAPTER 4. EFFECTS OF CARBOHYDRATE AVAILABILITY ON SUSTAINED SHIVERING: II. RELATING MUSCLE RECRUITMENT TO FUEL SELECTION

Based in part on

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INTRODUCTION

Shivering is an involuntary process generating heat through rhythmic, asynchronous muscle contractions. To sustain shivering thermogenesis over prolonged periods of cold exposure, muscle recruitment and fuel metabolism must be tightly coordinated. Previous research in this field falls in two broad categories dealing either with muscle metabolism (Haman *et al.*, 2002, Jacobs *et al.*, 1994) or with electrophysiological aspects of muscle recruitment (Bell *et al.*, 1992, Meigal, 2002, Tikuisis *et al.*, 2000a). These two complementary perspectives on the same problem have not been traditionally integrated and this study is a first attempt at doing so. In the first part (Chapter 3), we have shown that the size of glycogen reserves has a considerable effect on fuel selection. When CHO reserves were low (LO), total heat production was partitioned between CHO (28% $\dot{H}_{prod}$), lipids (53% $\dot{H}_{prod}$), and proteins (19% $\dot{H}_{prod}$), but the fuel selection pattern was strikingly different when CHO reserves were high (HI): CHO (65% $\dot{H}_{prod}$), lipids (23% $\dot{H}_{prod}$), and proteins (12% $\dot{H}_{prod}$). In particular, intramuscular glycogen was used at very different rates for LO and HI (168 ± 43 vs 380 ± 58 $mg \cdot min^{-1}$), but the effects of such large metabolic changes on shivering activity and muscle fiber recruitment have never been addressed. Fuel selection of contracting muscles can be modified in two ways: i) mobilizing different metabolic pathways within the same fibers, or ii) recruiting distinct fiber populations specialized for different fuels.

Over the last several decades, mechanisms of motor unit (MU) recruitment during voluntary contractions have received a lot of attention (see Gardiner, 2001, Linnamo *et al.*, 2003, Wakeling *et al.*, 2002 for review), but very few studies have investigated this process during shivering (Meigal, 2002, Meigal *et al.*, 1993, Meigal *et al.*, 1995, Petajian and Williams, 1972). Previous work shows that all fiber types can be involved in shivering (Jacobs *et al.*, 1994, Meigal, 2002). For example, Jacobs *et al.* (Jacobs et al., 1994) reported decreased glycogen concentration in all fiber types of the *vastus lateralis* after cold exposure. In addition, EMG recordings reveal two distinct patterns of shivering: i) thermoregulatory muscle tone, or continuous, low-intensity shivering (at 4-8 Hz), and ii) bursts of high-intensity shivering occurring at much lower frequencies (0.1-0.2 Hz or 8-16 times/min) (Israel and Pozos, 1989, Meigal, 2002). These two patterns are associated with the recruitment of specific MU (Meigal, 2002, Meigal *et al.*, 1993, Petajian and Williams, 1972). While continuous, low-intensity shivering is linked to low-threshold MU (type I, slow-oxidative, fatigue-resistant fibers), shivering bursts are associated with high-threshold MU (type II, fast-glycolytic, more fatigable fibers). Human type II fibers (IIA and IIX) show lower activities for oxidative enzymes than type I fibers (-60%) and much higher activities for glycolytic enzymes and creatine kinase (+300-400%) (Gardiner, 2001). Because of these large biochemical differences, the two shivering patterns observed may reflect the use of distinct metabolic substrates, type I being mostly geared towards lipid use and type II towards CHO use. Together, these observations suggest that fast-glycolytic type II fibers, and therefore, burst shivering activity, may be significantly affected by changes in glycogen reserves.

The first objective of this paper was to quantify the effects of changes in glycogen stores on whole-body shivering activity. More specifically, we monitored the recruitment levels of 8 large muscles representing >90% of total shivering muscle mass in men with low or high CHO reserves during sustained cold exposure. The second objective was to characterize the detailed shivering pattern and spectral parameters of individual muscles in an attempt to uncover more subtle effects of CHO availability on the shivering response. The chosen experimental design allowed us to investigate whether changes in fuel selection were achieved by recruiting "fuel-specific fibers" as previously suggested (Roberts et al., 1996). According to this idea, the recruitment of fast-glycolytic MU would be directly correlated with CHO utilization. At the whole-organism level, we predicted that total shivering activity would not be affected by changes in CHO availability, although the relative contributions of individual muscles could be altered to maintain heat production. Within each muscle, we anticipated that the shivering pattern and EMG spectral parameters would change with CHO availability because distinct fuel-specific fiber populations would be recruited.

METHODS

Subjects

Six healthy and physically active men participated in this study conducted with the approval of the Health Sciences Ethics Committee of the University of Ottawa. Description of volunteers was given in part one of this study (Chapter 3, Table 3.1).

Experimental procedures

Five to six days prior to the experiments, a 1-h session was held to familiarize the subjects with the equipment and the level of cold exposure faced in the experiments. For the actual experiments, subjects were exposed to the cold on two separate occasions following: i) a diet low in CHO and heavy exercise bouts (LO; last glycogen-depletion exercises were conducted at least 20 h before the experiment), and ii) a diet high in CHO without exercise bouts (HI; no exercise was performed at least 2.5 day before the experiment). A detailed description of the diet and exercise regimen has been given in Chapter 4. Upon their arrival in the laboratory (8:00 AM; 12h post-absorptive), subjects were instrumented with thermal probes (oesophageal and skin thermocouples, heat flux transducers), EMG electrodes and an indwelling catheter (18G, 32 mm, Medical Inc., Arlington, TX). They were then fitted with a LCS (Three piece Delta Temax, Pembroke, ON, Canada). After voiding their bladder (T= 0 min), subjects remained seated comfortably for the next 2-h at 23.2 ± 0.01°C (758 ± 2 mmHg, 39.8 ± 3.6% RH). Following this period, they were transferred to an environmental chamber (10.5 ± 0.01°C, 755 ± 3 mmHg, 61 ± 2 % RH) and a 10°C water perfusion was started through the LCS

using a temperature controlled circulation bath (Endocal, NESLAB and Model 200-00, Micropump, Vancouver, WA). Average environmental conditions in laboratory and thermal chamber were the same for LO and HI experiments. Thermal response was monitored continuously at 23°C and during the subsequent 2-h cold exposure using a Hewlett Packard data acquisition and control unit (model 3497A). Metabolic and thermal comfort data were collected periodically throughout the experimental session.

Shivering EMG signals were recorded from eight muscles located on the right side of the body: *trapezius* (TR), *latissimus dorsi* (LA), *pectoralis major* (PE), *rectus abdominis* (RA), *vastus lateralis* (VL), *rectus femoris* (RF), *vastus medialis* (VM) and *gastrocnemius* (GA). These muscles were selected to represent the largest possible fraction of total muscle mass involved in shivering (>90%) based on a previous study by Bell *et al.* (1992). Surface electrodes (Blue Sensor, Medicotest Inc., USA) were positioned 2 cm apart over the bellies of each muscle and their exact position was identified with an indelible skin marker to allow consistent placement between measurement sessions (i.e. two cold exposure experiments and maximal voluntary contraction (MVC) measurements). Surface electrodes were connected using pre-amplified and grounded EMG wires (375X) to a ME-3000 Professional EMG system (Mega Electronics, Kuopio, Finland). Raw EMG signals were collected at 1000 Hz and downloaded via fiber optic to a desktop computer. Shivering activity of the eight individual muscles was monitored 30 min before and six times during cold exposure between 5-20 min, 25-40 min, 45-60 min, 65-80 min, 85-100 min and 105-120 min (see Fig. 4.1A). Intervals between sampling periods (5 min) were used to save the recorded

data to the computer's hard drive. Voluntary muscle activity was minimized as much as possible throughout cold exposure by asking subjects to avoid voluntary movements during recording periods.

Normalization of EMG amplitude

Three to four days before the experiments, maximal EMG signals of each muscle (RMS_{mvc}) were determined from maximal isometric contractions (MVC) performed using a muscle testing and training system (KIN COM 500H, Chattecx corporation, Chattanooga, TN, USA). MVC protocols were comprised of a ramp-and-hold protocol that involved gradually increasing force from baseline to maximum over 2-3 s and then holding the maximum force for an additional 2-3 s (Kern et al., 2001). Participants were verbally encouraged to achieve maximum force during the three trials and were given at least 30 s rest between each MVC measurement. The following procedures were used to determine RMS_{mvc} of individual muscle: i) for TR, MVC was measured while subjects were standing up grasping the load cell with the right hand and arm in full extension. Subjects were then asked to raise their shoulder with maximal force while keeping their arm as close to the body as possible; ii) for PE and LA, subjects were asked to sit upright in a secured chair and measurements were performed with their arm extended away from the body at a 90° angle. For PE, the load cell was placed on the front, slightly over the elbow, and subjects were asked to push maximally forward against it. For LA, the load cell was positioned under the elbow and subjects pushed downward against it with maximal force; iii) for AB, while sitting upright in a secured chair, the load cell was

attached to a strap located around the subjects' chests. Subjects were curled forward using their abdominal muscle while keeping their backs straight and applying maximal force against the strap; iv) for VL, RF and VM, MVC measurements were performed with subjects sitting securely in an upright position and the load cell was attached slightly above the ankle. The load cell arm was placed at a 45° angle from its vertical position and subjects performed a maximal knee extension pushing against the load cell and; v) for GA, MVCs were measured lying down with the load cell was attached on the front of the foot and the leg was secured to the table with straps and subjects performed a maximal ankle flexion against the load cell.

Determination of shivering intensity and pattern

Raw EMG signals were analyzed using custom-designed MATLAB algorithms (Mathworks, Natick, MA, USA). EMG signals were filtered to remove below 20 Hz and above 500 Hz as well as 60Hz contamination (and associated harmonics).

Shivering intensity of individual muscles (EMG^{m}_{shiv}, where the index *m* identifies the muscle) was determined from root-mean-square values (RMS) calculated from EMG signals using a 50 ms overlapping-window (50%). Baseline RMS values ($RMS_{baseline}$; 15 min RMS average measured prior to cold exposure) were subtracted from RMS_{shiv} as well as RMS_{mvc} values. EMG^{m}_{shiv} was normalized to RMS_{mvc} using the following equation:

$$EMG^{m}_{shiv}\ (\%MVC) = \frac{RMS_{shiv} - RMS_{baseline}}{RMS_{mvc} - RMS_{baseline}} \times 100. \qquad (4.1)$$

In addition, shivering activity of individual muscles ($AEMG^{m}_{shiv}$) was determined from the area under the EMG^{m}_{shiv} curve using the Riemann sum.

$$AEMG^{m}_{shiv} = \sum EMG^{m}_{shiv}\Delta t,\ m = 1\ to\ 8, \tag{4.2}$$

where the summation is taken over all samples and $\Delta t = 1$ ms. The relative contribution of muscle m to shivering activity was determined by dividing $AEMG^{m}_{shiv}$ by the sum of shivering activities for all muscles.

EMG^{m}_{shiv} and $AEMG^{m}_{shiv}$ were subsequently quantified with 11 outcome variables corresponding to changes in overall shivering, continuous low-intensity shivering and burst shivering. The 11 outcome variables - determined for the three latter EMG components unless indicated otherwise - are i) total shivering time: total amount of time for which EMG^{m}_{shiv} was greater than 0% MVC (i.e. above $RMS_{baseline}$, *Eq.* 4.1) over the entire recording time (~90 min); ii) summed area, in %MVC·min; iii) mean intensity, in %MVC; iv) percent shivering time, in min: summed area of individual EMG components as a percentage of total recording time (%RT); v) steady-state intensity, in %MVC: average overall intensity reached in the last 15 min of cold exposure; vi) summed duration, in min: total duration continuous low-intensity shivering and burst shivering; vii) number of bursts: total number of bursts during the recording period; viii) mean burst duration, in s: mean duration of all bursts; ix) burst rate, in bursts·min^{-1}: mean number of bursts per min; x) Percent contribution to total whole body shivering

($\%Shiv_{total}$), relative contribution of continuous low-intensity shivering and burst shivering to total shivering activity calculated based on WBI (see below); xi) percentage of burst shivering activity to continuous low-intensity shivering activity: percentage of burst summed area (see variable 1) to total continuous low-intensity shivering area ($\%EMG_{burst}/EMG_{cont}$). A shivering burst was arbitrarily defined in the present study as an EMG interval with a duration greater than 0.2 s, an inter-burst interval greater than 0.75 s and an amplitude higher than the amplitude threshold at each recording period (5-20, 25-40, 45-60, 65-80, 85-100 or 105-120 min). Intensity threshold was determined by first calculating the average shivering intensity ($\overline{A}_{EMG}$) over the entire recording period. Remaining values above $\overline{A}_{EMG}$ were then averaged again ($\overline{B}_{EMG}$) and the intensity threshold was set at $\overline{B}_{EMG}$ (see Fig. 4.5).

To obtain a whole body index of shivering activity ($Shiv_{WBI}$) that can be related to total heat production in the cold (measured by indirect calorimetry), shivering activities of all muscles were summed taking into account the relative mass of the body region they represent (Bell *et al.*, 1992, Tikuisis *et al.*, 2000a):

$$Shiv_{WBI} = \int \left(f_{UT} EMG_{shiv}^{UT} + f_{LT} EMG_{shiv}^{LT} + f_{UL} EMG_{shiv}^{UL} + f_{LL} EMG_{shiv}^{LL} \right) dt, \qquad (4.3)$$

where EMG_{shiv}^{UT}, EMG_{shiv}^{LT}, EMG_{shiv}^{UL} and EMG_{shiv}^{LL} are upper trunk (average of TR, LA and PE), lower trunk (AB), upper leg (average of VL, RF and VM) and lower leg (GA) shivering activities. The coefficients f_{UT} (0.34), f_{LT} (0.19), f_{UL} (0.29) and f_{LL} (0.085) correspond to the relative muscle masses of each body regions (UT, LT, UL and LL) to total muscle mass (Bell *et al.*, 1992, Tikuisis *et al.*, 2000a). This whole body index

represents ~91% of total muscle mass and excludes upper limbs, head and feet which contribute minimally to total heat production (Bell et al., 1992). Following the approach proposed by Bell *et al.* (1992) and Tikuisis *et al.* (2000a), we have verified the linearity of the EMG vs force relationship by performing sub-maximal contractions at 10, 25, 50 and 75%MVC using a KIN COM 500H. This relationship was linear for all muscles selected in this study (r^2 = 0.85-0.98). Therefore, the calibration factor developed by these authors (Bell et al., 1992) to account for non-linearity under 20%MVC was not used.

EMG spectral analysis

Mean frequency of the EMG power spectrum (MPF) as a function of time were estimated by applying a short-time Fourier transform to the raw data. A Hanning window consisting of 4096 samples (or 4 s wide for a spectral resolution of 0.25 Hz) was applied with a 50% overlap, thereby producing a spectral estimate every 2 s. Consistent with the computations for the intensity, the spectral components below 20 Hz and above 500 Hz were removed. The raw data was also slightly contaminated with 60 Hz noise; the corresponding samples and associated harmonics were removed (set to zero) from the discrete spectrum. A MPF was then obtained by taking the mean of each spectral estimate. Finally, MPF data was smoothed using a running average with a 7 sample wide (14 s) window.

Statistical analyses

Changes in shivering intensity and MPF as a function of time for individual muscles as well as for UT, LT, UL and LL were assessed by two-way analysis of variance with replication. For each sampling time, a Bonferroni *t*-test was used to detect potential differences with control vaues observed before cold exposure. Differences in MPF (UT, LT, UL, LL) between LO and HI were determined using a paired *t*-test. Statistical differences were considered significant when $p \leq 0.05$. All values presented are means ± SE ($n = 6$) unless indicated otherwise.

RESULTS

Changes in $\dot{H}_{loss}$, $\dot{H}_{prod}$, T_{es} and $\overline{T}_{skin}$ before and during cold exposure for LO and HI have been reported in Chapter 3. A total of 90 min of EMG signal was collected from 8 large muscles corresponding to four regions of the body: UT, LT, UL and LL and representing >90% of total muscles mass involved in shivering (Bell et al., 1992). An example of raw EMG signal (Fig. 4.1A) as well as calculated shivering intensities (Fig. 4.1B) and mean power frequencies (Fig. 4.1C) are shown in Fig. 4.1.

Whole body shivering

Respective contributions of CHO (RG_{ox}), lipid and protein oxidation to total $\dot{H}_{prod}$ under LO and HI conditions have been presented in detail in part one of this study (Chapter 3). In the last 30 min of cold exposure, CHO, lipids and proteins contributed respectively 28 ± 5%, 53 ± 5%, and 19 ± 2% $\dot{H}_{prod}$ for LO and 65 ± 5%, 23 ± 5% and 12 ± 5% $\dot{H}_{prod}$ for HI. Changes in $\%RG_{ox}$, $\dot{H}_{prod}$ and $Shiv_{WBI}$ during cold exposure are shown in Fig. 4.2. While $\%RG_{ox}$ was 114% higher for HI than for LO (71.4±5.3 vs 33.4 ± 5.1 % $\dot{H}_{prod}$) (Fig 4.2A), $\dot{H}_{prod}$ (Fig. 4.2B) and $Shiv_{WBI}$ (Fig. 4.2C) were not different between LO and HI. Relationships between changes in $Shiv_{WBI}$ and $\dot{H}_{prod}$ for LO and HI are presented in Fig. 4.3 and were not different between treatments. $\dot{H}_{prod}$ increased 2.2-fold from control values prior to cold exposure reaching a maximum of 210.0±10.4W for LO and 186.9±11.0W for HI by the end of cold exposure. $Shiv_{WBI}$ also increased

continuously from an average area of 75 ± 17 and 254 ± 38 %MVC·s in the first sampling interval (T= 5-20 min) to 112 ± 40 and 214 ± 47 %MVC·s by the end of cold exposure (T= 105-120 min) for LO and HI, respectively.

Changes in shivering intensity for each muscle (%MVC) in LO and HI are presented in Fig 4.4. Shivering intensity increased continuously during cold exposure but no difference was observed between LO and HI. Maximal intensities reached by the end of cold exposure averaged 3.5±0.9, 6.3±1.1, 5.4±1.5, 3.8±1.3, 4.0±1.6, 3.8±0.7, 4.1±1.6 and 4.2±1.4 %MVC for LO and 3.2±0.8, 5.8±1.0, 3.8±0.6, 3.5±0.7, 4.2±1.7, 5.6±2.0, 3.6±1.6 and 8.7±2.4 %MVC for HI (for TR, LA, PE, RA, VL, RF, VM and GA, respectively). The relative contributions of individual muscles to total shivering activity were also found to be the same between LO and HI. Shivering intensity frequency distributions were calculated for each muscle and each subject separately, over the entire period of cold exposure. Even though minor differences between LO and HI were detected for individual muscles in some subjects, no significant differences between mean values could be established.

Shivering pattern and EMG spectral characteristics

The method used to distinguish burst shivering from continuous, low-intensity shivering is schematized in Fig. 4.5. Separating these 2 patterns was achieved based on large differences in intensity (2-5 vs 7-15% MVC) and rate of occurrence (8-10 vs 0.1-0.2 Hz). Table 4.1 summarizes all parameters calculated for burst shivering and continuous, low-intensity shivering for UT, LT, UL and LL muscles. Results show the 2

patterns were the same between LO and HI for all body regions. The effect of changes in fuel selection on burst shivering activity is emphasized in Fig. 4.6. Burst shivering intensity, burst shivering rate and relative contribution of burst shivering to total recording time were not different between LO and HI. Burst shivering intensity increased from 7.0 ± 0.8 (7.0 ± 1.7) %MVC in the first recording interval to 12.1 ± 1.1 (11.8 ± 2.4) %MVC by the end of cold exposure for LO (HI). Burst shivering rate increased from 3.1 ± 0.5 (3.0 ± 0.5) burst·min^{-1} to 4.6 ± 0.5 (4.5 ± 0.5) burst·min^{-1} for LO (HI) and the relative contribution of burst shivering to total recording time from 7.0 ± 1.6 (7.0 ± 1.4) %RT to 10.7 ± 0.8 (11.2 ± 0.6) %RT LO (HI).

Changes in MPF for each muscle for LO and HI are presented in Fig. 4.7. MPF remained constant in cold exposure averaging and was not different between LO and HI. As for shivering intensities, frequency distributions of MPF were calculated for each muscle and each subject separately, over the entire period of cold exposure. Minor differences between LO and HI were detected for individual muscles in some subjects but no significant differences between mean values could be established.

Figure 4.8 summarizes the effect of a very large change in fuel selection on changes in spectral characteristics of the EMG signal (MPF). Averages were calculated over the last 15 min of cold exposure. Although the relative contribution of lipids and proteins was 132 and 60% higher for LO than for HI, CHO provided 58% less to total heat production for LO than for HI. Independently of these differences, MPF for UT, LT, UL and LL was found to be the same for both conditions. The effect of a very large change in CHO oxidation rate on mean whole body MPF is emphasized in Fig. 4.8C.

While a 2.4-fold difference in $\%RG_{ox}$ between LO (27.7±5.2 $\%\dot{H}_{prod}$) and HI (65.3 ± 5.4 $\%\dot{H}_{prod}$), MPF was identical for both groups (78.4 ± 5.2 vs 82.5 ± 6.9 Hz).

Table 4.1. Overall, continuous and burst EMG activity of upper trunk, lower trunk, upper leg and lower leg in subjects with low- (LO) and high-CHO (HI) reserves exposed to 10°C for 120 min.

	Upper trunk		Lower trunk		Upper leg		Lower leg	
	LO	HI	LO	HI	LO	HI	LO	HI
Overall								
Summed area, %MVC·min	400 ± 33	435 ± 52	187 ± 74	193 ± 59	357 ± 152	423 ± 195	94 ± 32	222 ± 100
Mean intensity, %MVC	3.1 0.5	2.8 ± 0.6	5.6 ± 1.1	6.2 ± 1.1	4.2 ± 1.9	4.2 ± 1.7	3.0 ± 1.2	4.0 ± 1.7
Steady-state intensity, %MVC	4.9 ± 1.4	3.8 ± 0.7	4.4 ± 1.3	4.2 ± 0.9	4.0 ± 1.4	4.2 ± 1.9	4.6 ± 1.7	7.6 ± 2.7
%RT	88.5 ± 3.4	89.5 ± 4.3	73.8 ± 9.0	82.5 ± 8.2	90.9 ± 5.6	95.4 ± 1.9	70.4 ± 10.3	71.8 ± 10.4
Continuous shivering								
Summed area, %MVC·min	375 ± 30	409 ± 49	209 ± 73	213 ± 50	334 ± 143	391 ± 185	86 ± 30	204 ± 95
Mean intensity, %MVC	2.1 ± 0.1	2.3 ± 0.2	2.8 ± 0.7	2.4 ± 0.4	2.2 ± 0.9	2.5 ± 1.2	2.6 ± 0.8	5.4 ± 2.0
Summed duration, min	70.5 ± 2.2	71.3 ± 3.1	58.5 ± 7.0	65.0 ± 6.0	74.8 ± 4.3	78.0 ± 1.4	58.0 ± 8.4	58.3 ± 7.6
$\%Shiv_{time}$	88.7 ± 0.9	88.7 ± 0.7	88.6 ± 2.6	88.0 ± 10.4	91.5 ± 1.4	91.0 ± 0.8	91.6 ± 1.7	91.3 ± 1.6
Bursts								
Number of bursts	363 ± 40	379 ± 31	380 ± 99	459 ± 77	291 ± 68	302 ± 63	188 ± 44	226 ± 78
Burst rate, number·min^{-1}	4.0 ± 0.4	4.2 ± 0.3	4.2 ± 1.1	5.1 ± 0.9	3.2 ± 0.8	3.4 ± 0.7	2.1 ± 0.5	2.5 ± 0.9
Mean duration, s	2.0 ± 0.2	1.7 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	2.1 ± 0.4	2.3 ± 0.6	2.4 ± 0.6
Summed area, %MVC·min	25.0 ± 2.6	26.4 ± 3.5	15.6 ± 5.1	18.6 ± 5.0	23.1 ± 8.9	32.0 ± 11.3	7.5 ± 2.4	18.3 ± 6.0
Mean intensity, %MVC	8.3 ± 0.8	7.8 ± 0.7	10.6 ± 2.2	8.3 ± 1.7	8.5 ± 2.9	10.1 ± 4.2	13.1 ± 2.8	22.3 ± 4.6
Summed duration, min	9.1 ± 1.0	9.3 ± 0.9	7.9 ± 1.8	9.2 ± 1.8	7.1 ± 1.4	7.8 ± 0.8	5.4 ± 1.4	6.3 ± 1.9
$\%Shiv_{time}$	10.3 ± 1.1	10.5 ± 0.9	10.5 ± 2.3	10.6 ± 1.8	8.0 ± 1.5	8.7 ± 0.9	6.8 ± 1.5	7.5 ± 1.9
$\%EMG_{burst}/EMG_{cont}$	6.7 ± 0.3	6.4 ± 0.3	7.5 ± 1.2	8.2 ± 1.6	7.8 ± 0.5	12.7 ± 5.0	11.4 ± 2.9	13.7 ± 3.3

EMG, electromyograph; Mean s-s amplitude, shivering intensity measured in the last 15 min of cold exposure under quasi steady-state; %RT, percentage of total recording time (~90 min); $\%Shiv_{time}$, Percentage of total shivering time; MVC, maximal voluntary contraction; $\%EMG_{burst}/EMG_{cont}$ of shivering burst summed area to continuous shivering summed area.

Figure 4.1. Example of electromyographic recording in *pectoralis major* before and during cold exposure for one subject (A). Gray areas show the time intervals when EMG was not being recorded. The black arrow indicates the area used to schematize the shivering burst determination procedure (Fig. 4.5). Shivering intensities (B) and mean power frequencies (C) calculated from the raw EMG signal are also shown.

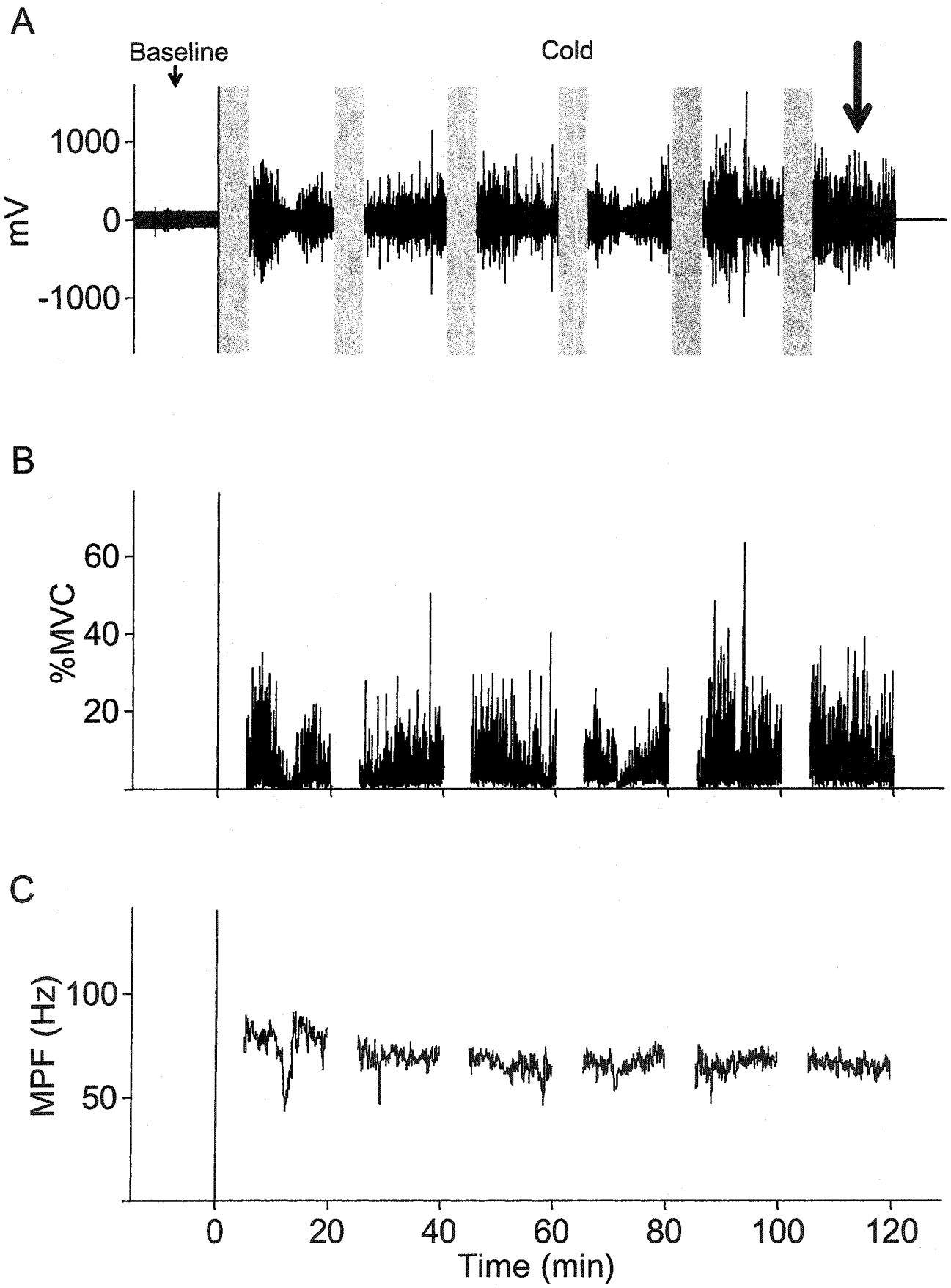

A
Baseline
Cold
1000
0
-1000
mV
B
60
40
20
%MVC
C
100
50
MPF (Hz)
0
20
40
60
80
100
120
Time (min)

Figure 4.2. Changes in the relative contribution of carbohydrates to total heat production (A; $\%RG_{ox}$), heat production (B; $\dot{H}_{prod}$) and whole body shivering activity (C; $Shiv_{WBI}$; *ref. Eq.* 4.3) in glycogen-depleted (closed circles) and glycogen-loaded (open circles) men exposed to 10°C for 120 min.

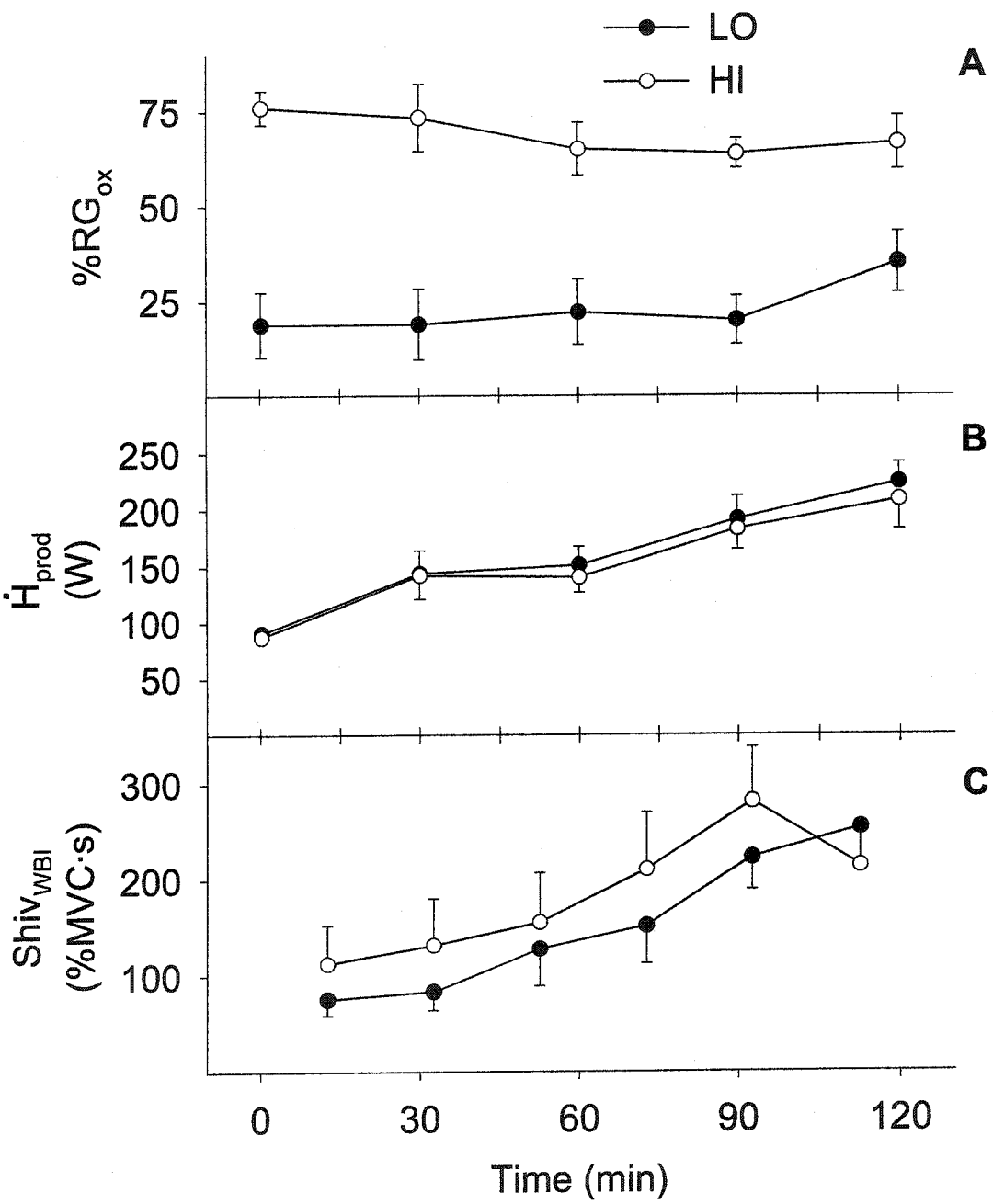

LO
HI
A
B
C
%RG$_{ox}$
75
50
25
$\dot{H}_{prod}$ (W)
250
200
150
100
50
Shiv$_{WBI}$ (%MVC·s)
300
200
100
0
30
60
90
120
Time (min)

Figure 4.3. Relationship between heat production ($\dot{H}_{prod}$) and whole body shivering intensity ($Shiv_{WBI}$; *ref. Eq.* 4.3) in glycogen-depleted (closed circles) and glycogen-loaded (open circles) men exposed to 10°C for 120 min. Values are presented for all subjects (n=6) and were averaged at 4 sampling intervals during cold exposure (T = 25-30, 55-60, 85-90 and 115-120 min). Linear regressions for LO (filled line) and HI (dotted line) are also presented.

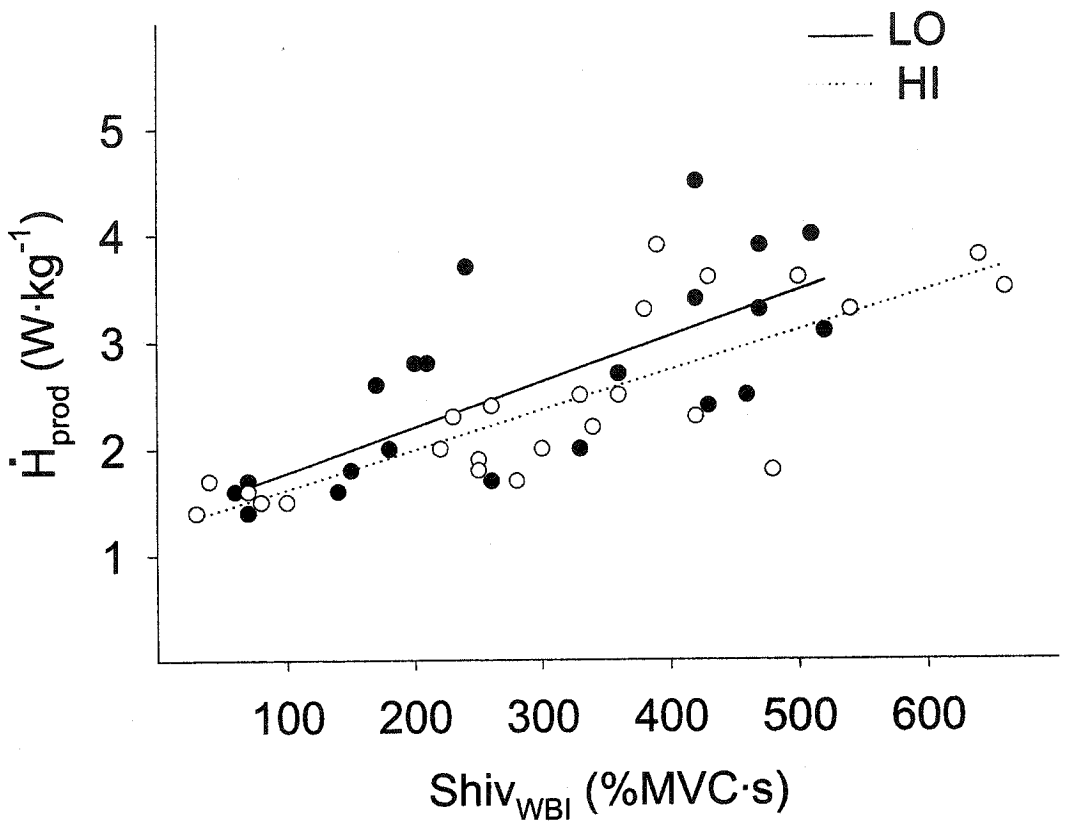

LO
HI
5
4
3
2
1
$\dot{H}_{prod}$ (W·kg^{-1})
100
200
300
400
500
600
Shiv$_{WBI}$ (%MVC·s)

Figure 4.4. Changes in individual muscle shivering intensity of glycogen-depleted (LO; closed circles) and glycogen-loaded (HI; open circles) men exposed to 10°C for 120 min. TR, *trapezius*; LA, *latissimus dorsi*; PE, *pectoralis major*; RA, *rectus abdominis*; VL, *vastus lateralis*; RF, *rectus femoris*; VM, *vastus medialis*; GA, *gastrocnemius* .

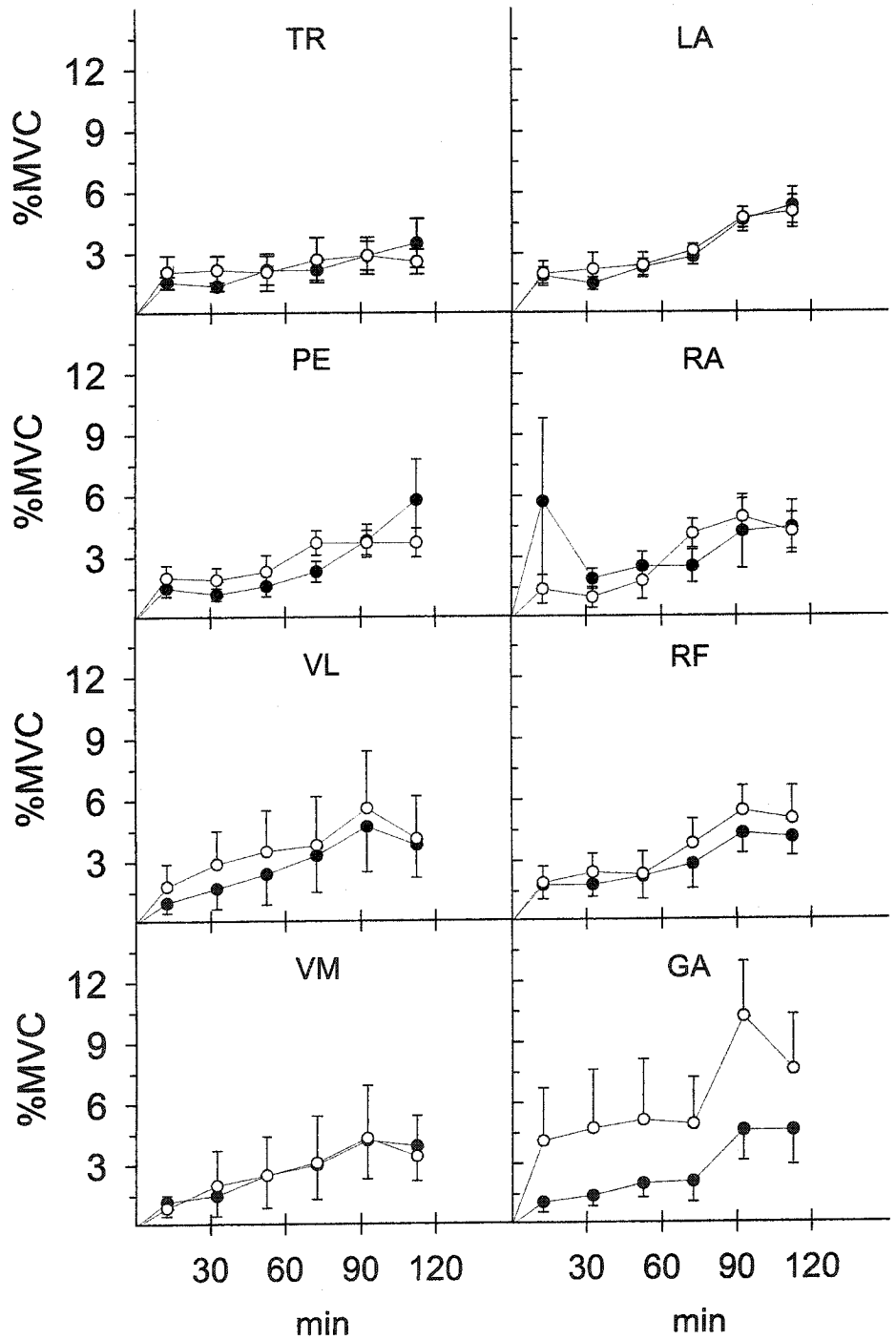

TR
LA
PE
RA
VL
RF
VM
GA
%MVC
12
9
6
3
30
60
90
120
min

Figure 4.5. Schematization of the procedure for shivering burst identification. The time interval presented here was taken from the example of EMG signal given in Fig. 4.1 (see black arrow). $\overline{A}_{EMG}$ (dashed line) represents the average shivering intensity for the 15 min recording interval (in this case T=105-120 min) and $\overline{B}_{EMG}$ (filled line) indicates the shivering intensity threshold for burst determination. A shivering burst was arbitrarily defined in the present study as an EMG interval with a duration greater than 0.2 s, an inter-burst interval greater than 0.75 s and an amplitude higher than the amplitude threshold. These criteria were used to identify bursts in each recording period (5-20, 25-40, 45-60, 65-80, 85-100 or 105-120 min). Gray boxes indicate location and duration of the shivering bursts found in this example. $\overline{A}_{EMG}$ and $\overline{B}_{EMG}$ were defined for each subject and for each 15 min recording interval (5-20, 25-40, 45-60, 65-80, 85-100 and 105-120 min).

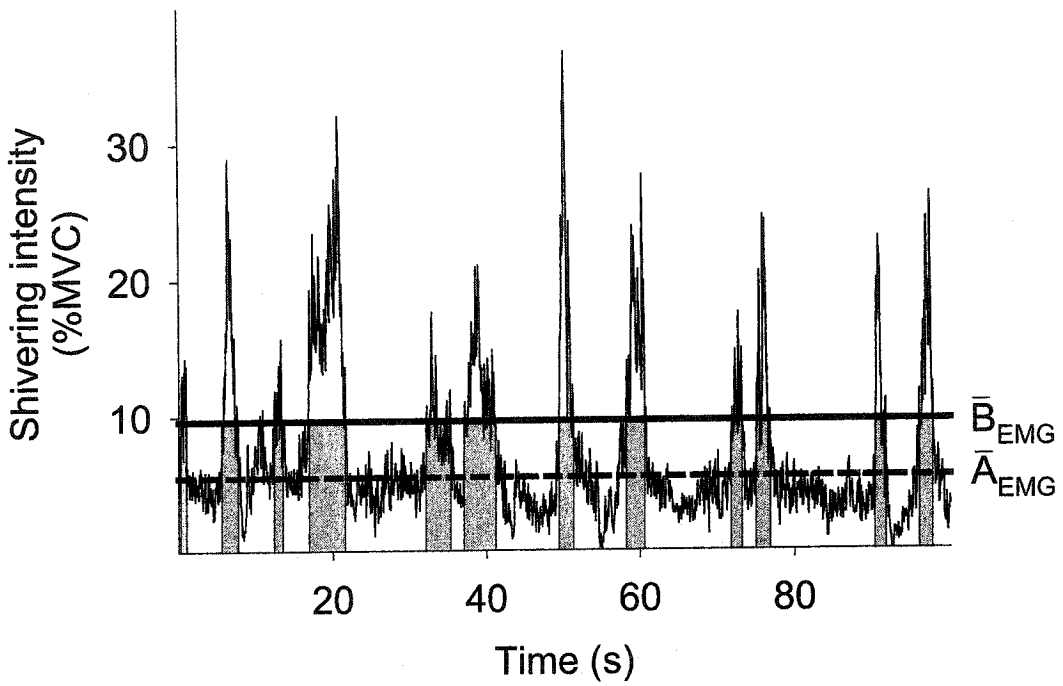

Shivering intensity
(%MVC)
30
20
10
20
40
60
80
Time (s)
$\bar{B}_{EMG}$
$\bar{A}_{EMG}$

Figure 4.6. Changes in burst intensity (A), burst shivering rate (B) and relative contribution to total recording time (C) averaged at each recording interval of glycogen-depleted (black bars) and glycogen-loaded (white bars) men exposed to 10°C for 120 min. *Significantly different from baseline values (Two-way ANOVA with replication and Bonferroni *post-hoc t*-test, $P \leq 0.05$, n=6).

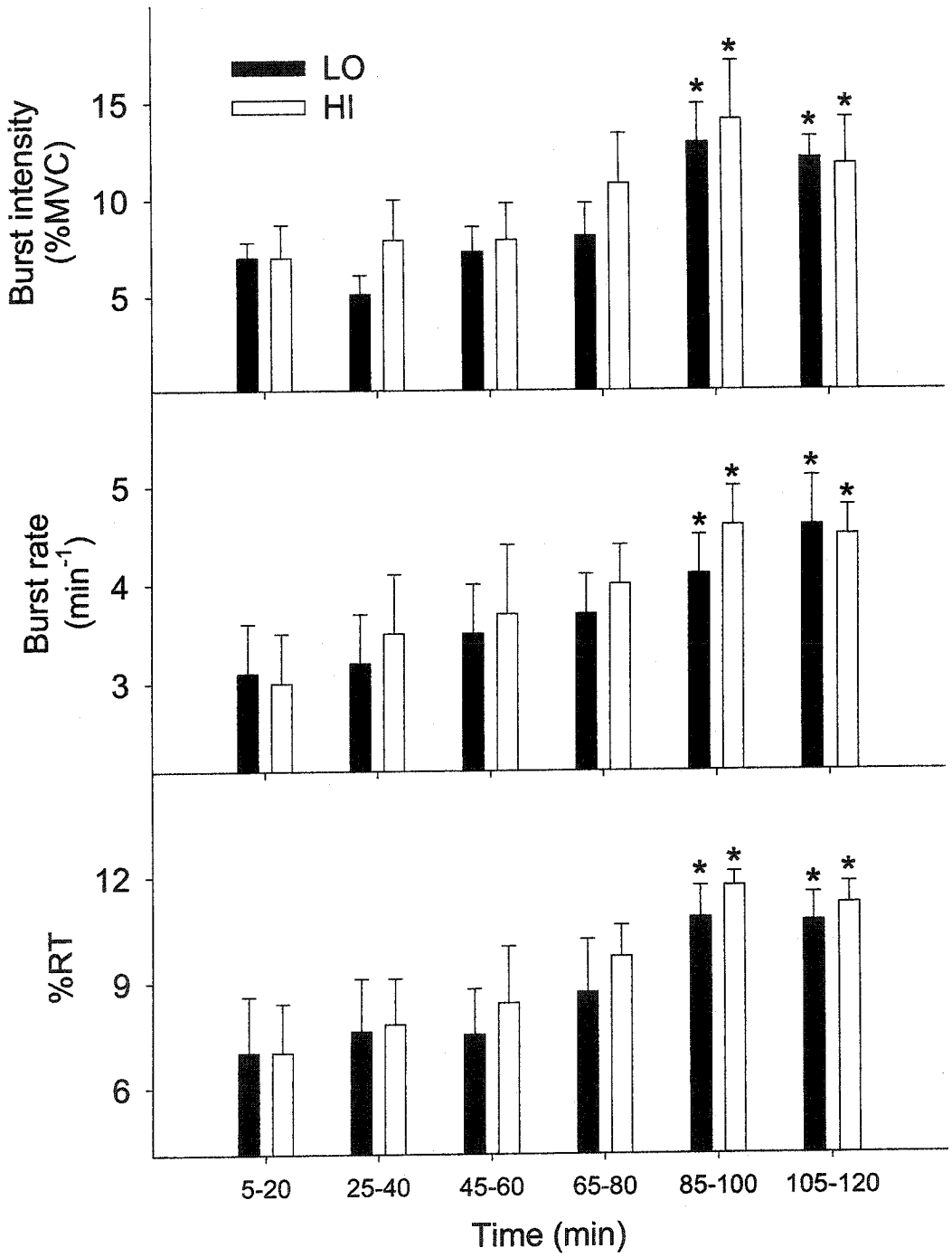

LO
HI
Burst intensity (%MVC)
15
10
5
Burst rate (min-1)
5
4
3
%RT
12
9
6
5-20
25-40
45-60
65-80
85-100
105-120
Time (min)

Figure 4.7. Changes in individual muscle mean power frequency (MPF) of glycogen-depleted (closed circles) and glycogen-loaded (open circles) men exposed to 10°C for 120 min. TR, *trapezius*; LA, *latissimus dorsi*; PE, *pectoralis major*; RA, *rectus abdominis*; VL, *vastus lateralis*; RF, *rectus femoris*; VM, *vastus medialis*; GA, *gastrocnemius* .

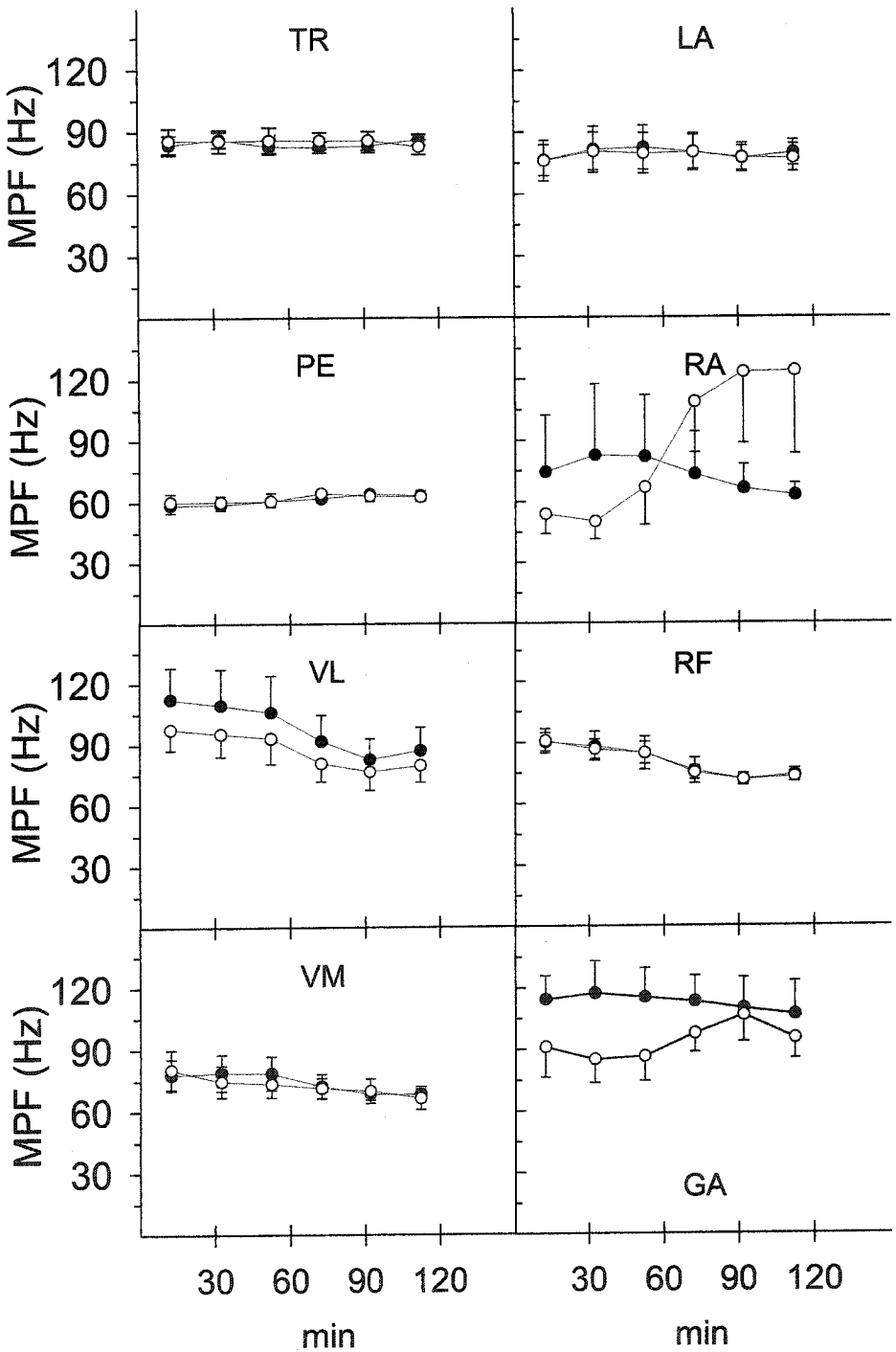
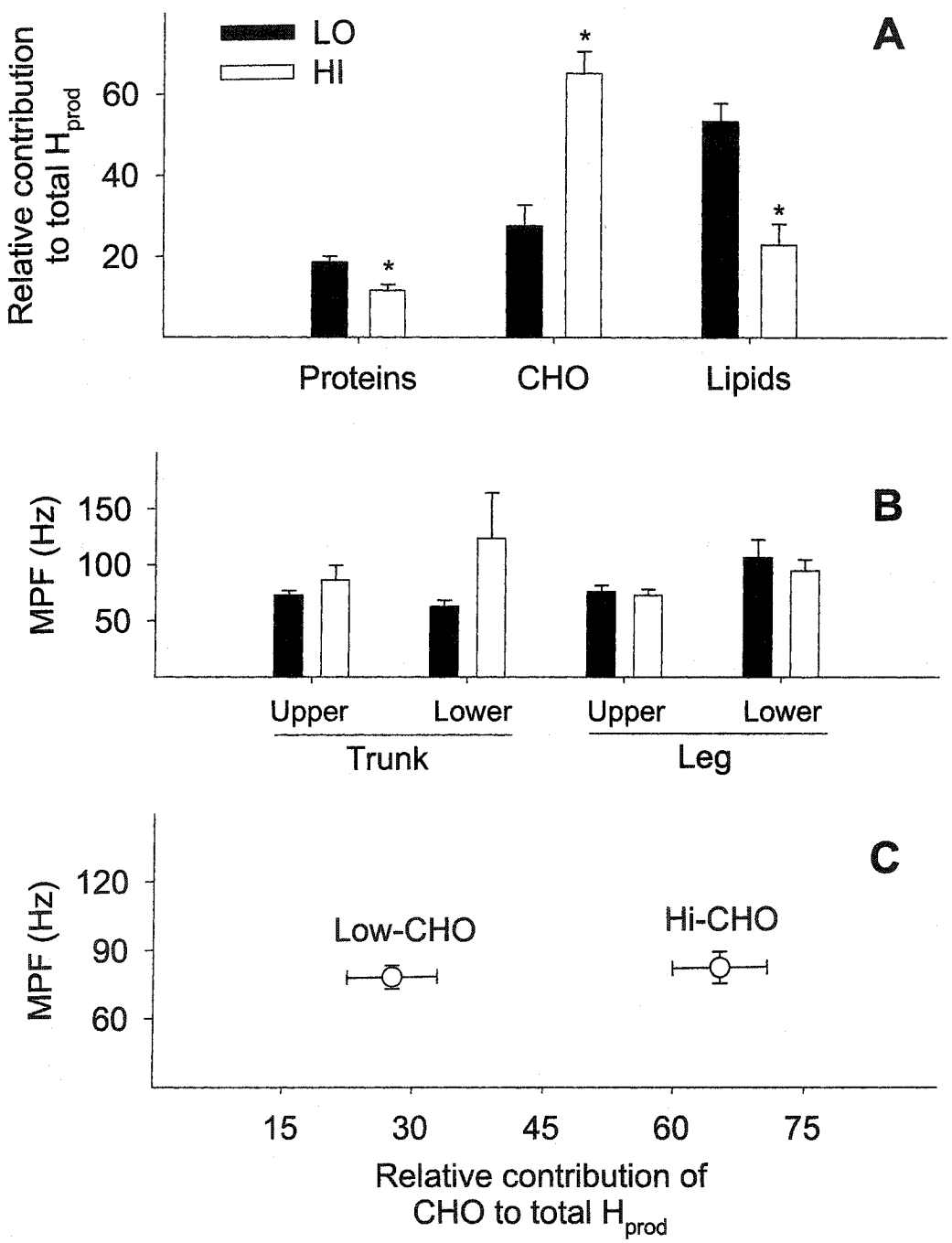

TR
LA
PE
RA
VL
RF
VM
GA
MPF (Hz)
120
90
60
30
30
60
90
120
min

Figure 4.8. A. Relative contribution of CHO, lipids and proteins to total heat production as well as B. mean power frequencies for upper trunk (UT), lower trunk (LT), upper leg (UL) and lower leg (LL) muscles determined for LO (black bars) and HI (white bars) in the last 15 min of cold exposure. C. Whole body MPF average for LO and HI as a function of the relative contribution of CHO to total heat production. *Significantly different from LO. (Two-way ANOVA, $P \leq 0.05$, n=6)


A
LO
HI
Relative contribution to total H_{prod}
60
40
20
*
*
*
Proteins
CHO
Lipids
B
MPF (Hz)
150
100
50
Upper
Lower
Upper
Lower
Trunk
Leg
C
MPF (Hz)
120
90
60
Low-CHO
Hi-CHO
15
30
45
60
75
Relative contribution of CHO to total H_{prod}

DISCUSSION

Contrary to expectation, this study shows that drastic changes in oxidative fuel selection are achieved without modifying the EMG signature of shivering muscles. Results support the notion that the same population of muscle fibers is responsible for oxidizing widely different fuel mixtures, but they are not compatible with the hypothesis that changing fuel selection is realized by recruiting fuel-specific motor units. EMG signal analyses were performed at two levels to assess potential differences in: i) whole body shivering activity (monitoring >90% of total shivering muscle mass) based on the recruitment intensity of 8 large muscles, and, ii) detailed EMG activity of individual muscles based on their shivering pattern and spectral characteristics.

Whole body shivering

Total shivering activity ($Shiv_{WBI}$, Fig. 4.2) and the respective contributions of specific muscles to $Shiv_{WBI}$ were not affected by large changes in fuel selection. Consequently, the contribution of each muscle to total heat production was the same for LO and HI. The normalized EMG amplitudes found here (%MVC) were small and consistent with the only 2 other studies reporting this parameter for low intensity shivering (3-10 %) (Bell *et al.*, 1992, Tikuisis *et al.*, 2000a). Inter-individual and inter-trial differences in the shivering response are mainly responsible for the high variability observed at each time point in this and previous studies. Because such temporal variability could mask subtle differences between treatments, we have eliminated

temporal effects by calculating frequency distributions of shivering intensities for each muscle and each subject separately, over the entire period of cold exposure. Even though minor differences between LO and HI were detected for individual muscles in some subjects, no significant differences between mean values could be established. This additional analysis strengthens the conclusion that shivering intensity is not modified by changes in fuel selection.

Shivering activity (EMG) and heat production (indirect calorimetry) provide two different estimates of thermogenesis, and their divergence over time could be used to detect possible contributions from non-shivering thermogenesis (brown adipose tissue, substrate cycles) (Olson, 1994). In this study, changes in shivering activity and heat production were closely correlated for LO and HI (Fig. 4.3), and the same observation was previously made for individuals with normal CHO reserves (Bell et al., 1992). These results were somewhat expected because non-shivering thermogenesis is generally assumed to be negligible in adult humans (Himms-Hagen and Ricquier, 1998). However, the tight correlation between shivering activity and heat production suggests that non-shivering thermogenesis probably remains unimportant for thermoregulation, even when fuel selection is drastically modified.

Shivering pattern and EMG spectral characteristics

A detailed characterization of shivering pattern and spectral parameters was performed for each muscle to try detecting subtle effects of changes in fuel selection. The EMG signal was analyzed by separating the patterns for "burst shivering" and

"continuous, low-intensity shivering" (Table 4.1). Distinguishing these two patterns was based on large differences in intensity (2-5 vs 7-15%MVC) and rate of occurrence (8-10 vs 0.1-0.2 Hz) (Fig. 4.5). Results clearly show that both shivering patterns remained unaffected by large changes in fuel selection. In addition, we found that burst shivering rate, relative contribution of burst activity to total shivering, and burst shivering intensity were the same between LO and HI (Fig. 4.6). Previous studies have associated continuous low-intensity shivering with the recruitment of slow-oxidative MU (Type I) and burst shivering with fast-glycolytic MU (Type II) (Meigal, 2002, Meigal *et al.*, 1993, Petajian and Williams, 1972). The lack of difference in shivering pattern between LO and HI suggests that MU recruitment is the same under both conditions and, more importantly, that the recruitment of fast-glycolytic fibers is not affected by the size of CHO reserves. Very little is known on the relative importance of continuous, low-intensity shivering and of burst shivering to total heat generation. How they are partitioned may have important implications on cold endurance and additional research is needed to determine the physiological significance of this dual pattern.

The second part of our detailed analysis dealt with spectral parameters of the EMG signal. Over the last few decades, changes in EMG frequency spectra have been used extensively to investigate MU recruitment and muscle fatigue (Andearssen and Arendt-Nielsen, 1987, Bonato *et al.*, 2001, De Luca *et al.*, 1982, Elert *et al.*, 1992, Gerdle *et al.*, 1988, Komi *et al.*, 2000, Kupa *et al.*, 1995, Moritani *et al.*, 1985, Wakeling *et al.*, 2002, Wakeling *et al.*, 2001). Using surface EMG, these studies have shown that the recruitment of fast-glycolytic fibers results in higher myoelectric frequencies than slow-

oxidative fibers (Wakeling et al., 2002). During shivering, however, information on changes in spectral parameters is scarce, and it was only obtained from a limited number of muscles (Bell *et al.*, 1992, Muza *et al.*, 1986). Here, time-frequency analysis was used to quantify potential changes in the spectral signature of 8 muscles (Fig. 4.7 and 4.8). Mean power frequency (MPF) remained the same under all conditions, showing that the frequency spectrum was unaffected by the size of CHO stores. Furthermore, the frequency distributions of MPF over the entire cold exposure period were identical for LO and HI, as we had found for shivering intensity. Therefore, detailed spectral analysis of EMG signals also shows that the pattern of MU recruitment is not modified.

During sustained isometric contractions, the onset of fatigue is known to be associated with a decrease in mean or median frequency of the surface EMG power spectrum (Bonato et al., 2001). However, the MPF of all the muscles selected in our study remained constant throughout cold exposure, suggesting that no fatigue occurs. Using a similar approach, two other studies have investigated whether prolonged shivering could cause fatigue. While no downward shift in the EMG power spectrum was found for *masseter*, *rectus abdominis*, *biceps brachii*, *brachioradialis*, *rectus femoris* and *gastrocnemius*, a decrease in the median frequency of *pectoralis major* was reported (Bell *et al.*, 1992, Muza *et al.*, 1986). It is worth noting here that a decrease in MPF cannot be unequivocally interpreted as muscle fatigue, because a clear link between these two parameters has not been established for shivering. For example, a shift in MPF can occur when changes in recruitment take place in the absence of fatigue (Wakeling et al., 2002). While some authors have provided evidence supporting the idea that prolonged

shivering can lead to fatigue (Tikuisis et al., 2002), further research is needed to clarify this issue.

It could be argued that the surface EMG method used here provides information on the recruitment of superficial MU, and may not detect changes taking place in deeper regions. However, such a scenario is very unlikely because the surface EMG signal provides action potential information from a large number of motor units (Kupa et al., 1995), and all fiber types are represented in superficial regions (Sjöström et al., 1986). For the muscles where superficial-to-deep gradients in fiber composition have been reported (*vastus lateralis*, *tibialis anterior*), more fast-glycolytic fibers were found in the periphery (Henriksson-Larsén *et al.*, 1983, Lexell *et al.*, 1983). In our context, such a gradient would improve the ability to detect potential changes in the recruitment of fast-glycolytic fibers. In addition, to minimize the effects of variability in fiber composition between individuals and between locations along the muscles, the same subjects and the same EMG sampling sites were used for LO and HI. In the absence of measurable changes in shivering intensity (Fig. 4.2-4.4, Table 4.1), shivering pattern (Table 4.1, Fig. 4.6) and EMG spectral parameters (Fig. 4.7 and 4.8), failing to detect differences in MU recruitment between LO and HI is, therefore, highly improbable.

Fuel selection within the same muscle fibers

We had anticipated that changing fuel selection would be achieved by recruiting different populations of muscle fibers. However, this hypothesis can be rejected for sustained shivering because no change in fiber recruitment was observed between LO and

HI. It appears that most of the heat is generated within type I fibers, because burst shivering only represents <10% of total shivering activity (Table 4.1). What are the mechanisms responsible for regulating such a large change in fuel mixture within these same fibers? Fuel selection has not been investigated in shivering muscle, but a lot of information is available on the reciprocal regulation of CHO and lipid metabolism during exercise (Jeukendrup, 2002, Spriet, 2002). A combination of many factors is responsible for controlling fuel oxidation. They include fuel availability (size of glycogen reserves), circulating hormones (e.g. catecholamines), trans-membrane transporters (GLUT and fatty acid transporters), and a large series of intracellular metabolites (acetyl-CoA, malonyl-CoA, Ca^{2+}, ADP, AMP, P_i and AMPK 1). The relative importance of these factors is far from being understood for exercise and fuel selection may be regulated differently during shivering.

Conclusion

Together with Chapter 3, this study provides the first integrated analysis of fuel metabolism and muscle fiber recruitment during shivering. It shows that shivering intensity, shivering pattern, and EMG spectral parameters are not modified by extreme changes in oxidative fuel selection. During low-intensity shivering, humans are able to sustain thermogenic rate by oxidizing widely different fuel mixtures within the same muscle fibers. Whether this selection strategy is also used during high intensity shivering, rather than recruiting different "fuel-specific" fibers, remains to be established.

CHAPTER 5. OXIDATIVE FUEL SELECTION DURING HIGH INTENSITY SHIVERING: EARLY RELIANCE ON CARBOHYDRATES

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INTRODUCTION

In cold exposed humans, CHO can provide up to 60% of all the heat generated (Haman *et al.*, 2002, Jacobs *et al.*, 1994). Two separate sources of CHO are available: muscle glycogen and plasma glucose provided to working muscles via the circulation. During high-intensity shivering, the exact contribution of these two fuel sources is unknown because whole-body oxidation rates have never been quantified. Recent studies have reported that during low intensity shivering the role of plasma glucose was always minor, independent of changes in CHO availability (Haman *et al.*, 2004b, Haman *et al.*, 2002). In contrast, muscle glycogen stores played a more prominent role, providing at least ~3 times more glucose units for oxidation than glucose in the circulation. Indirect evidence suggests that, at higher thermogenic rates, the relative importance of plasma glucose could be increased substantially. Glucose production (R_aGLU) and disappearance rates (R_dGLU) were both reported to double from low- to high-intensity shivering suggesting that the rate of plasma glucose oxidation could also be increased (Tipton *et al.*, 1997); however, non-oxidative glucose disposal may be important at such low metabolic rates (Friedlander *et al.*, 1997, Haman *et al.*, 2002). At the highest metabolic rate observed during maximal shivering (~45% $\dot{V}O_{2max}$ or 5 times RMR) (Eyolfson *et al.*, 2001), exercise studies have also shown that plasma glucose oxidation may be stimulated by as much as 5-fold (Friedlander *et al.*, 1997). Considering the low metabolic rates reached previously during low-intensity shivering (<2.3 fold rise in $\dot{H}_{prod}$

or ~20% $\dot{V}O_{2max}$), further research is needed to establish whether plasma glucose plays a more prominent role at higher thermogenic rates.

During cold exposure, one other important issue remains unresolved. The effect of changes in shivering intensity on the respective importance of total CHO, lipids and proteins has never been established (Haman *et al.*, 2002, Weller *et al.*, 1998). A number of studies have reported CHO and lipid oxidation rates at either low-intensity (Glickman-Weiss *et al.*, 1993, Glickman-Weiss *et al.*, 1994, Haman *et al.*, 2002, MacNaughton *et al.*, 1990, Vallerand *et al.*, 1995, Vallerand and Jacobs, 1989, Vallerand and Jacobs, 1990, Vallerand *et al.*, 1989, Vallerand *et al.*, 1993, Vallerand *et al.*, 1999a, Weller *et al.*, 1998) or high-intensity shivering (Martineau and Jacobs, 1988, Martineau and Jacobs, 1989a, Martineau and Jacobs, 1989b, Tikuisis *et al.*, 2002, Tikuisis *et al.*, 2000b) and only a few of these account for the contribution of proteins (Haman *et al.*, 2002, Vallerand *et al.*, 1995, Vallerand *et al.*, 1999a). In these studies, the reported dominance of either CHO or lipids has no clear link with differences in shivering intensity but seem to be correlated with the cooling protocol. While subjects exposed to cold air used CHO preferentially (Glickman-Weiss *et al.*, 1993, Glickman-Weiss *et al.*, 1994, MacNaughton *et al.*, 1990, Vallerand *et al.*, 1995, Vallerand and Jacobs, 1989, Vallerand and Jacobs, 1990, Vallerand *et al.*, 1989, Vallerand *et al.*, 1993, Vallerand *et al.*, 1999a), those cooled by water immersion or LCS favored lipid utilization (Haman *et al.*, 2002, Martineau and Jacobs, 1988, Martineau and Jacobs, 1989a, Martineau and Jacobs, 1989b, Tikuisis *et al.*, 2002, Tikuisis *et al.*, 2000b, Weller *et al.*, 1998). The size of CHO reserves has also been shown to have a major effect on fuel selection before and during shivering (Haman *et al.*,

2004b, Martineau and Jacobs, 1989b, Young *et al.*, 1989). In the cold, a shift from lipid oxidation when glycogen reserves are low to CHO-based metabolism when they are high is observed. Further research is needed to determine the effect of shivering intensity on the respective roles of oxidative fuels during cold exposure.

Consequently, the purpose of this study was to quantify the relative contributions of plasma glucose, muscle glycogen, lipids and proteins to total heat production during low- (2.5-fold rise in $\dot{H}_{prod}$ or 40%$Shiv_{peak}$) and high-intensity shivering (3.5-fold rise in $\dot{H}_{prod}$ or 60%$Shiv_{peak}$). More specifically, using a combination of stable isotope and indirect calorimetry methods, fuel selection was measured in men exposed to 5°C for 90 min and compared to values previously obtained at 10°C (combination of LCS and thermal chamber) (Haman *et al.*, 2002). We hypothesize that, during high-intensity shivering, 1) the role of plasma glucose will be higher than during low-intensity shivering and, 2) lipids will remain the major pathway for heat generation because of the small change in metabolic rate observed in the cold.

METHODS

This study compares metabolic and oxidative fuel selection parameters for two groups of adult male subjects exposed to either 10°C (LOW) (Haman *et al.*, 2002) or 5°C (HIGH) using a combination of LCS and thermal chamber. All experimental procedures for LOW have been described in detail previously (Haman *et al.*, 2002) and consequently, only the experimental procedures for HIGH will be described in this study.

Subjects

Eight healthy and trained men volunteered for this study approved by the Health Sciences Ethical Committee of the University of Ottawa, and written consent was obtained from the participants. Physical characteristics of the subjects are presented in Table 5.1. Percent body fat (underwater weighing; Brosek *et al.* (1963)) and maximal oxygen consumption (progressive treadmill protocol) were measured 5-7 days prior to the experiments.

Experimental protocol

Experiments were conducted between 8:00 and 12:00, following 36-h without heavy physical activity. The last evening meal was standardized (~988 kcal, ~52% CHO, ~18% lipids, ~30% proteins) and subjects were asked to report to the laboratory the next morning (8:00 AM) after a 12-14h fast. Ingestion of carbohydrates from plants naturally rich in ^{13}C (C_4 photosynthetic cycle) was avoided to maintain low ^{13}C background

Table 5.1. Physical characteristics of subjects for LOW (n=6) and HIGH (n=8). Values are presented as means ± SE.

	LOW	HIGH
Age, yr	24.7 ± 1.5	23.3 ± 0.4
Body mass, kg	78.1 ± 4.8	71.7 ± 3.0
Height, cm	178.2 ± 2.5	174.0 ± 1.0
Body surface area, m^2	2.0 ± 0.1	1.9 ± 0.1
Percent body fat, %	13.3 ± 1.9	12.7 ± 0.8
$\dot{V}O_{2max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	56.4 ± 2.9	53.1 ± 2.5

enrichment in plasma glucose and expired CO_2. Care was taken to minimize thermal stimuli between awakening and the start of the experiment (i.e. avoid exposure to hot or cold temperatures, very low intensity exercise during transit from home to the laboratory). Upon their arrival in the laboratory, subjects were instrumented with thermal probes and an indwelling catheter (18G, 32 mm, Medical Inc., Arlington, TX) placed in an antecubital vein (left arm) for blood sampling, and fitted with a LCS (One-piece CORETEC, Delta Temax, Inc. Pembroke, ON, Canada). Subjects were then asked to empty their bladder (t = 0 min) and sit quietly for two hours at 25.5 ± 0.2°C (759 ± 2 mmHg, 45 ± 4% RH). Following this habituation period, they were transferred to an environmental chamber (5.7 ± 0.1°C, 759 ± 2 mmHg, 69 ± 2% RH) and a 5°C water perfusion was started through the LCS using a temperature controlled circulation bath (Endocal, NESLAB and Model 200-00, Micropump, Vancouver, WA). Thermal response, metabolic rate and fuel utilization rate were measured at 26°C and during the subsequent 90 min at 5°C.

Thermal response

Changes in whole body $\dot{H}_{loss}$ and $\dot{H}_{prod}$ as well as T_{es} and $\overline{T}_{skin}$ were determined as described previously (Chapter 2). $\dot{H}_{loss}$ (in watts) was estimated using the following equation:

$$\dot{H}_{loss} = (\dot{R} + \dot{C}) + (\dot{E}_{resp} + \dot{C}_{resp}) \quad (5.1)$$

$\dot{R}$ and $\dot{C}$ were estimated using heat flux transducers (Concept Engineering, Old Saybrook, CT) placed on the surface of the skin at 11 sites (i.e. forehead, chest, biceps, forearm, abdomen, lower and upper back, front and back calf, quadriceps, hamstrings) and calculated using an area-weighed equation (Dubois and Dubois, 1916). Evaporative heat loss from the skin was assumed to be negligible at 23°C and 10°C (Nishi, 1981). $\dot{H}_{prod}$ was calculated by indirect respiratory calorimetry corrected for protein oxidation (see below). Percent of shivering peak ($\%Shiv_{peak}$) was determined by dividing $\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$) in the cold by the estimated shivering peak value, as described by Eyolfson et al. (2001). T_{es} and $\overline{T}_{skin}$ were monitored continuously prior and during cold exposure using a pediatric esophageal probe (Mon-a-therm general purpose, Mallinckrodt Medical Inc, St-Louis, MO) and heat flux transducer (area-weighed equation from 12 sites: finger tip + 11 sites mentioned above for the determination of $\dot{H}_{loss}$; (Dubois and Dubois, 1916).

Metabolic rate and fuel utilization

$\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$ were determined by open-circuit spirometry (250 l, chain-compensated gasometer, Warren Collins inc., Braintree, MA) as described in Chapter 2. Expired gases were collected for 5 min every 15 min before and during cold exposure. Oxygen and carbon dioxide concentrations in dry expired gases were determined using calibrated electrochemical gas analyzers (AMETEK Model S-3A/1 and CD 3A, Applied Electrochemistry, Pittsburg, PA).

RP_{ox}, RG_{ox} and RF_{ox} oxidation rates (in $g \cdot min^{-1}$) were calculated using the following equations (Livesey and Elia, 1988):

$$RP_{ox}(g \cdot min^{-1}) = 2.9 \times UREA_{urine}(g \cdot min^{-1}) \quad (5.2)$$

$$RG_{ox}(g \cdot min^{-1}) = 4.59 \dot{V}CO_2(l \cdot min^{-1}) - 3.23 \dot{V}O_2(l \cdot min^{-1}) \quad (5.3)$$

$$RF_{ox}(g \cdot min^{-1}) = -1.70 \dot{V}CO_2(l \cdot min^{-1}) + 1.70 \dot{V}O_2(l \cdot min^{-1}) \quad (5.4)$$

where $\dot{V}CO_2$ ($l \cdot min^{-1}$) and $\dot{V}O_2$ ($l \cdot min^{-1}$) were corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1.010 and 0.843 $l \cdot g^{-1}$, respectively).

RP_{ox} was estimated from $UREA_{urine}$ in urine samples collected for a period of 90 min at 26°C and 5°C. Urinary concentrations were determined on a Synchron Clinical System (CX7, Beckman, Anaheim, CA). Energy potentials of 16.3 $kJ \cdot g^{-1}$, 40.8 $kJ \cdot g^{-1}$ and 19.7 $kJ \cdot g^{-1}$ were used to calculate the relative contributions of glucose, lipid and protein oxidation to total heat production, respectively (Elia, 1991, Péronnet and Massicotte, 1991).

Plasma glucose oxidation

Plasma glucose oxidation rate was estimated by repeated ^{13}C-glucose ingestion (Chapter 2). On the evening of the experiment, 6 g of glucose ($^{13}C/C = 0.01098$) artificially enriched with ^{13}C (1.66 µg/kg body mass; U ^{13}C-glucose, $^{13}C/C > 99\%$, Isotec, Miamisburg, OH) to obtain a final $^{13}C/C$ ratio ranging between 1.66 and 2.20 (R_{exo}) were diluted into 700 ml of water and split into seven equal doses (100 ml). Following the measurement of baseline $^{13}C/C$ in plasma and expired CO_2 (t = 15 min), subjects ingested

the first dose of ^{13}C-glucose. To improve isotopic steady-state prior to cold exposure, a ^{13}C-bicarbonate priming dose (0.8 mg/kg) was given in combination with the first ^{13}C-glucose dose. Furthermore, baseline measurements were made between 105 and 135 min prior to cold exposure to allow sufficient time for ^{13}C-glucose equilibration in plasma glucose. Subsequent doses were then taken every 30 min until the end of the experiment. Isotopic composition of plasma glucose and expired CO_2 were determined *–prior to the ingestion of the next ^{13}C-glucose dose* – in blood and expired gas samples at 60, 90, 120, 135 and 150 prior to cold exposure and at 180, 210, 225 and 240 min in the cold. Upon collection, blood samples were put on ice, spun in a refrigerated centrifuge, separated, and the plasma was kept frozen at –20°C until analyzed.

Plasma glucose was isolated by double-bed ion exchange chromatography with superimposed columns (resins: AG 50W-X8 H^+, 200-400 mesh, and AG 1-X8 chloride, 200-400 mesh). Following evaporation, glucose was combusted (60 min at 400°C) in the presence of CuO, and CO_2 was recovered. Measurements of $^{13}C/^{12}C$ in expired CO_2 (R_{exp}) and in CO_2 obtained from glucose combustion (R_{glu}) were determined in a Prism mass spectrometer (VG, Manchester, UK). Isotopic composition was expressed as $\%^{13}C/C$.

$RG_{ox\text{-}plasma}$ was calculated from $^{13}CO_2$ production at the mouth and plasma glucose isotopic composition (Fig. 5.1) using the following equation (Derman *et al.*, 1996, Wolfe, 1992):

$$RG_{ox\text{-}plasma} = \dot{V}CO_2\ (R_{exp}\text{-}R_{ref}/R_{glu}\text{-}R_{ref})(1/k1 \cdot k2) \qquad (5.5)$$

where $\dot{V}CO_2$ is in $l \cdot min^{-1}$ (STPD), R_{ref} is the isotopic composition of expired CO_2 prior to the ingestion of the first ^{13}C-glucose dose, k1 ($0.7426\ l \cdot g^{-1}$) is the volume of CO_2 produced from the complete oxidation of glucose and k2 is the fractional recovery at the mouth of CO_2 produced in tissues (Pallikarakis et al., 1991). A fractional recovery of $^{13}CO_2$ at the mouth (k2) of 0.8 and 1 was used before and during cold exposure, respectively (Wolfe, 1992). Due to the large size of the bicarbonate pool, only values in the last 30 min before and during cold exposure were used in the calculation of $RG_{ox\text{-}plasma}$. In addition, $RG_{ox\text{-}plasma}$ was corrected to account for the fraction of plasma glucose oxidized from exogenous sources (Chapter 2). Oxidation of glucose derived from muscle glycogen stores ($RG_{ox\text{-}mus}$; $g \cdot min^{-1}$), directly or through the lactate shuttle (Brooks et al., 1999), was calculated by subtracting $RG_{ox\text{-}plasma}$ from RG_{ox}.

$$RG_{ox\text{-}mus} = RG_{ox} - RG_{ox\text{-}plasma} \quad (5.6)$$

Blood analysis

Plasma glucose and lactate concentrations were measured spectrophotometrically at 340 nm on a Beckman DU 640 (Bergmeyer, 1985) while total NEFA concentration were determined using an analytical assay kit (NEFA C, Wako Chemicals, Osaka, Japan and Sigma Kit #310, Sigma-Aldrich Canada, Oakville, ON, Canada). Insulin concentration was measured using a radioimmunoassay (#KTSP-11001, Medicorp Inc, Montréal, Qc, Canada).

Statistical analyses

Overall changes in T_{es}, $\overline{T}_{skin}$, $\dot{H}_{loss}$, $\dot{H}_{prod}$, blood metabolite concentrations, expired CO_2 and plasma glucose isotopic enrichments, and gas exchange over time were assessed using a one-way analysis of variance (ANOVA) with replication. For each sampling time, a Bonferroni t-test was used to detect potential differences with control values before cold exposure. Differences in $\dot{H}_{prod}$, fuel utilization for CHO (RG_{ox}, $RG_{ox\text{-}plasma}$, $RG_{ox\text{-}mus}$), lipids (RF_{ox}) and proteins (RP_{ox}) as well as plasma metabolite concentrations over the last 30 min before and during cold exposure were determined using a one-way analysis of variance to verify the main effect of shivering intensity (LOW vs HIGH). Statistical differences were considered significant when $P \leq 0.05$. All values given are means ± S.E. (n=6) for low-intensity and means ± S.E. (n=8) for high-intensity shivering.

RESULTS

Thermal response

Changes in $\dot{H}_{loss}$ and $\dot{H}_{prod}$ are presented in Fig. 5.2. $\dot{H}_{loss}$ increased by a maximum of 3.3-fold for LOW (77.7 ± 0.6 to 258.4 ± 10.6 W) and 4.2-fold for HIGH (75.1 ± 3.9 W to 315.1 ± 14.3 W). After reaching a maximum in the first 10 to 20 min after the onset of cold exposure, $\dot{H}_{loss}$ decreased thereafter by 16% for LOW (238.3 ± 0.6 W) and 9% for HIGH (289.1 ± 13.7 W). $\dot{H}_{prod}$ increased progressively by a maximum of 2.6-fold for LOW (95.3 ± 2.2 to 243.8 ± 4.2 W) and 3.5-fold for HIGH (89.5 ± 3.5 W to 292.1 ± 18.2 W). While observed shivering for LOW appeared to be minimal in the first 60 min and increased progressively in the last hour, onset of shivering occurred within the first 5 min of cold exposure for HIGH. Changes in T_{es} and $\overline{T}_{skin}$ are presented in Fig. 5.3. Whereas T_{es} remained constant at 36.4 ± 0.1°C throughout cold exposure for LOW, a transient increase in T_{es} was observed for HIGH in the first 60 min (36.6 ± 0.1°C at 26°C to 37.0 ± 0.1°C) before returning to baseline values (36.8 ± 0.2°C) (Fig. 5.3A). $\overline{T}_{skin}$ was reduced by 20% for LOW (34.0 ± 0.02 to 27.2 ± 0.02°C) and 25% for HIGH (34.2 ± 0.2°C to 25.8 ± 0.4°C) by the end of cold exposure (Fig. 5.3B). In the last 15 min in the cold (t = 75 to 90 min), shivering intensity was equivalent to 42.0 ± 3.4 $\%Shiv_{peak}$ for LOW and 57.4 ± 3.1 $\%Shiv_{peak}$ for HIGH.

Metabolic response and fuel utilization

RG_{ox}, RF_{ox} and RP_{ox} and their respective contribution to total $\dot{H}_{prod}$ during low- and high-shivering intensity are plotted in Fig. 5.4 and Fig. 5.5, respectively. Total glucose oxidation increased 2.2-fold for LOW (Fig. 5.4A; 165 ± 9 mg glucose·min^{-1} to 358 ± 41 mg glucose·min^{-1}) and 5-fold for HIGH (Fig. 5.4A; 138 ± 12 mg glucose·min^{-1} to 694 ± 118 mg glucose·min^{-1}). Total lipid utilization increased 3.8-fold for LOW (39 ± 2 mg fatty acids·min^{-1} to 141 ± 34 mg fatty acids·min^{-1}) and 3.3-fold for HIGH (43 ± 5 mg fatty acids·min^{-1} to 141 ± 34 mg fatty acids·min^{-1}). Protein utilization was not affected significantly by the change in temperature and averaged 62 ± 3 and 76 ± 13 mg·min^{-1} before and, 78 ± 5 and 70 ± 11 mg·min^{-1} during cold exposure for LOW and HIGH, respectively. While RG_{ox} for HIGH was 1.9-fold higher than for LOW in the last 30 min in the cold, no difference was observed for RF_{ox} and RP_{ox} between the two groups. During cold exposure, the relative contribution of CHO to total $\dot{H}_{prod}$ was also 1.5-fold higher for HIGH than for LOW (54.8 ± 5.9 vs 36.5 ± 4.9% $\dot{H}_{prod}$) whereas, it was 1.4-fold higher for LOW than for HIGH for both lipids (Fig. 5.5B; 51.5 ± 5.1 vs 36.8 ± 5.4% $\dot{H}_{prod}$) and proteins (Fig. 5.5C; 12.0 ± 1.6 vs 8.4 ± 1.0 % $\dot{H}_{prod}$).

Plasma concentrations

Changes in plasma concentrations of insulin, glucose, lactate and NEFA during low- and high-shivering intensity are presented in Fig. 5.6. As a result of cold exposure, insulin and glucose concentrations were not affected by the change in temperature (Fig. 5.6A and B) whereas, a transient increase in insulin (from 96.9 ± 5.2 at 26°C to

109.5 ± 5.6 pmol·l^{-1} at 5°C) and glucose levels (4.8 ± 0.1 at 26°C to 5.3 ± 0.2 mM at 5°C) were observed at 60 and 75 min, respectively. Plasma lactate increased more rapidly for HIGH (Fig. 5.6C; from 0.8 ± 0.1 to 1.6 ± 0.5 mM within 60 min) than for LOW (from 0.9 ± 0.11 to 1.61 ± 0.15 mM) but reached the same level by the end of the experiment. In contrast, NEFA levels were the same during cold exposure for LOW (0.21 ± 0.03 before to 0.52 ± 0.01 mM by the end of cold exposure) and for HIGH (0.39 ± 0.04 before to 0.51 ± 0.02 mM by the end of cold exposure.

CHO oxidation: plasma glucose vs. muscle glycogen

$RG_{ox\text{-}plasma}$ and $RG_{ox\text{-}mus}$ and their relative contribution to total heat production are presented in Fig. 5.7. The respective contribution of these two glucose sources was the same for the two groups. For LOW and HIGH, plasma glucose provided 25 ± 6 and 29 ± 3% of the total glucose oxidized in the cold whereas, muscle glycogen supplied 75 ± 6 and 71 ± 3%, respectively.

Table 5.2 summarizes average values measured for LOW and HIGH for all the parameters of fuel utilization estimated between 60 and 90 min of cold exposure (RG_{ox}, $RG_{ox\text{-}plasma}$, $RG_{ox\text{-}mus}$, RF_{ox} and RP_{ox}). RG_{ox}, $RG_{ox\text{-}plasma}$ and $RG_{ox\text{-}mus}$ were 2.2-, 2.2- and 2.1-fold higher for HIGH than for LOW, while no difference was observed between the two groups for RF_{ox} and RP_{ox}. Increasing shivering intensity also resulted in an increased contribution of total CHO, plasma glucose and muscle glycogen to total heat production (a 50, 48 and 51% increase for each fuel, respectively). In contrast, a tendency towards a

decrease in the relative contribution of lipids ($p = 0.08$) and proteins ($p = 0.06$) was observed from low- to high-intensity shivering.

Table 5.2. Absolute oxidation ($mg \cdot min^{-1}$) and relative ($\%\dot{H}_{prod}$) contributions of glucose (Total: RG_{ox}; plasma glucose: $RG_{ox\text{-}plasma}$ and muscle glycogen: $RG_{ox\text{-}mus}$), lipid (RF_{ox}) and protein oxidation (RP_{ox}) to total heat production during low-intensity (LOW, 2.5 times RMR, n=6) and high-intensity (HIGH, 3.5 times RMR, n=8) shivering. *Significantly different from LOW (Two-way ANOVA, $P \leq 0.05$).

	LOW	HIGH
Heat production rate ($kJ \cdot kg^{-1} \cdot h^{-1}$)	10.5 ± 0.9	14.7 ± 0.9*
Total glucose (RG_{ox}), $mg \cdot kg^{-1} \cdot h^{-1}$	233 ± 32	503 ± 71*
$\%\dot{H}_{prod}$	36.5 ± 4.9	54.8 ± 5.9*
Plasma glucose ($RG_{ox\text{-}plasma}$), $mg \cdot kg^{-1} \cdot h^{-1}$	58 ± 3	129 ± 9*
$\%\dot{H}_{prod}$	9.9 ± 1.1	14.6 ± 1.1*
Muscle glycogen ($RG_{ox\text{-}mus}$), $mg \cdot kg^{-1} \cdot h^{-1}$	175 ± 32	374 ± 67*
$\%\dot{H}_{prod}$	26.6 ± 4.6	40.2 ± 5.7*
Lipids (RF_{ox}), $mg \cdot kg^{-1} \cdot h^{-1}$	133 ± 22.4	138 ± 24.6
$\%\dot{H}_{prod}$	51.5 ± 5.1	36.8 ± 5.4
Proteins (RP_{ox}), $mg \cdot kg^{-1} \cdot h^{-1}$	60.0 ± 5.6	59.4 ± 6.9
$\%\dot{H}_{prod}$	12.0 ± 1.6	8.4 ± 1.0

Figure 5.1. Expired CO_2 (closed-circle) and plasma glucose (open-circle) isotopic enrichment (% $^{13}C/^{12}C$) before and during high intensity shivering.

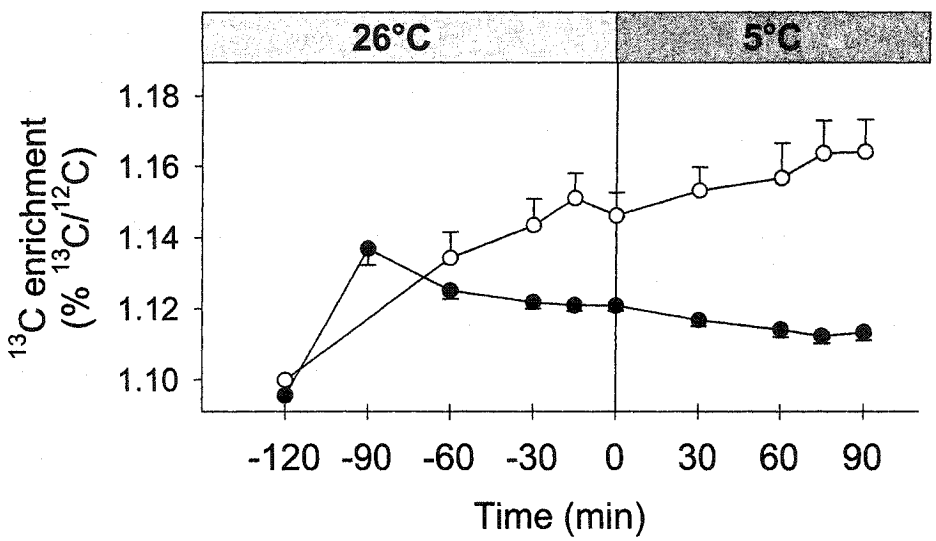

26°C
5°C
1.18
1.16
1.14
1.12
1.10
^{13}C enrichment
(% ^{13}C/^{12}C)
-120
-90
-60
-30
0
30
60
90
Time (min)

Figure 5.2. Absolute heat loss (A, $\dot{H}_{loss}$, W, *Eq. 5.1*) and heat production (B, $\dot{H}_{prod}$, W) before and during low- (LOW, open circles, n=6) and high-intensity (HIGH, closed circles, n=8) shivering. *Significantly different from control values before cold exposure (Two-way ANOVA with replication and Bonferroni *post-hoc t*-test, $P \leq 0.05$) † Significantly different from LOW (Two-way ANOVA with replication and Bonferroni *post-hoc t*-test, $P \leq 0.05$).

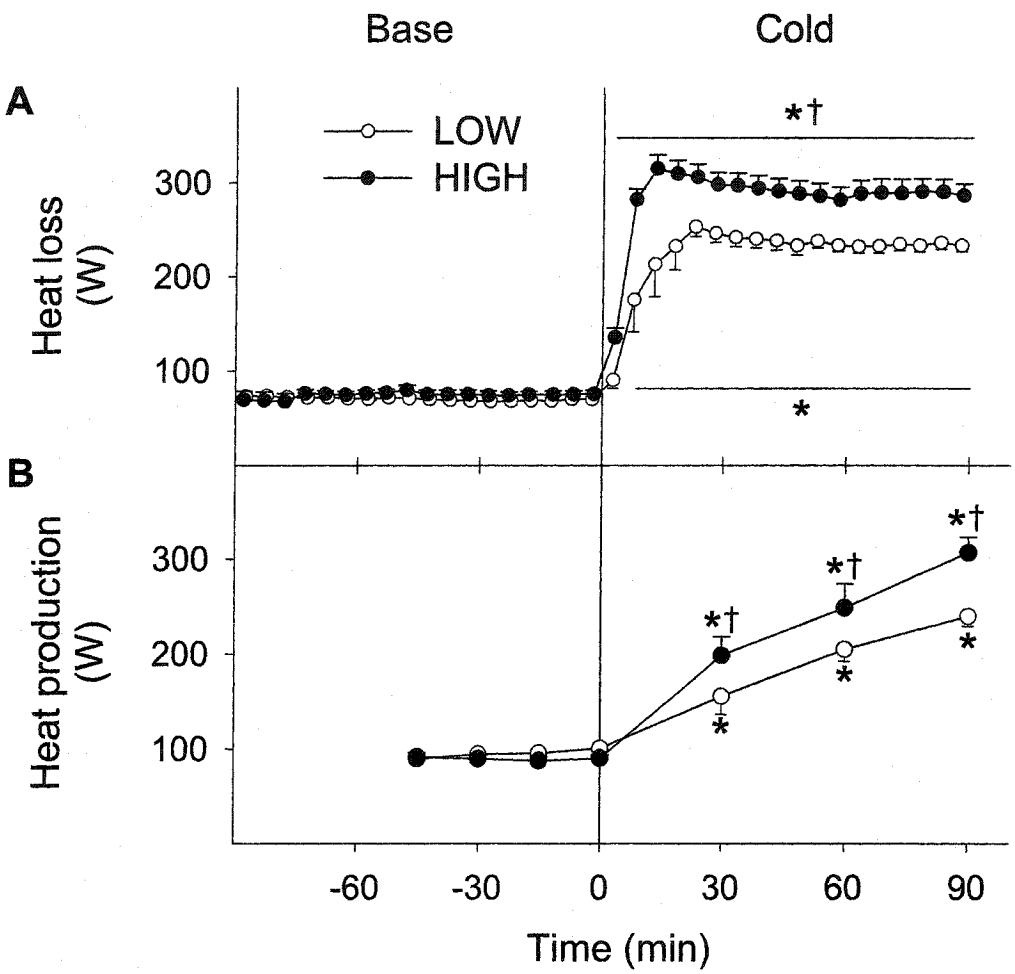

Base
Cold
A
LOW
HIGH
Heat loss (W)
300
200
100
*†
*
B
Heat production (W)
300
200
100
*†
*
-60
-30
0
30
60
90
Time (min)

Figure 5.3. Esophageal (T_{es}) and mean skin ($\overline{T}_{skin}$) temperature before and during low- (LOW, open circles, n=6) and high-intensity (HIGH, closed circles, n=8) shivering. Arrows indicate the times at which ^{13}C-glucose solutions were ingested. *Significantly different from control values before cold exposure (Two-way ANOVA with replication and Bonferroni *post-hoc* *t*-test, $P \leq 0.05$) † Significantly different from LOW (Two-way ANOVA with replication and Bonferroni *post-hoc* *t*-test, $P \leq 0.05$).

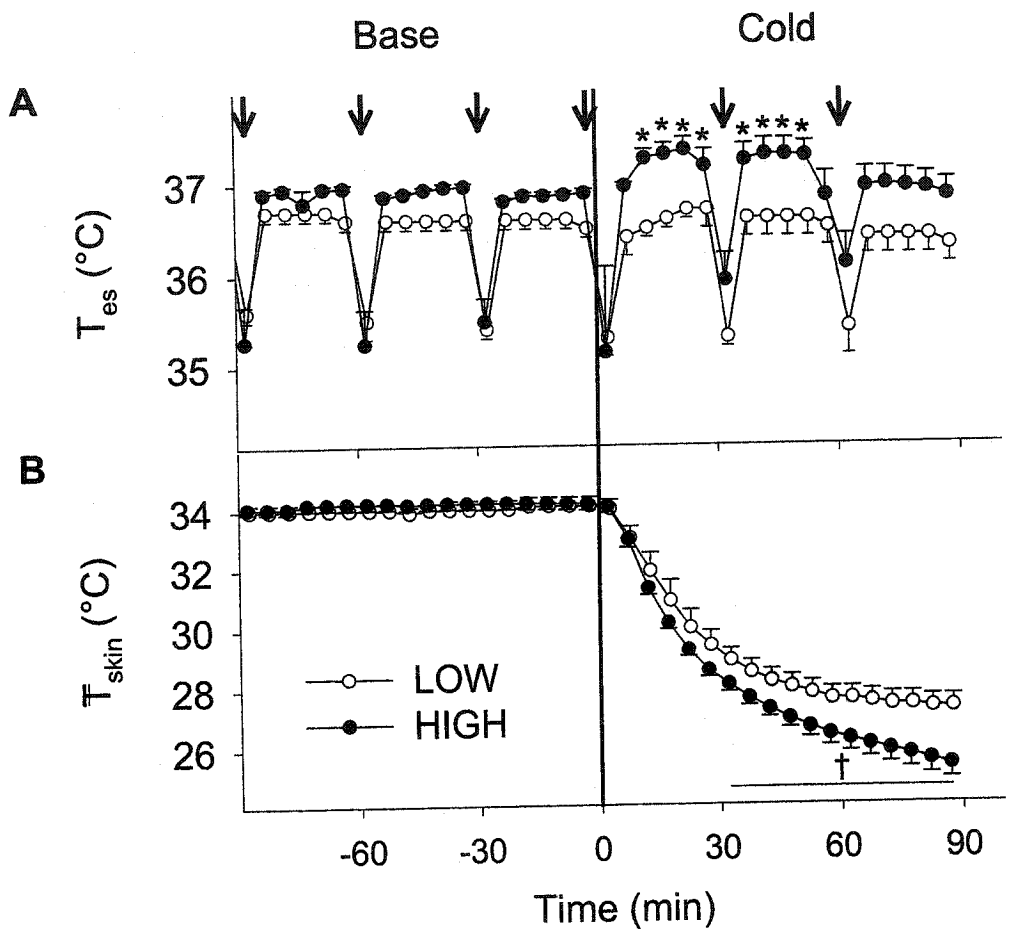

Base
Cold
A
B
T_{es} (°C)
T_{skin} (°C)
37
36
35
34
32
30
28
26
LOW
HIGH
-60
-30
0
30
60
90
Time (min)

Figure 5.4. CHO (A) and lipid (B) utilization rates before and during low- (LOW, open circles, n=6) and high-intensity (HIGH, closed circles, n=8) shivering. *Significantly different from control values before cold exposure (Two-way ANOVA with replication and Bonferroni *post-hoc* *t*-test, $P \leq 0.05$, n=6) † Significantly different from LOW (Two-way ANOVA with replication and Bonferroni *post-hoc* *t*-test, $P \leq 0.05$, n=8).

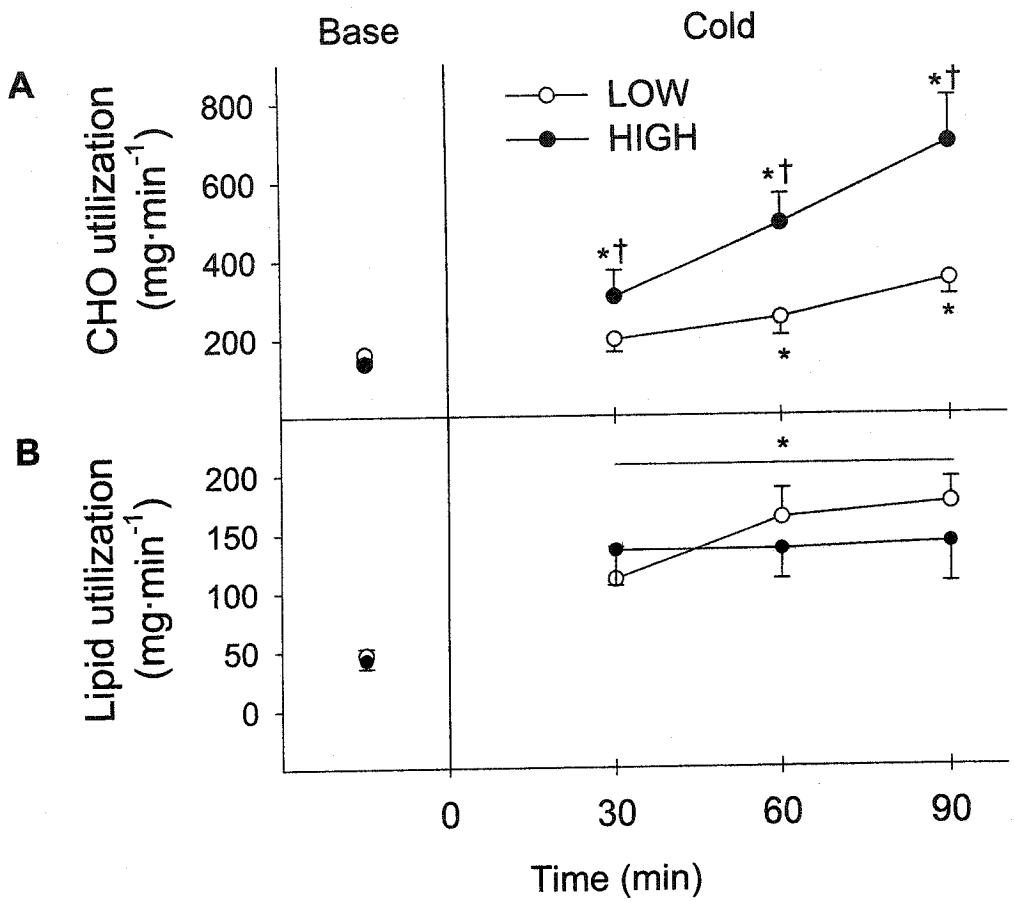

Base
Cold
A
CHO utilization
(mg·min-1)
800
600
400
200
LOW
HIGH
*†
*†
*†
*
*
B
Lipid utilization
(mg·min-1)
200
150
100
50
0
*
0
30
60
90
Time (min)

Figure 5.5. Relative contribution of CHO (A), lipids (B) and proteins (C) to total heat production before and during low- (LOW, open circles, n=6) and high-intensity (HIGH, closed circles, n=8) shivering. *Significantly different from control values before cold exposure (Two-way ANOVA with replication and Bonferroni *post-hoc* *t*-test, $P \leq 0.05$) † Significantly different from LOW (Two-way ANOVA with replication and Bonferroni *post-hoc* *t*-test, $P \leq 0.05$).

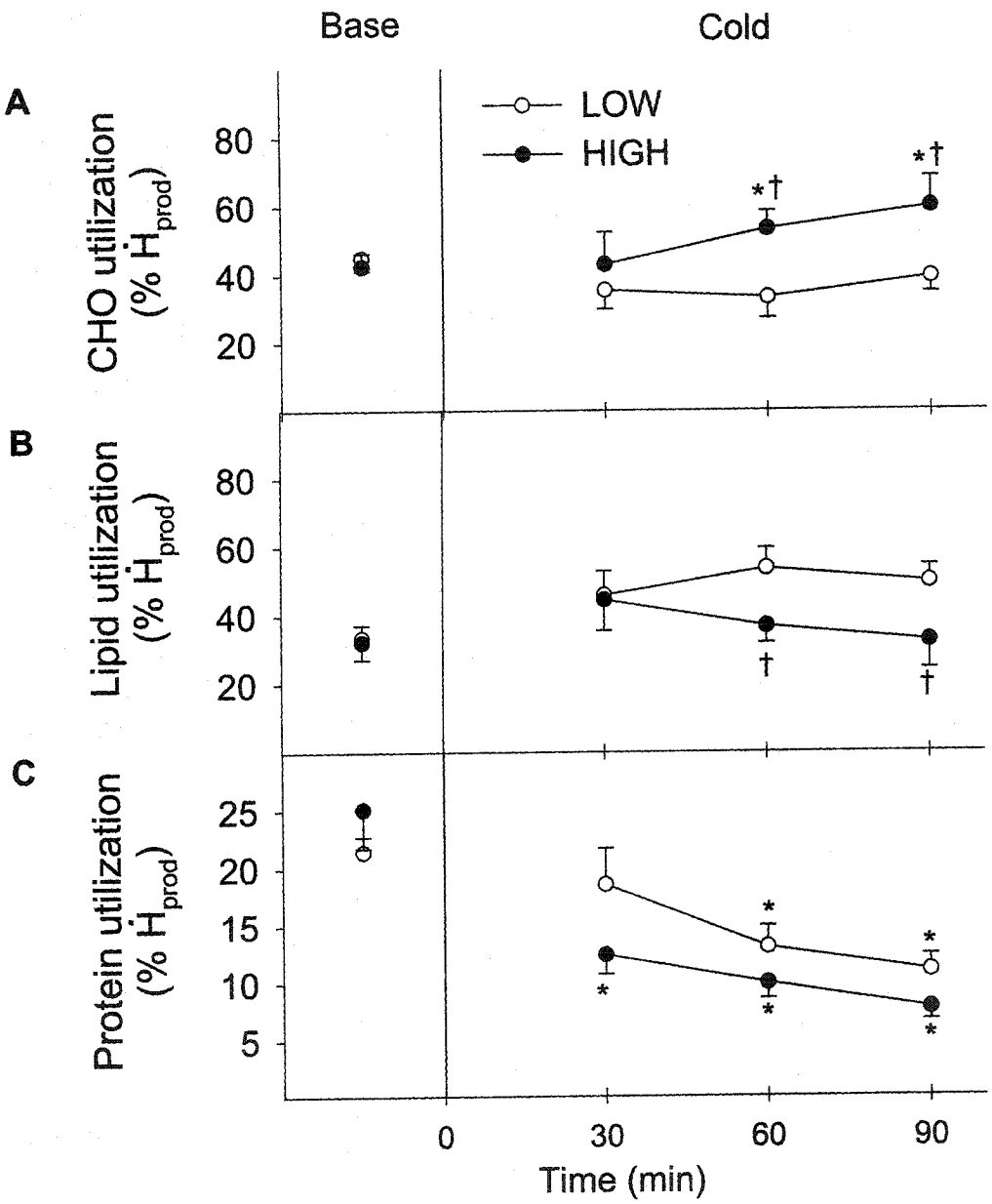

Base
Cold
A
B
C
LOW
HIGH
CHO utilization (% $\dot{H}_{prod}$)
Lipid utilization (% $\dot{H}_{prod}$)
Protein utilization (% $\dot{H}_{prod}$)
80
60
40
20
25
15
10
5
0
30
60
90
Time (min)
*†
†
*

Figure 5.6. Plasma insulin, glucose, lactate and nonesterified fatty acid (NEFA) concentrations before and during low- (LOW, open circles, n=6) and high-intensity (HIGH, closed circles, n=8) shivering. *Significantly different from control values before cold exposure (Two-way ANOVA with replication and Bonferroni *post-hoc* *t*-test, $P \leq 0.05$) † Significantly different from LOW (Two-way ANOVA with replication and Bonferroni *post-hoc* *t*-test, $P \leq 0.05$).

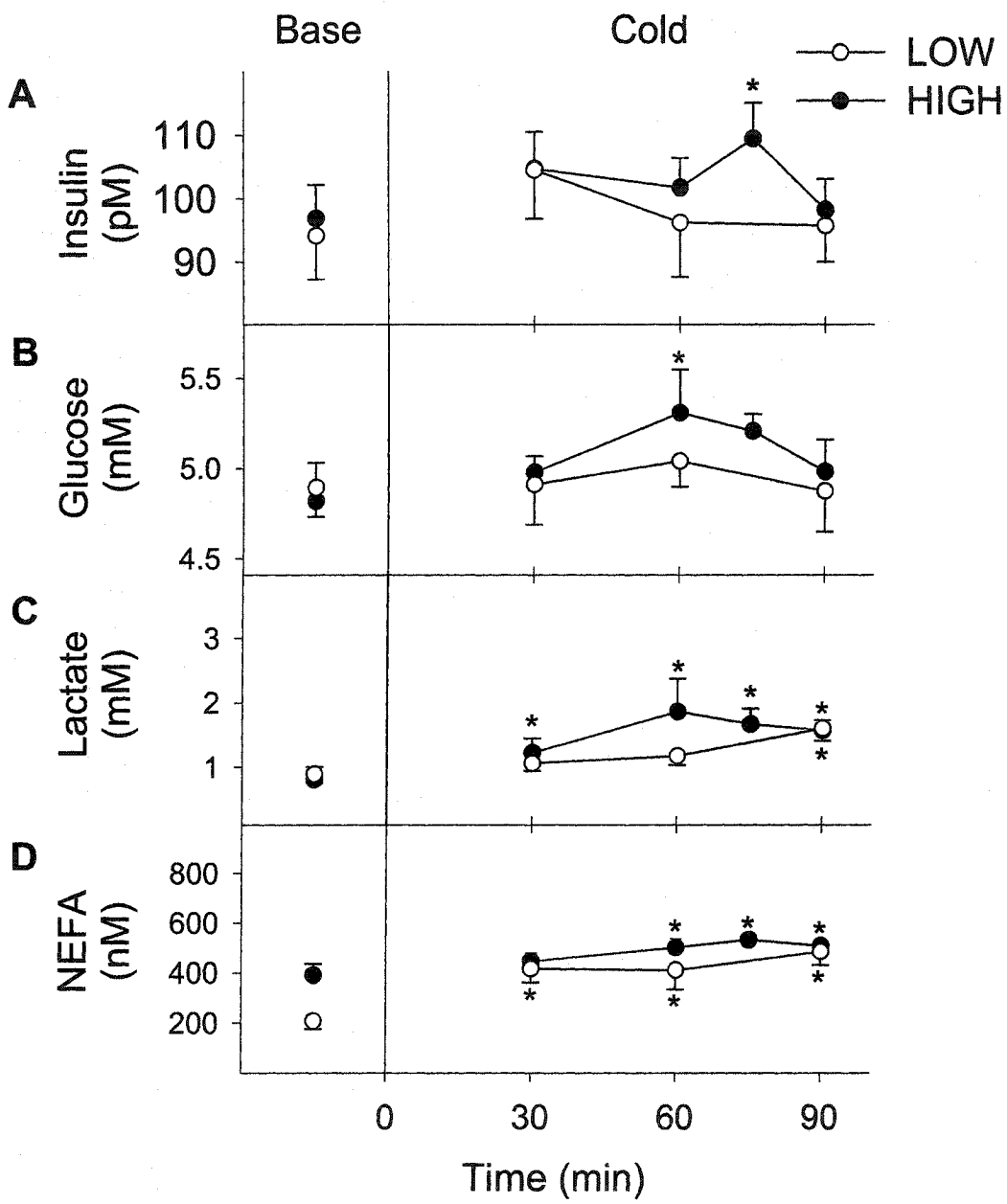
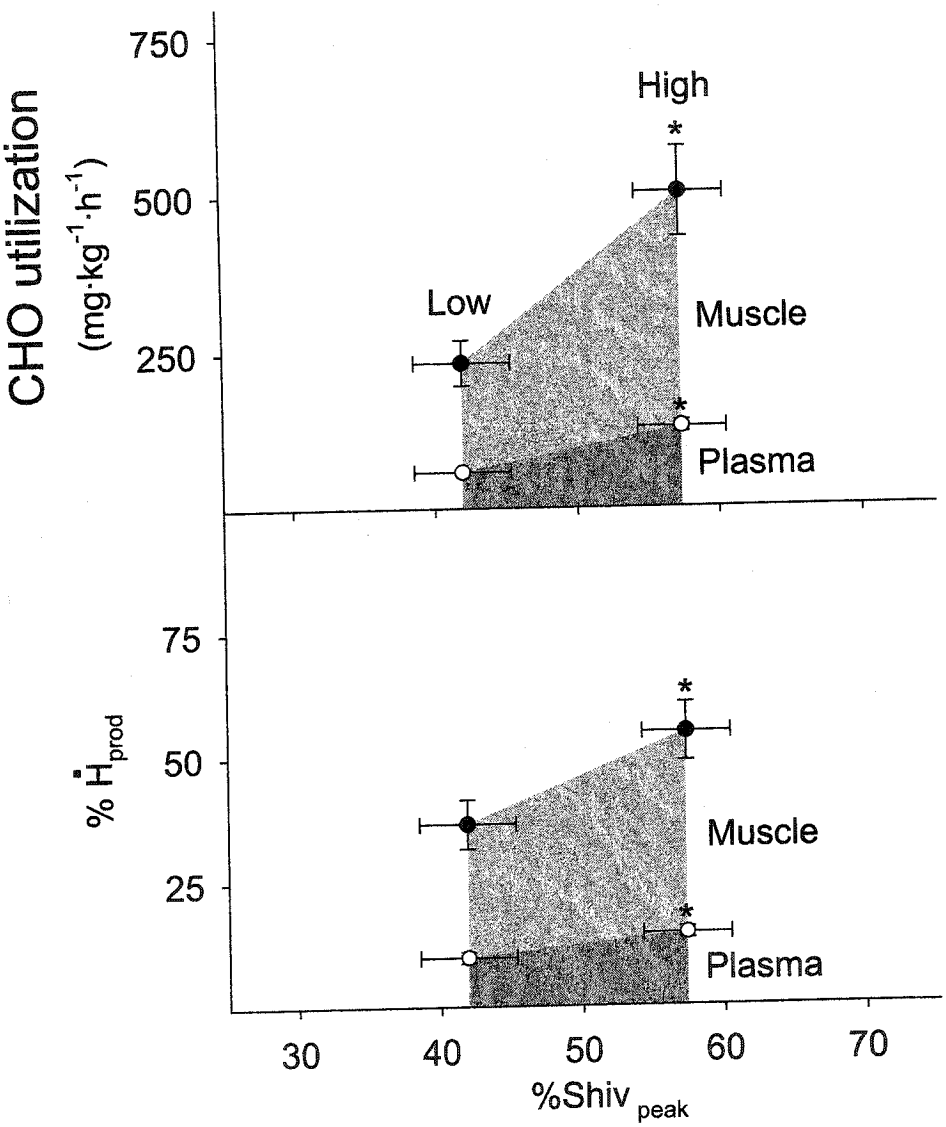

Base
Cold
LOW
HIGH
A
Insulin (pM)
110
100
90
B
Glucose (mM)
5.5
5.0
4.5
C
Lactate (mM)
3
2
1
D
NEFA (nM)
800
600
400
200
0
30
60
90
Time (min)

Figure 5.7. Total CHO (RG_{ox}, closed circles) and plasma glucose ($RG_{ox\text{-}plasma}$, open circles) utilization rates and their relative contribution to total heat production measured after 90 min of low-intensity (Low, n=6) and high-intensity shivering (High, n=8). The difference between RG_{ox} and $RG_{ox\text{-}plasma}$ represents the contribution of muscle glycogen ($RG_{ox\text{-}mus}$). * Significantly different from LOW (Two-way ANOVA with replication and Bonferroni *post-hoc t*-test, $P \leq 0.05$).


CHO utilization
(mg·kg-1·h-1)
750
500
250
High
*
Low
Muscle
*
Plasma
75
50
25
% Ḣprod
*
Muscle
*
Plasma
30
40
50
60
70
%Shiv peak

DISCUSSION

This study quantifies the relative importance of plasma glucose, muscle glycogen, lipids and proteins at low and high thermogenic rates. Even though plasma glucose oxidation rate was strongly stimulated from low- to high-intensity shivering (+122%), results show that this fuel remained a minor source of heat (<15% $\dot{H}_{prod}$) (Table 5.2, Fig. 5.7). In contrast, muscle glycogen was always the main CHO source at least doubling its oxidation rate and providing 75-80% of all the glucose oxidized. Contrary to expectations, lipids were *not* the dominant fuel source during high-intensity shivering. Instead, CHO oxidation increased by as much as 403% and sustained alone the 50% increase in metabolic rate (100W) observed from low- to high-intensity shivering (Table 5.2, Fig. 5.2, 5.4 and 5.5). This early reliance of CHO at such low relative metabolic rates indicates that the patterns of fuel selection are not the same for shivering and exercise.

Blood glucose oxidation

Using a [^{13}C]-glucose ingestion method, this study allowed us to quantify the role of circulatory glucose as an oxidative fuel to support high-intensity shivering. As suggested by the previously reported increase in R_dGLU (Tipton *et al.*, 1997), $RG_{ox\text{-}plasma}$ more than doubled from low- to high-intensity shivering (58 ± 3 mg·kg^{-1}·hr^{-1} to 129 ± 9 mg·kg^{-1}·hr^{-1}) (Table 5.2). However, this substantial increase in $RG_{ox\text{-}plasma}$ had little effect on the relative importance of plasma glucose to total heat production, which

was only increased by 5% (from 10% $\dot{H}_{prod}$ for LO to 15% $\dot{H}_{prod}$ for HIGH, Table 5.2, Fig. 5.7). Maintaining a low contribution of plasma glucose may be key in preventing hypoglycemia, and safeguarding this fuel for glucose-dependent tissues (i.e. brain and nerves) (Thanasis *et al.*, 1996). Most importantly during cold exposure, animal (Cassidy *et al.*, 1925, Cassidy *et al.*, 1926, Dworkin and Finney, 1927, Silva and Boulant, 1984) and human (Gale *et al.*, 1983, Haight and Keating, 1973, Thanasis *et al.*, 1996) studies have reported that hypoglycemia inhibits shivering thermogenesis. For example, Thanasis *et al.* (1996) showed that $\dot{H}_{prod}$ is decreased by 20% in hypoglycemic humans (induced by an insulin clamping method). It is still uncertain whether this inhibitory effect is central (i.e. inhibition of cold-sensitive neurons within the pre-optic anterior hypothalamus) and/or peripheral in nature (i.e. lack of substrate for maintaining shivering). Considering the relatively low metabolic rates reached even at maximal shivering intensity (<45 % $\dot{V}O_{2max}$), it is also unclear whether significant increases in $RG_{ox\text{-}plasma}$ and/or its relative contribution to total $\dot{H}_{prod}$ could result in hypoglycemia. During prolonged high-intensity shivering (3-4 h at ~60-70% $Shiv_{peak}$), Tikuisis *et al.* (2002) found recently that glycemia not only is unaffected by cold exposure but actually increases slightly (+13%). Further research is needed to determine whether a substantial decrease in plasma glucose level could develop following several hours (>5h) or even days of cold exposure.

During low-intensity shivering, the stimulation of $RG_{ox\text{-}plasma}$ was not accompanied by changes in plasma glucose or insulin concentrations indicating that i) R_aGlu and R_dGlu increased in parallel and ii) the increase in glucose uptake was not

dependent upon changes in insulin levels. As discussed previously, this insulin-independent control may allow delivery of glucose specifically to shivering muscles rather than indiscriminately to all insulin-sensitive tissues (Haman *et al.*, 2002). In contrast, during high-intensity shivering, our results show that the increase in $RG_{ox\text{-}plasma}$ was associated with a transient rise in glucose and insulin concentrations (at 60 min and 75 min, respectively) (Fig. 5.4A and B). This increase in glycemia is consistent with another study reporting values during high-intensity shivering (Tikuisis *et al.*, 2002) and indicates a temporary imbalance between R_aGlu and R_dGlu. However, in the only study where these rates were measured directly at high thermogenic rates, no such difference was found and consequently, no change in glucose levels was observed (Tipton *et al.*, 1997). It is also important to note that several other studies have reported changes in glucose and insulin levels during high-intensity shivering (Martineau and Jacobs, 1988, Martineau and Jacobs, 1989a, Martineau and Jacobs, 1989b), but, unfortunately, values were corrected for hemoconcentration making comparisons with this and other studies extremely difficult.

Muscle glycogen oxidation

Muscle glycogen was the major source of CHO during cold exposure. At high thermogenic rate, results show that $RG_{ox\text{-}mus}$ was more than twice as high than for LOW (374 ± 67 vs 175 ± 32 $mg \cdot kg^{-1} \cdot hr^{-1}$) and accounted for 75% of all the glucose oxidized (vs. 80% for LOW) (Table 5.2). Muscle glycogen was also the most important substrate for $\dot{H}_{prod}$ providing more heat than all other metabolic fuels (40% vs 37% for lipids, 15%

for plasma glucose and 8% for proteins) (Table 5.2). This strong mobilization of muscle glycogen for thermogenesis is consistent with previous studies reporting significant decreases in glycogen levels from the *vastus lateralis* after 90 min of high intensity shivering (Jacobs *et al.*, 1985, Martineau and Jacobs, 1988). Substantial increases in muscle glycogen oxidation rates have also been demonstrated during low-intensity shivering in glycogen-depleted (LO) and glycogen-loaded individuals (HI) (Haman *et al.*, 2004b). Even though $RG_{ox\text{-}mus}$ was shown to be more than 2 times lower for LO (~170 $mg \cdot min^{-1}$) than for HI (~380 $mg \cdot min^{-1}$), muscle glycogen remained an important fuel source for heat production (20% and 50% $\dot{H}_{prod}$ in LO and HI, respectively) supplying 75 to 80% of all the CHO oxidized (Haman *et al.*, 2004b). Interestingly, the greatest relative change caused by cold exposure was observed when glycogen reserves were reduced (+445% for LO vs +80% for HI). Unfortunately, the effect of low- and high-CHO stores on the relative importance of $RG_{ox\text{-}plasma}$ and $RG_{ox\text{-}mus}$ has never been quantified during high-intensity shivering. In view of the major role played by muscle glycogen reserves for thermogenesis found herein, further research is needed to address this issue.

Protein oxidation

At both shivering intensities, absolute rates of protein oxidation remained unaffected by cold exposure, and therefore, the 2.3-fold and 3.5-fold increases in metabolic rate observed respectively for LOW and for HIGH resulted in a proportional decrease in the relative contribution of proteins (12% in LOW and 8% in HIGH). However, proteins do not always play such a minor role in heat generation. In glycogen

depleted individuals exposed to low-intensity shivering, previous results have shown that the relative importance of proteins increases substantially (~19% $\dot{H}_{prod}$) (Haman *et al.*, 2004b). Under such a condition, failing to account for proteins oxidation in fuel oxidation budget may lead to a significant overestimation of CHO and lipid oxidation rates. Whether this is also the case at higher thermogenic rates when glycogen reserves are low remains to be established because protein oxidation rate has never been quantified.

Lipid oxidation

The importance of lipid for heat generation has been reported previously during cold exposure (Haman *et al.*, 2002, Jacobs, 1997, Martineau and Jacobs, 1988, Martineau and Jacobs, 1989a, Martineau and Jacobs, 1989b, Tikuisis *et al.*, 2002, Tikuisis *et al.*, 2000b, Weller *et al.*, 1998). Here, results show that the increase in RF_{ox} was similar for LOW and HIGH (230% vs 300%) resulting in almost identical oxidation rates after 90 min in the cold (133±22 for LOW vs 138±25 $mg \cdot kg^{-1} \cdot min^{-1}$) (Table 5.2). Clearly, however, RF_{ox} is not limited to this 3-4 fold increase because much higher oxidation rates have been reported during exercise (~13-fold increase or ~600 $mg \cdot min^{-1}$) (Achten *et al.*, 2002). Exact reasons why RF_{ox} was not stimulated further to compensate for the increase in metabolic rate from LOW to HIGH, instead of switching to CHO based metabolism, should be addressed in future studies.

Stimulation of RF_{ox} (Table 5.2) for LOW and HIGH was also accompanied by a respective 2 and 1.5-fold increase in circulatory NEFA levels (Fig. 5.4D). Such changes

are consistent with several other studies where RF_{ox} was increased during cold exposure (Haman *et al.*, 2002, Tipton *et al.*, 1997, Vallerand *et al.*, 1995, Vallerand and Jacobs, 1990, Vallerand and Jacobs, 1992, Vallerand *et al.*, 1999b, Weller *et al.*, 1998) and may reflect the link found previously between plasma NEFA concentration and NEFA flux (Vallerand *et al.*, 1999a).

Contrary to expectation, lipids were *not* the main fuel source during high intensity shivering accounting for only 37% of all the heat produced (vs 55% $\dot{H}_{prod}$ for CHO and 8% for proteins, Table 5.2). This finding contradicts previous results obtained during high intensity shivering where this fuel was shown to provide 60% of all the heat generated (Jacobs, 1997, Martineau and Jacobs, 1988, Martineau and Jacobs, 1989a, Martineau and Jacobs, 1989b, Tikuisis *et al.*, 2002, Tikuisis *et al.*, 2000b). Interestingly, such a discrepancy in fuel use is also observed during low-intensity shivering. Our results show that RFox provides 52% of all the heat generated for LOW (vs 37% $\dot{H}_{prod}$ for CHO and 12% $\dot{H}_{prod}$ for proteins, Table 5.2). This dominance on lipids is consistent with another study (Weller *et al.*, 1998) while most others report a higher reliance on CHO (Glickman-Weiss *et al.*, 1993, Glickman-Weiss *et al.*, 1994, MacNaughton *et al.*, 1990, Vallerand *et al.*, 1995, Vallerand and Jacobs, 1989, Vallerand and Jacobs, 1990, Vallerand *et al.*, 1989, Vallerand *et al.*, 1993, Vallerand *et al.*, 1999a). Physiological reasons for such discrepancies in fuel use are unknown but, as mentioned previously (see Introduction), may be related to differences in cooling protocol (Haman *et al.*, 2002) and/or nutritional state (Haman *et al.*, 2004b, Martineau and Jacobs, 1989b, Young *et al.*, 1989). For example, previous experiments in glycogen-depleted and glycogen-loaded

have shown that humans possess a remarkable flexibility in oxidative fuel selection to ensure that heat production is not compromised during cold exposure, even at high thermogenic rates (Haman *et al.*, 2004b, Martineau and Jacobs, 1989b, Young *et al.*, 1989). In the present study, a series of precautions were taken to minimize the effects of modifications in CHO availability, and possibly cooling procedure, on fuel selection during cold exposure. All individuals were submitted to the same pre-experimental exercise and dietary manipulations and cooled in the same manner using a combination of LCS and thermal chamber. Experiments were also conducted at the same time of the day in 12-h post-absorptive subjects. Finally, cold response was normalized as much as possible by selecting men of comparable age, morphology (surface to volume ratio) and body composition (percent body fat). These precautions were sufficient to elicit similar changes in metabolic rate among subjects: a 2.3-fold increase for LOW and 3.3-fold increase for HIGH.

Early reliance on CHO for thermogenesis

During high intensity shivering, RG_{ox} increased by more than 2-fold over values measured for LOW and accounted for more heat than all the other metabolic substrates combined (55 % $\dot{H}_{prod}$)(Table 5.2, Fig. 5.4 and 5.5). This early reliance on CHO was unexpected because i) all other studies had suggested that lipids were the primary fuel source during high intensity shivering and, ii) exercise studies had shown that lipids generally predominate at such low relative metabolic rates (<50% $\dot{V}O_{2max}$) (Bergman and Brooks, 1999, Brooks *et al.*, 1999, Roberts *et al.*, 1996). Figures 5.8 and 5.9 compare

absolute oxidation rates and relative contribution of plasma glucose, muscle glycogen, total CHO and lipids measured herein for LOW and HIGH to values obtained by van Loon *et al.*, (2001) at different exercise intensities. Even though this comparison reveals important distinctions between shivering and exercise, it also provides some striking similarities. As anticipated, $RG_{ox\text{-}plasma}$, $RG_{ox\text{-}mus}$, RG_{ox} and RF_{ox} were ~2 to 7 times lower during shivering than during exercise in accordance with the much lower metabolic rates reached in the cold (Fig. 5.8A and 5.9A). More interestingly, the switch from CHO dominance to lipid dominance (or "crossover concept" (Brooks and Mercier, 1994)) occurs at an absolute metabolic rate 4 times lower during shivering than during exercise (Fig. 5.9B). This observation represents a crucial distinction between both types of muscle work and provides strong evidence that patterns of fuel selection are not identical during shivering and exercise. Independently of these important differences, some interesting similarities in fuel selection were unexpected. At all shivering and exercise intensities, muscle glycogen is the main CHO source accounting for 70 to 80%RG_{ox} while the contribution of plasma glucose is always minor (10 to 18% of total energy expenditure, Fig. 5.8B and 5.9B). When the relative importance of plasma glucose, muscle glycogen, total CHO and lipids to total heat production are plotted as a function of $\%Shiv_{peak}$ or $\%VO_2max$, the similarity is even more remarkable (Fig. 5.8C and 5.9C). The relative contribution of these fuels to total energy expenditure at a given relative metabolic rate is almost identical during shivering and during exercise. If one considers the extremely different ranges of metabolic rates reached during shivering (100-500 W)

and exercise (100-2000 W), this observation is even more perplexing. Additional research is clearly needed to uncover the principles that govern fuel selection in the cold.

Conclusion

This study shows that total $\dot{H}_{prod}$ during high intensity shivering is unequally shared among CHO (55%), muscle glycogen (40%), lipids (37%), circulatory glucose (15%), and proteins (8%). While the importance of plasma glucose oxidation was increased at higher thermogenic rates, the relative contribution of this fuel remained low (<15% $\dot{H}_{prod}$). Contrary to expectations, total CHO oxidation played a significant thermogenic role, even at such low relative metabolic rates. This indicates that fuel selection patterns between shivering and exercise are not identical and future work should focus on the processes of fuel selection during cold exposure.

Figure 5.8. Comparison of A. absolute rates and B. relative contributions of plasma glucose and muscle glycogen to total heat production during low- (LOW, n=6) and high-intensity shivering (HIGH, n=8) with values reported previously at 3 different exercise intensities (van Loon *et al.*, 2001). C. Relative contributions of plasma glucose and muscle glycogen as a function of the relative heat production rate for shivering (filled line) and exercise (dotted line).

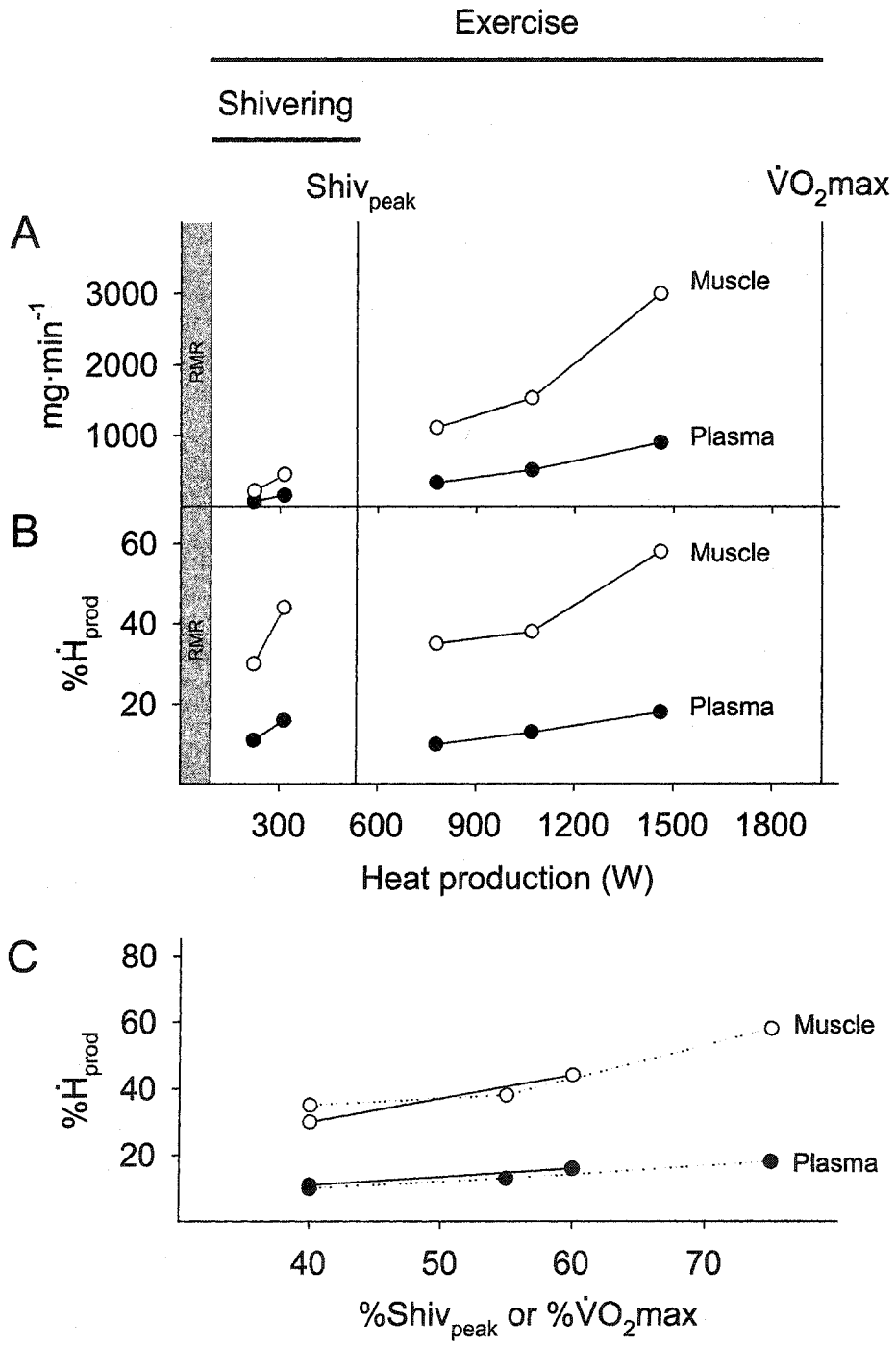
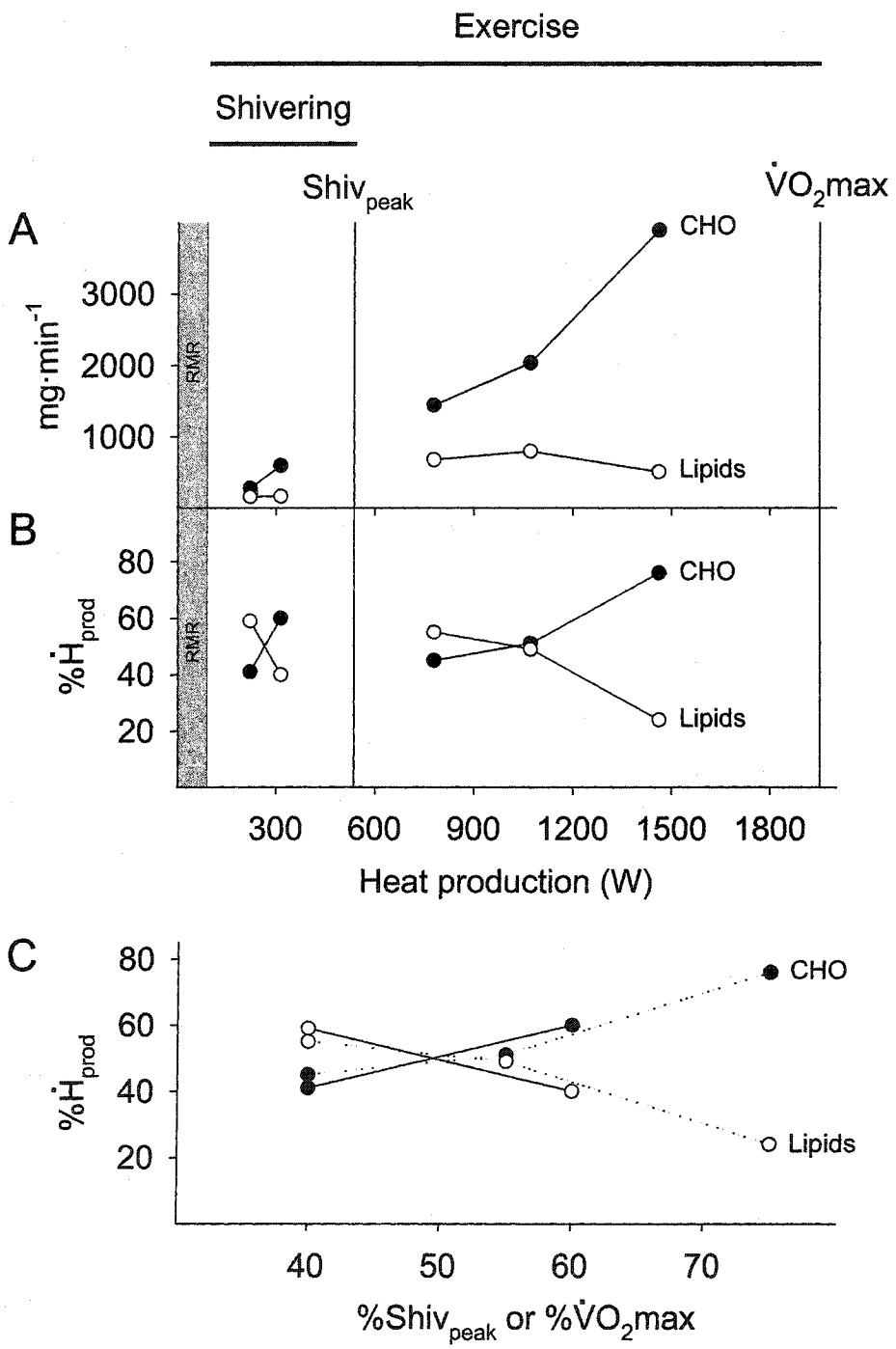

Exercise
Shivering
Shiv$_{peak}$
$\dot{V}O_2$max
A
mg·min^{-1}
3000
2000
1000
RMR
Muscle
Plasma
B
%$\dot{H}_{prod}$
60
40
20
RMR
Muscle
Plasma
300
600
900
1200
1500
1800
Heat production (W)
C
%$\dot{H}_{prod}$
80
60
40
20
Muscle
Plasma
40
50
60
70
%Shiv$_{peak}$ or %$\dot{V}O_2$max

Figure 5.9. Comparison of A. absolute rates and B. relative contributions of total carbohydrates (CHO) and lipids to total heat production during low- (LOW, n=6) and high-intensity shivering (HIGH, n=8) with values reported previously at 3 different exercise intensities (van Loon *et al.*, 2001). C. Relative contributions of CHO and lipids as a function of the relative heat production rate for shivering (filled line) and exercise (dotted line).


Exercise
Shivering
Shiv$_{peak}$
$\dot{V}O_2$max
A
mg·min^{-1}
3000
2000
1000
RMR
CHO
Lipids
B
%$\dot{H}_{prod}$
80
60
40
20
RMR
CHO
Lipids
300
600
900
1200
1500
1800
Heat production (W)
C
%$\dot{H}_{prod}$
80
60
40
20
CHO
Lipids
40
50
60
70
%Shiv$_{peak}$ or %$\dot{V}O_2$max

CHAPTER 6. FUEL SELECTION DURING INTENSE SHIVERING: EMG PATTERN REFLECTS CARBOHYDRATE OXIDATION

Based in part on

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INTRODUCTION

Shivering is essential for human survival in the cold and this thermogenic response depends critically on coordinating muscle fiber recruitment and oxidative fuel metabolism. Research on cold exposure has included electromyographic studies (EMG) (Haman *et al.*, 2002, Jacobs *et al.*, 1994) and measurements of energy metabolism in shivering muscles (Bell *et al.*, 1992, Meigal, 2002, Tikuisis *et al.*, 2000a), but these two complementary approaches have not been traditionally integrated (Haman *et al.*, 2004b). In active muscles, fuel selection can either be achieved by mobilizing different metabolic pathways within the same fibers, or by recruiting distinct fiber populations specialized for different fuels. Two EMG patterns associated with the recruitment of specific motor units (MU) have been identified during shivering: i) continuous, low-intensity shivering at 4-8 Hz (or thermogenic muscle tone), and, ii) bursts of high-intensity shivering at much lower frequencies (0.1-0.2 Hz) (Israel and Pozos, 1989, Meigal, 2002, Meigal *et al.*, 1993, Petajian and Williams, 1972). While continuous, low-intensity shivering is linked to low-threshold MU (type I, slow-oxidative, fatigue-resistant), high-intensity bursts are associated with high-threshold MU (type II, fast-glycolytic, fatigable). No information is available on the physiological significance of this dual pattern in relation to fuel selection or thermogenic rate. Adjusting the relative importance of *low-intensity shivering* and *shivering bursts* may be a key mechanism to modify oxidative fuel mix or total heat production.

Through simultaneous measurements of EMG and fuel metabolism during mild shivering, we have recently shown that glycogen-depleted and glycogen-loaded humans

can sustain the same thermogenic rate by oxidizing widely different fuel mixtures within the same muscle fibers (Haman *et al.*, 2004a). Drastic changes in fuel metabolism were achieved predominantly within type I, slow oxidative fibers (28 vs 65% of total heat production ($\dot{H}_{prod}$) from carbohydrates for glycogen-depleted and glycogen–loaded subjects, respectively; 53 vs 23% $\dot{H}_{prod}$ from lipids, and 19 vs 12% $\dot{H}_{prod}$ from proteins). However, it is not clear whether this fuel selection strategy is also used during high intensity shivering, rather than recruiting different populations of fuel-specific fibers. For example, slow-oxidative fibers used predominantly during mild shivering may not be able to produce enough heat for intense shivering, and higher heat production rates could require the recruitment of fast fibers specialized for carbohydrates.

The primary goal of this study was to determine whether the EMG pattern of muscle recruitment could provide metabolic information on oxidative fuel selection during high-intensity shivering. More specifically, we have simultaneously monitored burst shivering activity (in 8 large muscles representing >90% of total shivering muscle mass) and fuel metabolism during intense shivering. We hypothesized that burst shivering rate would be correlated with the oxidation of CHO for thermogenesis.

METHODS

Subjects

Eight healthy, trained men volunteered for this study approved by the Health Sciences Ethical Committee of the University of Ottawa and written consent was obtained from the participants. Physical characteristics of the subjects have been presented previously (Chapter 5, Table 5.1). Percent body fat (underwater weighing; Brosek *et al.*, (1963)) and maximal oxygen consumption (using a progressive treadmill protocol) were measured 5-7 days before the experiments.

Experimental protocol

Experiments were conducted between 8:00 and 12:00, following 36-h without heavy physical activity. The last evening meal was standardized (~950 kJ, ~51 % CHO, ~27 % lipids, ~22 % proteins) and subjects were asked to report to the laboratory the next morning (8:00 AM) after a 12-14h fast. Care was taken to minimize thermal stimuli between awakening and the start of the experiment. Upon arrival in the laboratory, subjects were instrumented with thermal probes and fitted with a LCS (One-piece CORETEC, Delta Temax, Inc. Pembroke, ON, Canada). EMG collection sites, located on the right side of the body, were shaved and cleaned using an ethanol swab. Following skin preparation, disposable surface electrodes (Blue Sensor, Medicotest Inc., USA) were placed 2 cm apart over the bellies of each muscle – parallel to the direction of muscle fibers - and secured in place with medical transpore tape (3M Canada, London, ON, CANADA). Shivering EMG was collected from eight muscles: *trapezius* (TR),

latissimus dorsi (LA), *pectoralis major* (PE), *rectus abdominis* (RA), *vastus lateralis* (VL), *rectus femoris* (RF), *vastus medialis* (VM) and *adductor magnus* (AD). Pre-amplified and grounded EMG wires (375X) were connected to the surface electrode of each muscle to a ME-3000 Professional EMG system (Mega Electronics, Kuopio, Finland). Subjects were then asked to empty their bladder (t = 0 min) and sit quietly for two hours at 25.5 ± 0.2°C (759 ± 2 mmHg, 45 ± 4% RH). Following this habituation period, they were transferred to an environmental chamber (5.7 ± 0.1°C, 759 ± 2 mmHg, 69 ± 2% RH) and a 5°C water perfusion was started through the LCS using a temperature-controlled circulation bath (Endocal, NESLAB and Model 200-00, Micropump, Vancouver, WA). Thermal, metabolic and electrophysiological parameters were quantified at 26°C (baseline) and during 90 min at 5°C.

Thermal response

Central body temperature (T_{es}) was monitored continuously using a pediatric esophageal temperature probe (Mon-a-therm general purpose, Mallinckrodt Medical Inc, St-Louis, MO) which was inserted through the nose to a depth placing the tip of the thermocouple at the level of the left atrium, or one quarter of the standing height of the subject (Mekjavic and Rempel, 1990). Heat flux transducers (Concept Engineering, Old Saybrook, CT) were used to estimate mean skin temperature ($\overline{T}_{skin}$) and non-evaporative heat flux from the forehead, chest, biceps, forearm, abdomen, lower and upper back, front and back calf, quadriceps, hamstrings and finger. $\overline{T}_{skin}$ and mean heat flux were calculated using an area-weighted equation (Dubois and Dubois, 1916) as described

previously (Chapter 2). Heat flux measurements were used to calculate whole body $\dot{R}$ and $\dot{C}$. $\dot{E}_{resp}$ and $\dot{C}_{resp}$ were determined from ventilation measurements by estimating water loss via the respiratory tract (2,411.3 J of heat per gram of evaporated water) (Brooks et al., 1999). It was assumed that evaporative heat loss from the skin was negligible under the LCS. Whole-body $\dot{H}_{loss}$ (in watts) was calculated as follows:

$$\dot{H}_{loss} = (\dot{R} + \dot{C}) + (\dot{E}_{resp} + \dot{C}_{resp}). \quad (6.1)$$

Metabolic rate and fuel selection

$\dot{V}O_2$ and $\dot{V}CO_2$ were determined by open-circuit spirometry (250 l, chain-compensated gasometer, Warren Collins inc., Braintree, MA). Expired gases were collected for 5 min, every 15 min before and during cold exposure. Oxygen and carbon dioxide concentrations in dry expired gases were determined using calibrated electrochemical gas analyzers (AMETEK Model S-3A/1 and CD 3A, Applied Electrochemistry, Pittsburg, PA). Total RP_{ox}, RG_{ox} and RF_{ox} oxidation rates (in $g \cdot min^{-1}$) were calculated using the following equations (Livesey and Elia, 1988):

$$RP_{ox}(g \cdot min^{-1}) = 2.9 \times UREA_{urine}(g \cdot min^{-1}) \quad (6.2)$$

$$RG_{ox}(g \cdot min^{-1}) = 4.59\,\dot{V}CO_2(l \cdot min^{-1}) - 3.23\,\dot{V}O_2(l \cdot min^{-1}) \quad (6.3)$$

$$RF_{ox}(g \cdot min^{-1}) = -1.70\,\dot{V}CO_2(l \cdot min^{-1}) + 1.70\,\dot{V}O_2(l \cdot min^{-1}) \quad (6.4)$$

where $\dot{V}CO_2$ ($l \cdot min^{-1}$) and $\dot{V}O_2$ ($l \cdot min^{-1}$) were corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1.010 and 0.843 $l \cdot g^{-1}$, respectively).

RP_{ox} was estimated from $UREA_{urine}$ in urine samples collected for a period of 90 min at 26°C and 5°C. Urinary concentrations were determined on a Synchron Clinical System (CX7, Beckman, Anaheim, CA). Energy potentials of 16.3 $kJ \cdot g^{-1}$, 40.8 $kJ \cdot g^{-1}$ and 19.7 $kJ \cdot g^{-1}$ were used to calculate the relative contributions of CHO ($\%RG_{ox}$), lipid ($\%RF_{ox}$) and protein ($\%RP_{ox}$) oxidation to total heat production, respectively (Elia, 1991, Péronnet and Massicotte, 1991).

EMG analysis

Raw EMG signals were collected at 1000 Hz, filtered to remove spectral components below 20 Hz and above 500 Hz as well as 60Hz contamination and analyzed using custom-designed MATLAB algorithms (Mathworks, Natick, MA, USA). EMG amplitude of individual muscles was determined based on root-mean-square values (RMS) calculated from EMG signals using a 50 ms overlapping-window (50%). Baseline RMS values ($RMS_{baseline}$: 15 min RMS average measured prior to cold exposure) were subtracted from shivering RMS values.

Burst shivering rate was determined as described previously (Haman *et al.*, 2004a). A shivering burst was defined as an EMG interval with a duration > 0.2 s, an inter-burst interval > 0.75 s and an amplitude higher than the amplitude threshold of the given recording period. Amplitude threshold for the identification of shivering bursts was done by: i) averaging shivering intensity ($\overline{A}_{EMG}$) over the entire recording period, ii) averaging the remaining values above $\overline{A}_{EMG}$ ($\overline{B}_{EMG}$) and, iii) setting the amplitude

threshold at $\overline{B}_{EMG}$. At each sampling interval, burst rate was calculated by dividing the number of bursts by total interval recording time.

Calculations and statistical analysis

Theoretical estimates of maximal time before muscle glycogen depletion ($GLY_{depletion}$) were calculated assuming that: i) the relative use of the different fuels would remain the same as after 90 min of shivering, ii) 80% of total muscle glycogen is available for oxidation, iii) active muscle mass during shivering is 70% of 36 kg, iv) mean muscle glycogen concentrations are 100 mmol glucosyl units/ kg wet mass as observed in Martineau *et al.*, (1989b) and, v) muscle glycogen oxidation rates range between 6.8 and 56.4 μmol·kg body $mass^{-1}\cdot min^{-1}$ based on unpublished data collected on this group of subjects. Overall changes in T_{es}, $\overline{T}_{skin}$, $\dot{H}_{loss}$, $\dot{H}_{prod}$ were assessed using a one-way analysis of variance (ANOVA) with replication. For each sampling time, a Bonferroni t-test was used to detect potential differences from control values observed at 26°C. Stepwise regression analyses were performed to determine the best predictors of changes in rates of CHO and lipid utilization. Statistical differences were considered significant when $p<0.05$. Values presented are means ± SE ($n = 8$).

RESULTS

Thermal response

Changes in $\dot{H}_{loss}$, $\dot{H}_{prod}$, T_{es} and $\overline{T}_{skin}$ are presented in Fig. 6.1. $\dot{H}_{loss}$ increased from 75.1 ± 3.9 W at 26°C to 315.1 ± 14.3 W during the first 10 min in the cold, and decreased thereafter to reach 289.1 ± 13.7 W by the end of the experiment. $\dot{H}_{prod}$ increased progressively from 89.5 ± 3.5 W at 26°C to 292.1 ± 18.2 W by the end cold exposure. A transient increase in T_{es} was observed in the first 60 min of cold exposure (36.6 ± 0.1°C at 26°C to 37.0 ± 0.1°C) before stabilizing at a value not different from baseline levels (36.8 ± 0.2°C). $\overline{T}_{skin}$ decreased by 25% averaging 34.2 ± 0.2°C at 26°C and 25.8 ± 0.4°C by the end of cold exposure.

Fuel selection: inter-individual variability

Table 6.1 summarizes individual values for metabolic rate ($\dot{V}O_2$, $\%Shiv_{peak}$) and relative contributions of CHO and lipids to total heat production measured in the last 15 min at 5°C (T=75-90 min). A large variability was observed in the relative changes in CHO and lipid oxidation rates from baseline values to the end of cold exposure. Depending on the subject, CHO oxidation rate increased 1.9 to 9.3-fold whereas lipid oxidation did not change or increased by as much as 23.4-fold. Average CHO and lipid oxidation were respectively 2.0 ± 0.2 $mg \cdot kg^{-1} \cdot min^{-1}$ and 0.6 ± 0.1 $mg \cdot kg^{-1} \cdot min^{-1}$ at 26°C and 8.9 ± 1.3 $mg \cdot kg^{-1} \cdot min^{-1}$ and 2.3 ± 0.4 $mg \cdot kg^{-1} \cdot min^{-1}$. Total amount of CHO used over the entire cold exposure (in $mmol \cdot kg^{-1}$ for T= 0 to 90 min) and $GLY_{depletion}$ (in hours) for

each subject are presented in Fig. 6.2. Total CHO use averaged 3.1 ± 0.5 mmol·kg^{-1} but was extremely variable among subjects ranging between 1.5 to 5.1 mmol·kg^{-1} (Fig. 6.2A) whereas, estimates of $GLY_{depletion}$ ranged between 7 and 50 h (Fig. 6.2B). Relationships between metabolic rate (based on $\dot{V}O_2$ measurements) and absolute utilization as well as relative contribution of CHO to total heat production are presented in Fig. 6.3. The large variation in CHO oxidation observed during high intensity shivering was not correlated with differences in metabolic rate.

EMG pattern and fuel selection

The relationship between mean burst shivering rate (averaged for the 8 muscles) and RG_{ox} are presented in Fig. 6.4. While mean burst shivering rate covaried with RG_{ox} (Fig. 6.4A), no significant correlation could be established for $\%RG_{ox}$ ($p=0.07$; Fig. 6.4B). Table 6.2 summarizes the correlation coefficients between BR and RG_{ox} and RF_{ox} for individual muscles. While no relationship between BR and RF_{ox} was observed, close correlations between BR and RG_{ox} were found for RA, VL, RF and VM, but not TR, LA, PE and AD. Correlations between mean BR averaged by body regions (upper trunk, lower trunk and upper leg) and RG_{ox} are presented in Fig. 6.5. While mean BR of upper trunk (TR, PE and LA; Fig. 6.5A) was not related with RG_{ox}, BR for lower trunk (RA; Fig. 6.5B) and upper leg muscles (average of VL, RF, VM and AD; Fig. 6.5C) were correlated with differences in RG_{ox}. Correlations between mean BR for muscles showing the highest correlation coefficients with RG_{ox} (RA, VL, RF and VM, Table 6.2) or $\%RG_{ox}$ are presented in Fig. 6.6.

Table 6.1. Metabolic rate (VO_2), shivering intensity (%$Shiv_{peak}$), CHO (%CHO) and lipid (%FAT) oxidation rate in men during sustained high-intensity shivering (~60%$Shiv_{peak}$). Physical caracteristics including body mass (Mb), percent body fat (BF), body surface area (BSA), maximal aerobic capacity (VO_2max) and maximal shivering capacity ($Shiv_{peak}$) are also presented.

Subjects	Mb (kg)	Height (cm)	Age (y)	BF (%)	BSA (m^2)	VO2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	Shivpeak* ($ml \cdot kg^{-1} \cdot min^{-1}$)	VO2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	%$Shiv_{peak}$	%CHO	%FAT
1	81.0	178	23	13.4	1.99	59.0	22.4	10.6	47.2	49.5	38.6
2	59.5	176	23	13.4	1.73	59.9	28.5	14.0	49.1	78.0	14.1
3	68.8	170	23	15.7	1.79	46.6	19.7	10.9	55.0	39.8	50.3
4	83.4	180	23	15.1	2.03	45.6	17.6	13.4	75.9	71.5	25.3
5	64.7	170	22	10.9	1.74	43.2	20.1	11.1	55.6	65.1	25.9
6	73.4	172	25	12.7	1.86	52.9	20.5	12.1	59.2	40.6	48.3
7	77.0	174	22	10.2	1.91	58.0	23.0	13.1	56.9	61.8	32.5
8	65.9	173	25	9.9	1.78	59.5	25.4	15.3	60.2	32.5	59.2
Mean	71.7	174	23.3	12.7	1.85	53.1	22.2	12.6	57.4	54.8	36.8
S.E. (n=8)	3.0	1.3	0.4	0.8	0.04	2.5	1.2	0.6	3.1	5.9	5.4

*$Shiv_{peak}$ was calculated as described by Eyolfson *et al*. (2001); $Shiv_{peak} = 30.5 + (0.348 \times \dot{V}O_{2max}) - (0.909 \times BMI) - (0.233 \times age)$

where BMI is the body mass index (weight/height^2)

Table 6.2. Pearson correlations between burst shivering rate and CHO and lipid (FAT) utilization rates for individual muscles

EMG sites	CHO		FAT
trapezius	0.14		0.09
latissimus dorsi	0.58		0.09
pectoralis major	-0.27		0.42
rectus abdominis	0.79	*	-0.52
vastus lateralis†	0.84	*	-0.65
rectus femoris	0.79	*	-0.43
vastus medialis†	0.87	*	-0.67
adductor magnus	0.09		-0.23

* Correlation is significant at $p < 0.05$ (Stepwise regression analyses)

† Best predictors of CHO utilization rate

Figure 6.1. Changes in A. heat loss ($\dot{H}_{loss}$) and heat production rates ($\dot{H}_{prod}$) as well as in B. esophageal (T_{es}) and mean skin ($\overline{T}_{skin}$) temperature at 26°C and during a 5°C cold exposure. Dotted lines indicate ±SE * Significantly different from baseline values at 26°C (One-way ANOVA with replication and Bonferroni *post-hoc* *t*-test, $P \leq 0.05$, n=8).

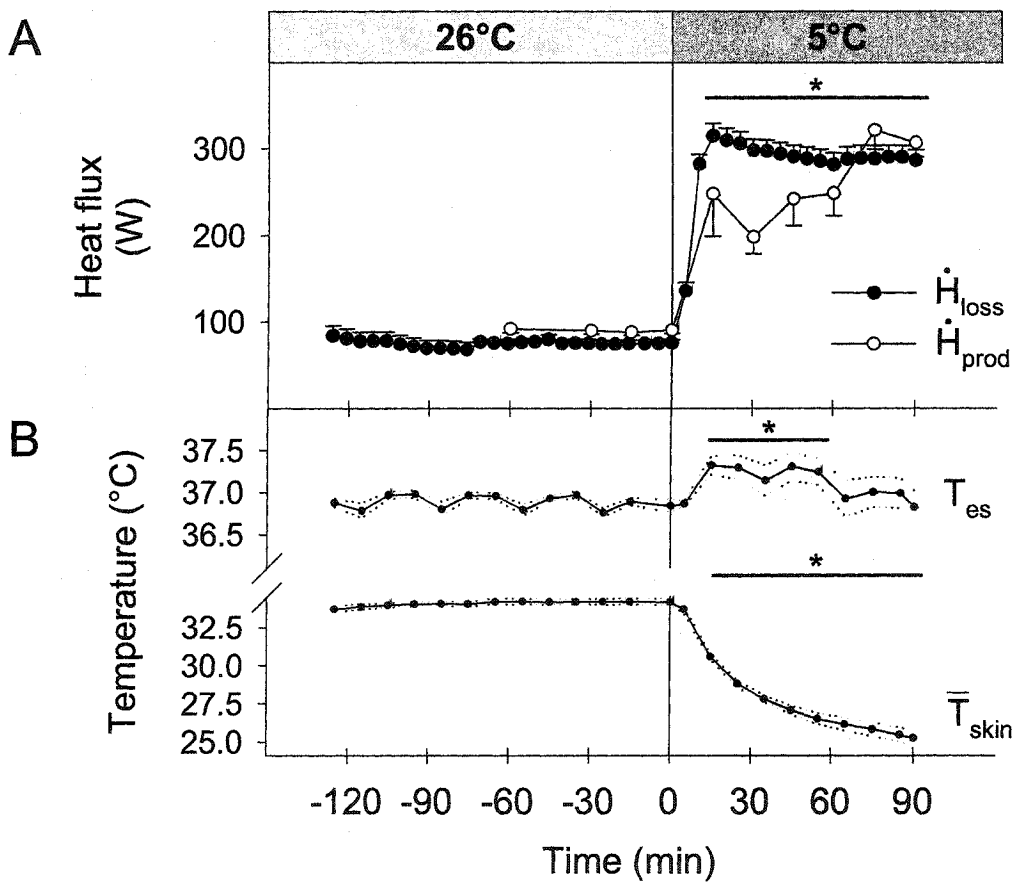
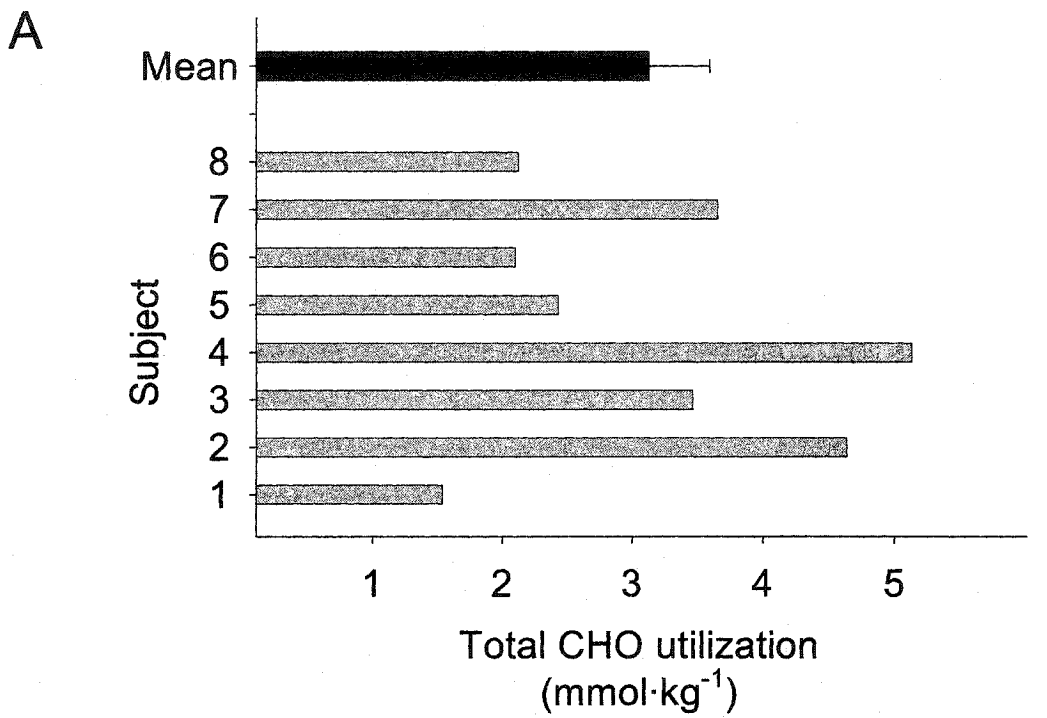

A
26°C
5°C
*
300
200
100
Heat flux (W)
$\dot{H}_{loss}$
$\dot{H}_{prod}$
B
37.5
37.0
36.5
*
T_{es}
*
32.5
30.0
27.5
25.0
$\overline{T}_{skin}$
Temperature (°C)
-120 -90 -60 -30 0 30 60 90
Time (min)

Figure 6.2. A. Average and individual total CHO utilization over the entire 90 min at 5°C and, B. theoretical estimates of the time before muscle glycogen depletion ($GLY_{depletion}$) under the conditions of our experiments, assuming that the relative use of the different fuels remains the same as measured after 90 min of high intensity shivering. An exponential regression line is also presented (black line). Subject numbers are the same as in Table 6.1.


A
Mean
8
7
6
5
4
3
2
1
Subject
1
2
3
4
5
Total CHO utilization
(mmol·kg-1)

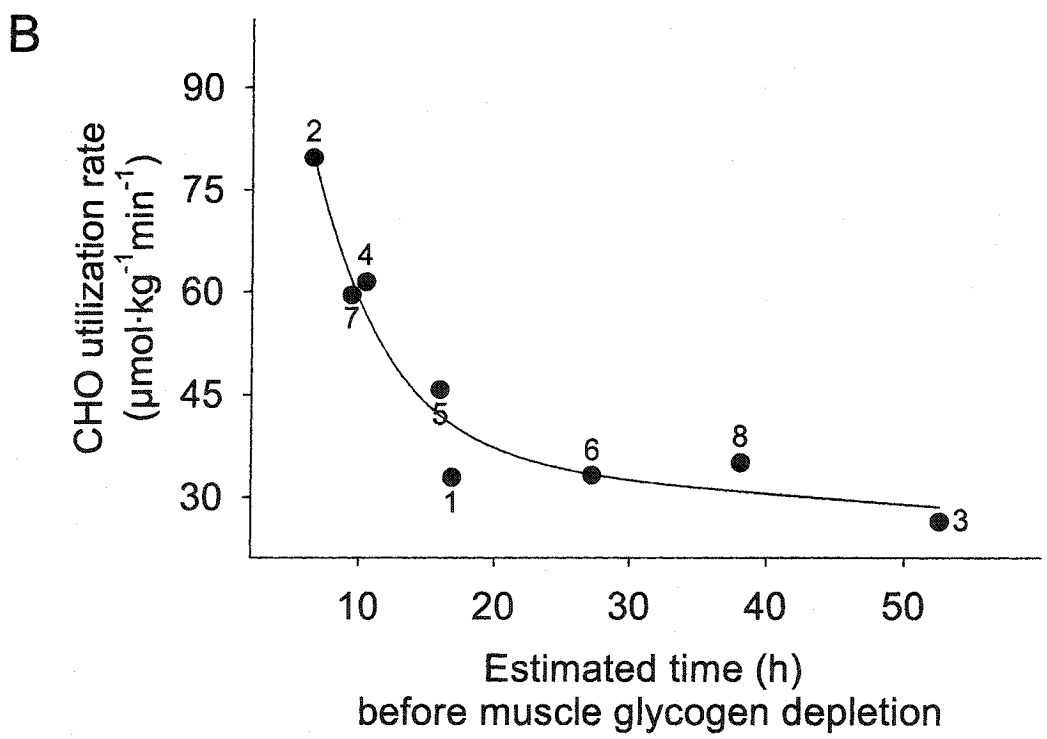

B
90
75
60
45
30
CHO utilization rate
(μmol·kg-1min-1)
2
4
7
5
1
6
8
3
10
20
30
40
50
Estimated time (h)
before muscle glycogen depletion

Figure 6.3. Relationship between shivering intensity ($\dot{V}O_2$) and the absolute oxidation rate (A, RG_{ox}) as well as the relative contribution of CHO to total heat production (B, $\%RG_{ox}$) in a group of men exposed to 5°C for 90 min. Dotted lines indicate the 95% confidence interval for the linear regression. Values were averaged in the last 15 min of cold exposure (T = 75-90 min).

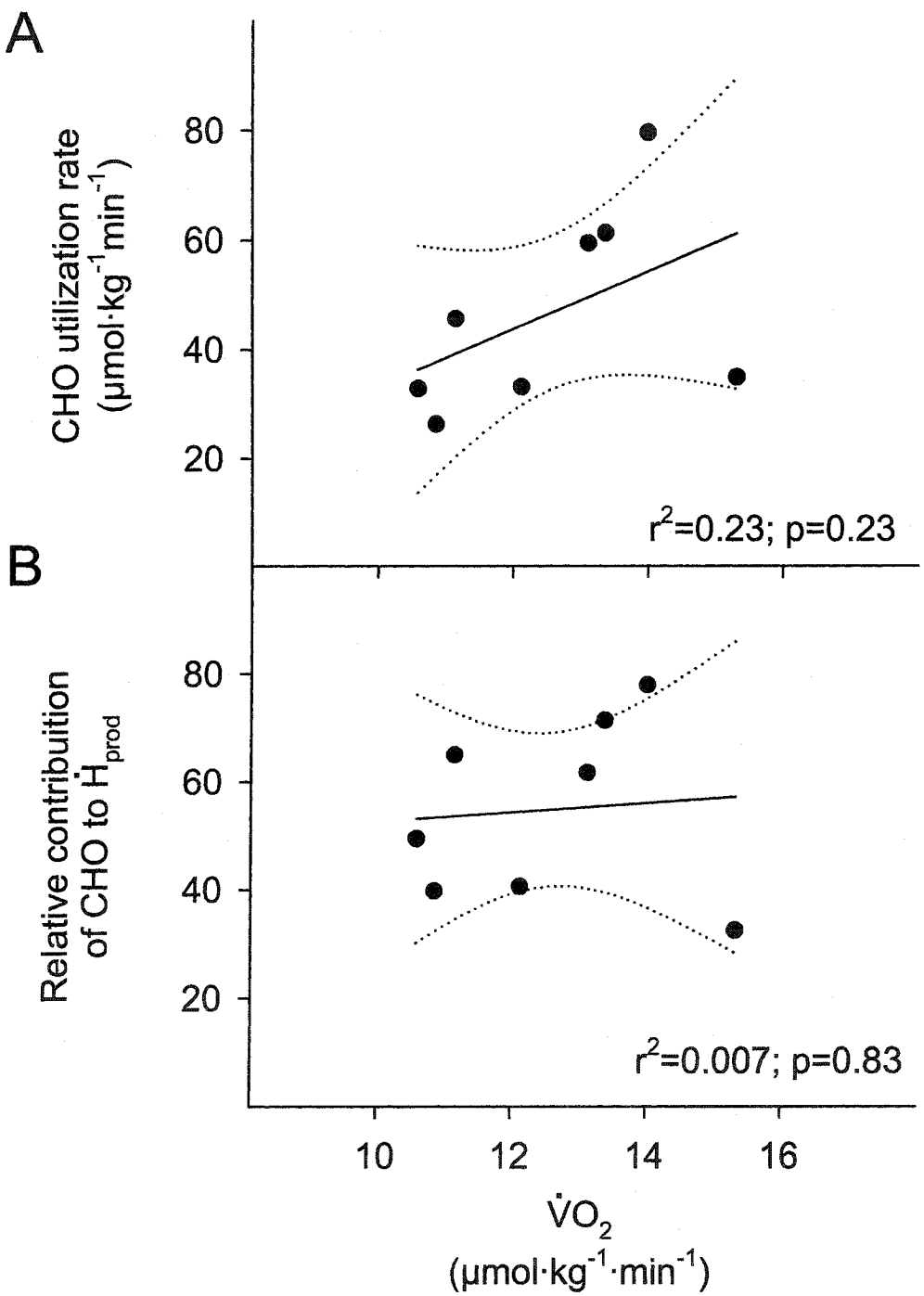

A
CHO utilization rate
(μmol·kg-1min-1)
80
60
40
20
r2=0.23; p=0.23
B
Relative contribuition
of CHO to Ḣprod
80
60
40
20
r2=0.007; p=0.83
10
12
14
16
V̇O2
(μmol·kg-1·min-1)

Figure 6.4. Relationship between burst shivering rate (BR, average of the 8 sampling sites) and the absolute oxidation rate (A, RG_{ox}) as well as the relative contribution of CHO to total heat production (B, $\%RG_{ox}$) in a group of men exposed to 5°C for 90 min. Dotted lines indicate the 95% confidence interval for the linear regression. Values were averaged in the last 15 min of cold exposure (T = 75-90 min).

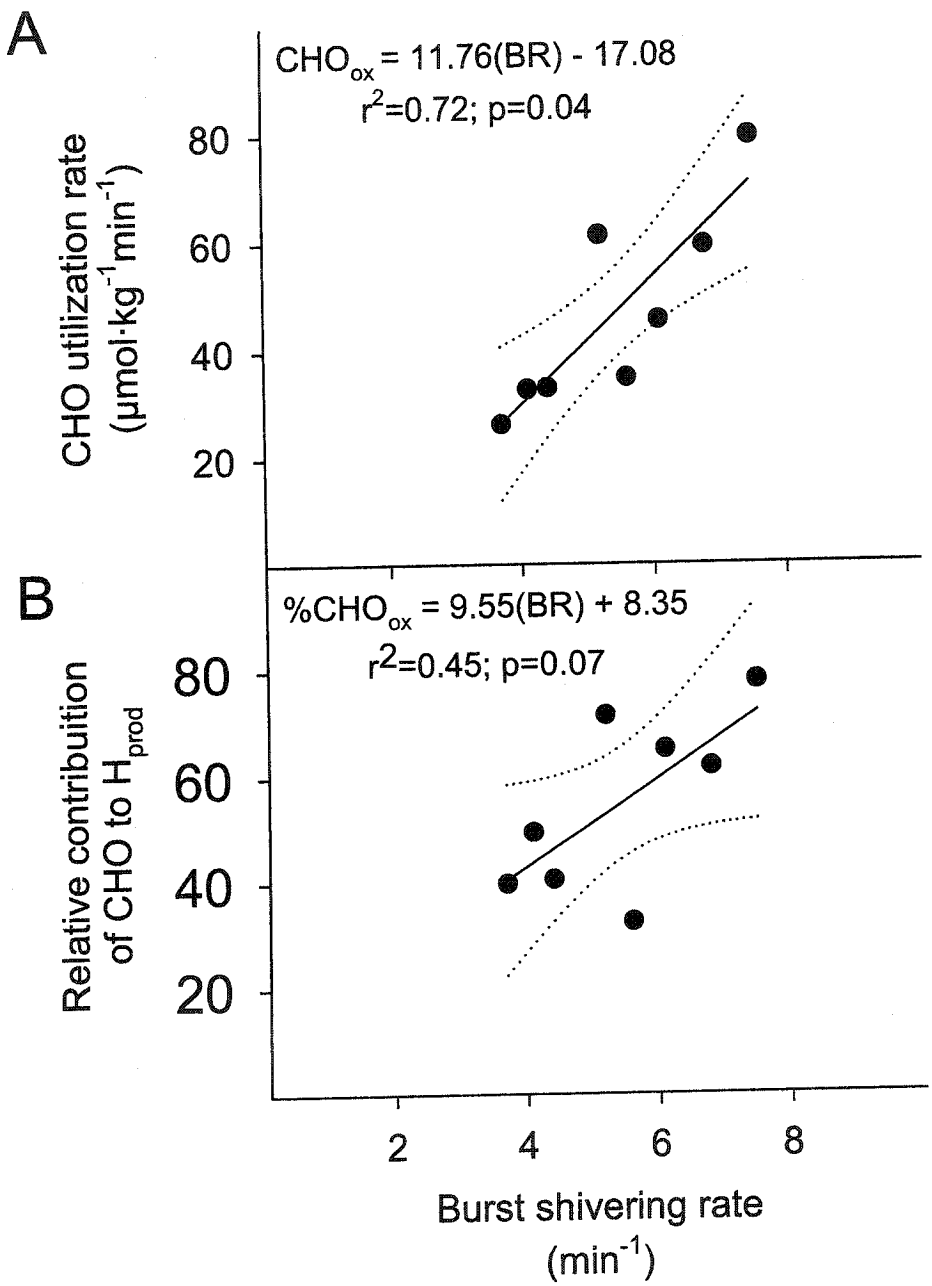

A
CHO_{ox} = 11.76(BR) - 17.08
r^2=0.72; p=0.04
CHO utilization rate
(µmol·kg^{-1}min^{-1})
80
60
40
20
B
%CHO_{ox} = 9.55(BR) + 8.35
r^2=0.45; p=0.07
Relative contribuition
of CHO to H_{prod}
80
60
40
20
2
4
6
8
Burst shivering rate
(min^{-1})

Figure 6.5. Relationship between burst shivering rate (BR) and the absolute CHO oxidation rate (RG_{ox}) of the upper trunk (A), lower trunk (B) and upper leg muscles (C) in a group of men exposed to 5°C for 90 min. Dotted lines indicate the 95% confidence interval for the linear regression. Values were averaged in the last 15 min of cold exposure (T = 75-90 min).

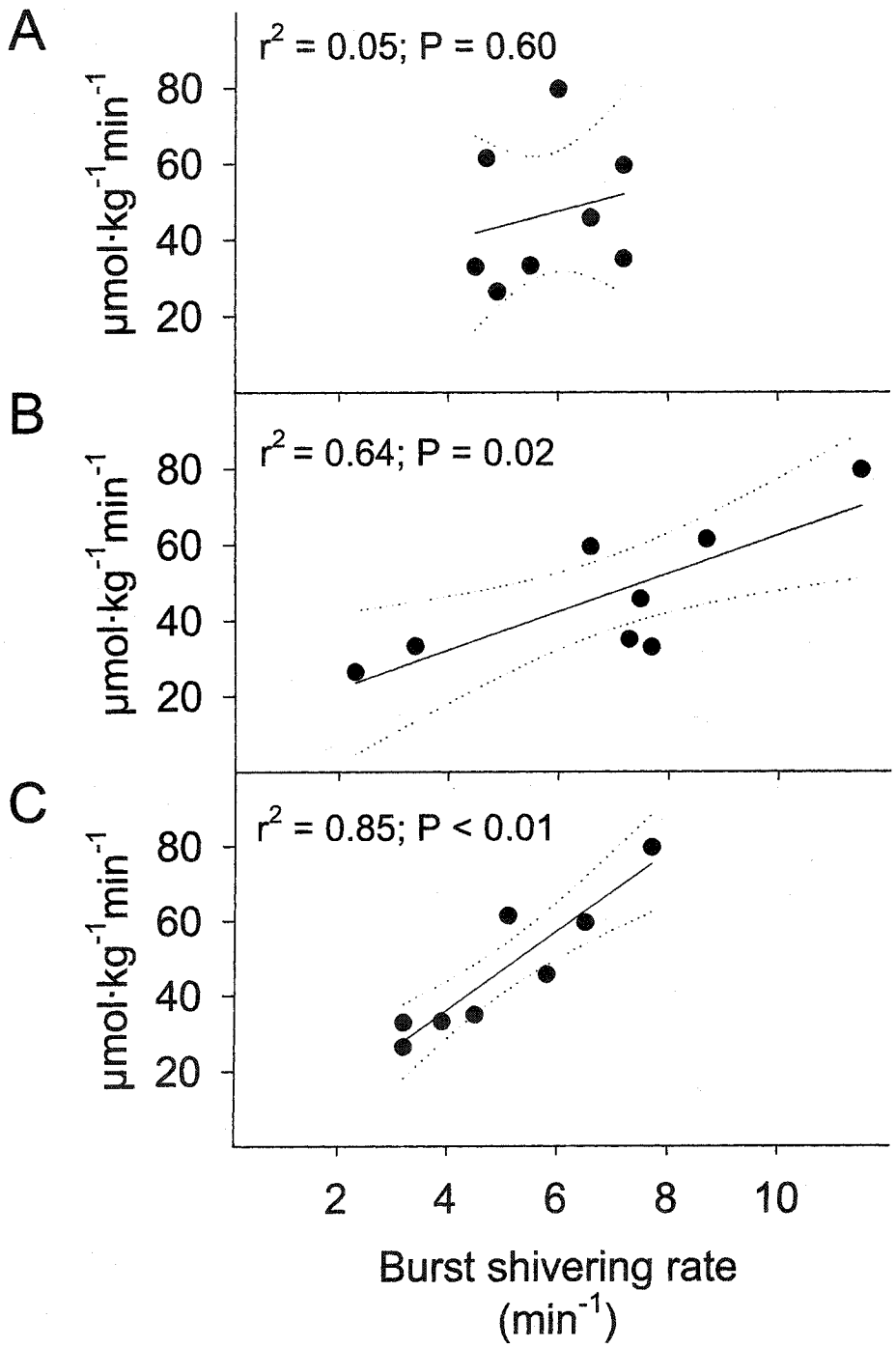

A
B
C
r^2 = 0.05; P = 0.60
r^2 = 0.64; P = 0.02
r^2 = 0.85; P < 0.01
µmol·kg^{-1}min^{-1}
20
40
60
80
2
4
6
8
10
Burst shivering rate
(min^{-1})

Figure 5

Figure 6.6. Relationship between burst shivering rate (BR, average of only the best predictors of RG_{ox} and $\%RG_{ox}$ from Table 6.2: *rectus abdominis*, *vastus lateralis*, *rectus femoris* and *vastus medialis*) and A. the absolute oxidation (RG_{ox}) as well as B. the relative contribution of CHO to total heat production ($\%RG_{ox}$) in a group of men exposed to 5°C for 90 min. Dotted lines indicate the 95% confidence interval for the linear regression. Values were averaged in the last 15 min of cold exposure (T = 75-90 min).

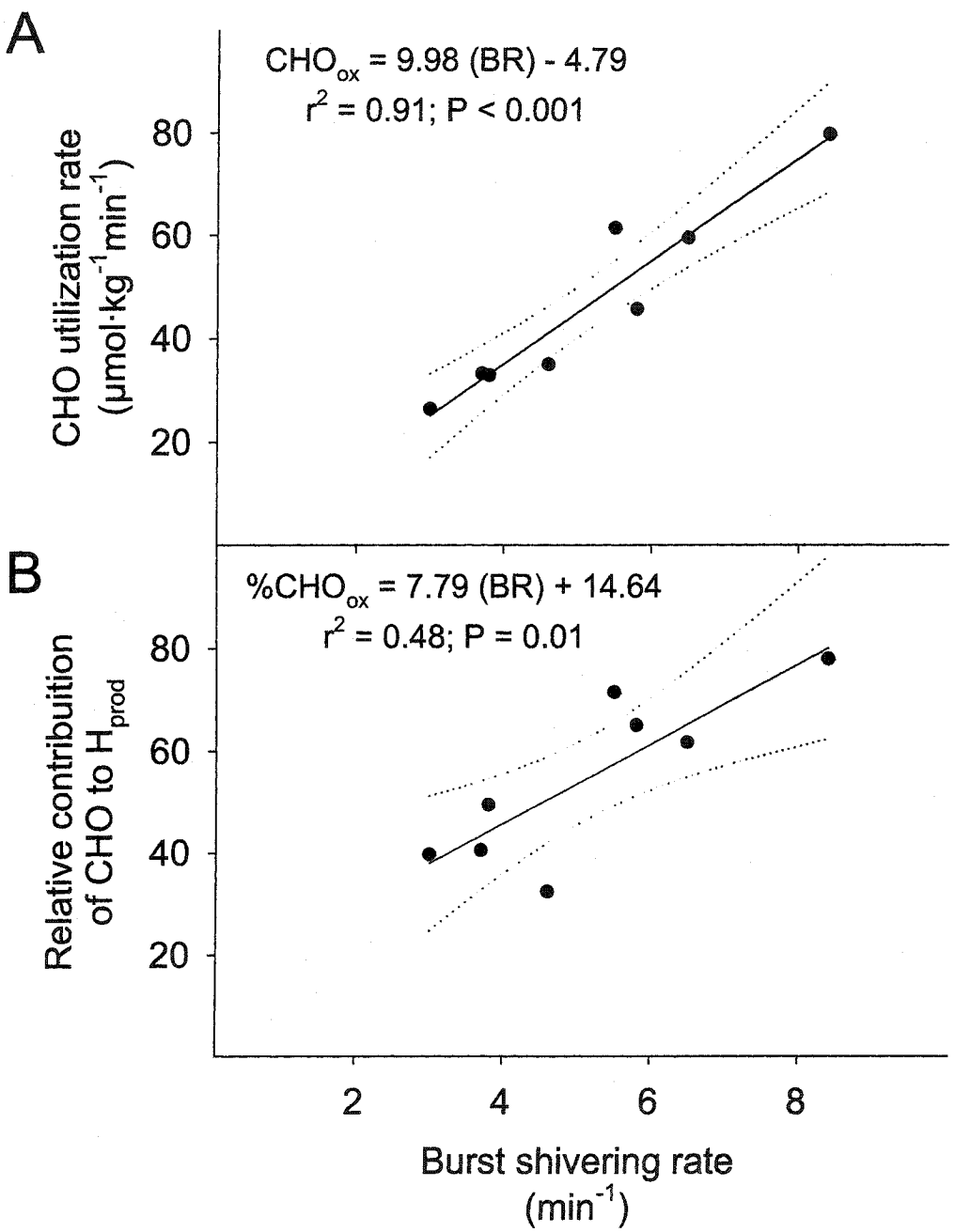
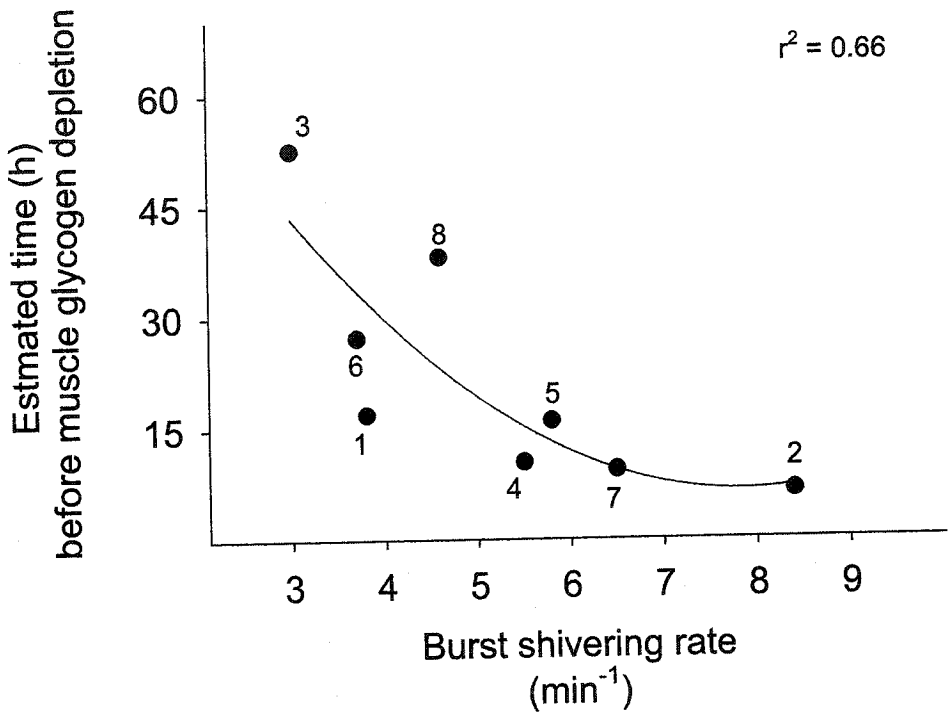

A
CHO_{ox} = 9.98 (BR) - 4.79
r^2 = 0.91; P < 0.001
CHO utilization rate
(µmol·kg^{-1}min^{-1})
80
60
40
20
B
%CHO_{ox} = 7.79 (BR) + 14.64
r^2 = 0.48; P = 0.01
Relative contribuition
of CHO to H_{prod}
80
60
40
20
2
4
6
8
Burst shivering rate
(min^{-1})

Figure 6.7. Relationship between burst shivering rate (BR, average of only the best predictors of RG_{ox} and $\%RG_{ox}$ from Table 6.2: *rectus abdominis*, *vastus lateralis*, *rectus femoris* and *vastus medialis*) and theoretical estimates of the time before muscle glycogen depletion ($GLY_{depletion}$) under the conditions of our experiments, assuming that the relative use of the different fuels remains the same as measured after 90 min of high intensity shivering. A second order regression is also presented (black line). Subject numbers are the same as in Table 6.1.


r^2 = 0.66
Estmated time (h)
before muscle glycogen depletion
60
45
30
15
3
8
6
5
1
2
4
7
3
4
5
6
7
8
9
Burst shivering rate
(min^{-1})

DISCUSSION

This study is the first to show that the pattern of muscle recruitment (EMG) can provide quantitative information on energy metabolism. Cold exposure was used as a tool to investigate muscle activity and oxidative fuel selection simultaneously. We reasoned that shivering thermogenesis would provide a better model than exercise for this purpose, because it eliminates electrical noise caused by limb movements. During high-intensity shivering, the relative use of lipids and carbohydrates was very different between subjects (Table 6.1), and this high variability in fuel selection was primarily explained by differences in burst shivering rate (Fig. 6.4).

Shivering pattern reflects fuel selection

We were particularly careful to minimize inter-individual variability in the response to cold. The group of male subjects selected for this study was controlled as much as possible (Table 6.1), for age, morphology (surface to volume ratio), body composition (percent body fat), and diet. These precautions were sufficient to elicit similar changes in metabolic rate among subjects: a 3.3-fold increase in the cold. However, surprisingly large inter-individual differences in fuel selection were observed. Depending on the subject, the cold-induced increase in CHO oxidation ranged between 2 and 8-fold, with CHO oxidation accounting for 33-78% $\dot{H}_{prod}$, and lipid oxidation for 14-60% $\dot{H}_{prod}$ (Table 6.1). In exercising muscles, such large differences in substrate metabolism are usually explained by differences in exercise intensity but here, during

shivering, there was no significant correlation between fuel selection and metabolic rate (Fig. 6.3). Therefore, we turned our attention to the possibility that differences in fuel metabolism were related to the selective recruitment of different fiber populations; a detailed characterization of shivering patterns was performed to quantify the relationship between the EMG signatures and fuel selection. EMG signals were analyzed by separating the patterns for *continuous, low-intensity shivering* (type I fibers) and *burst shivering* (type II fibers). Distinguishing the two types was based on large differences in intensity (2-5 vs. 7-15% MVC) and rate of occurrence (8-10 vs. 0.1-0.2 Hz). Results clearly show that mean burst shivering rate (averaged among subjects and for 8 muscles in each subject) co-varies closely with the rate of CHO oxidation (Fig. 6.4). This finding suggests that, during high-intensity shivering, the recruitment of type II fibers is linked to an increase in CHO use and plays a key role in orchestrating fuel selection.

During exercise, the relationship between type II fiber recruitment and CHO oxidation is generally accepted, even though no direct experimental evidence is available because no simultaneous measurement of EMG and fuel selection has been performed. However, indirect support is strong (Armstrong, 1988, Brooks *et al.*, 1999, Roberts *et al.*, 1996). At low exercise intensity, the recruitment of type I fibers is related to an increase in lipid use. When exercise intensity is increased, CHO oxidation becomes progressively more important with the gradual recruitment of more type II fibers. Through simultaneous monitoring of EMG signals and fuel metabolism in shivering muscles, this study shows that higher rates of CHO oxidation are achieved by recruiting more type II fibers.

Shivering pattern of individual muscles

After relating mean burst shivering rate to CHO oxidation at the whole-organism level, the same relationship was examined individually for each muscle. While the burst shivering rate of abdominal and quadriceps muscles were closely correlated with whole-body CHO use (r^2 = 0.79-0.87), no such relationship could be established for the upper trunk (*pectoralis major, trapezius* and *lattissimus dorsi*) or for *adductor magnus* (Table 6.2). Therefore, we found that burst shivering activity was not synchronized among muscles, and these results are not consistent with the only two previous studies dealing with this issue in humans (Bawa *et al.*, 1987, Israel and Pozos, 1989). The exact reasons for this discrepancy are unclear, but they may be related to differences in the fiber composition of the subjects used in the three studies. Interestingly, changes in burst shivering pattern have been associated with differences in fiber composition in birds. While more aerobic muscles shiver continuously (high in type I fibers), those depending more on anaerobic metabolism (high in type II fibers) have the tendency to shiver in bursts (Hohtola and Stevens, 1986). In humans, fiber composition varies significantly among muscles (Gardiner, 2001) and even more so between individuals (Simoneau and Bouchard, 1989). Unfortunately, because little attention has been dedicated to shivering bursts (Israel and Pozos, 1989), no information is available on their rate of occurrence in relation to fiber composition. While research on animals suggests that continuous low-intensity shivering (tonic) is controlled by the hypothalamic shivering centre, burst shivering (phasic) appears to be generated within the spinal cord (Gorke and Pierau,

1979, Herdman, 1978, Kosaka and Simon, 1968, Simon *et al.*, 1966). Whether burst shivering activity is controlled by a non-hypothalamic site in humans is still unclear. However, some evidence indicates that burst shivering frequency is modulated by thermosensitive segmental influences (i.e. fluctuations in skin temperature detected by cutaneous cold receptors) (Burton and Edholm, 1969) and/or by efferent signals from the hypothalamic center (Martin and Cooper, 1981). In view of our present results, additional work on shivering bursts is clearly needed. This seemingly minor component of the overall EMG signal appears to play the most prominent role in modulating fuel metabolism.

Changing fuel selection in the cold

Modifying fuel selection of contracting muscles can be achieved in 2 ways: i) by mobilizing different metabolic pathways within the same fibers or ii) by recruiting distinct fiber populations specialized for different fuels. Previous results for low-intensity shivering have shown that glycogen-depleted and glycogen-loaded humans are able to sustain the same rate of heat production by oxidizing broadly different fuels within the same muscle fibers (Haman *et al.*, 2004a). Here, during high-intensity shivering, the alternative mechanism of fuel selection is observed: large differences in fuel use are achieved by recruiting different "fuel-specific" fibers. Depending on the individual, thermogenesis was sustained by oxidizing mostly lipids (up to 59% H_{prod}) within predominantly type I fibers (4 bursts·min^{-1}), or by recruiting more type II fibers (8 bursts·min^{-1}) and use mostly CHO (up to 78% H_{prod}). Whether these large inter-subject

differences in fiber recruitment offer a selective advantage for cold survival is unknown and more research is needed to establish the physiological significance of individual shivering patterns. What are possible consequences of differences in burst shivering rate (and related CHO use) on cold endurance? Over the last few decades, CHO metabolism has often been considered as a limiting factor for heat production because the availability of this critical fuel is very low (~1% of total energy reserves) (Haman *et al.*, 2002, Jacobs *et al.*, 1994). Here (Fig. 6.6), we have plotted theoretical estimates of maximal cold endurance based on glycogen depletion, as a function of burst shivering rate ($GLY_{depletion}$; see Methods for details of calculations). According to these estimates, individuals with extremely high burst shivering rate (8 burst·min^{-1}) and low burst shivering rate (4 burst·min^{-1}) would be able to produce heat at 300 W for ~7 h and ~50 h respectively before depleting their muscle glycogen reserves. However, it is still unclear whether CHO are essential for maintaining such a high thermogenic rate (Haman *et al.*, 2004b, Martineau and Jacobs, 1989b, Young *et al.*, 1989). If CHO are essential, our calculations show that cold endurance varies greatly among morphologically similar individuals shivering at the same relative intensity and, consequently, the normalization of burst shivering rate will be required in future studies to decrease inter-subject variability. If CHO are not essential, lipids and proteins will have to compensate for varying contributions from CHO and the above estimates of cold endurance will have to be increased by at least an order of magnitude.

Shivering vs exercise

Our study reveals interesting differences between exercise and shivering, further supporting the notion that these two processes are not analogous (Tipton *et al.*, 1997). For the same metabolic rate, mean recruitment of type II fibers and variability in fuel selection are much higher during shivering than exercise. This suggests that the "crossover point" (i.e. the metabolic rate for which CHO and lipid oxidation contribute equally to oxygen consumption; see (Brooks and Mercier, 1994)) is significantly lower during shivering than exercise. Also, inter-individual differences in type II fiber recruitment are sufficient to affect the relative use of CHO (from 33 to 78% H_{prod}, greatly exceeding the expected range of values for exercise at such low metabolic rates, see Table 6.1). Patterns of fiber recruitment have received a lot of attention during exercise (see Gardiner, 2001, Linnamo *et al.*, 2003 for review, Wakeling *et al.*, 2002), but very few studies have investigated them during shivering (Israel and Pozos, 1989, Meigal, 2002, Meigal *et al.*, 1993, Meigal *et al.*, 1995, Petajian and Williams, 1972). In exercise, fibers are recruited according to the "size principle" (Henneman *et al.*, 1965) which stipulates that type I fibers are activated for low-force contractions and increasingly larger and faster fibers (type II) are then activated to supply greater force (Brooks *et al.*, 1999, De Luca *et al.*, 1982, Freund *et al.*, 1975, Linnamo *et al.*, 2003, Milner-Brown *et al.*, 1973, Moritani and Muro, 1987, Wakeling *et al.*, 2002). Clearly, further research is needed to establish what principle governs fiber recruitment as shivering intensity is modified.

Conclusion

Traditionally, EMG signals have provided information on overall muscle activity, on the recruitment of specific motor units, and on the onset of fatigue. Here, we show for the first time that the EMG signature of active muscles also includes important quantitative information on metabolic fuel utilization. During intense cold exposure, differences in burst shivering rate are directly linked to differences in CHO oxidation. All subjects have the ability to sustain the same high thermogenic rate, but each individual can do so by recruiting different combinations of "fuel-specific" fibers that oxidize widely different fuel mixtures. Further research should focus on understanding burst shivering because it plays a central role in orchestrating fuel selection and may provide essential clues on what limits human survival in the cold.

CHAPTER 7. GENERAL CONCLUSION: EFFECTS OF CARBOHYDRATE AND SHIVERING PATTERN ON METABOLIC FUEL SELECTION DURING COLD EXPOSURE IN HUMANS

THESIS OVERVIEW

The main purpose of this thesis was to determine the effects of changes in CHO availability and shivering intensity on oxidative fuel selection and muscle recruitment during cold exposure. All experiments were conducted in humans, an endotherm well adapted for dissipating heat in warm conditions, but particularly ill-suited at conserving it in cold environments. Previous research in this field falls into two broad categories dealing either with muscle metabolism (Haman *et al.*, 2002, Jacobs *et al.*, 1994) or with electrophysiological aspects of muscle recruitment (Bell *et al.*, 1992, Meigal, 2002, Tikuisis *et al.*, 2000a). These two complementary perspectives on the same problem have not been traditionally integrated and the experiments conducted herein were the first attempt at doing so. This work was sub-divided into six studies. First, the respective contributions of plasma glucose, muscle glycogen and lipid oxidation to total heat production was quantified during sustained, low-intensity shivering (CHAPTER 2). Second, the effects of changes in CHO availability on oxidative fuel selection were determined during sustained, low-intensity shivering (CHAPTER 3). Third, the effects of changes in glycogen stores on whole-body shivering activity, shivering pattern and spectral parameters were quantified during sustained, low-intensity shivering (CHAPTER 4). Fourth, the effect of shivering intensity on the relative importance of plasma glucose, muscle glycogen, lipid and protein to total heat production was determined (CHAPTER 5). Finally, the goal of the last study was to determine whether muscle recruitment pattern (EMG) could provide metabolic information on fuel selection during high-

intensity shivering (CHAPTER 6). The following conclusions can be drawn from this thesis.

SUMMARY OF PRINCIPAL FINDINGS

CHAPTER 2: EFFECT OF COLD EXPOSURE ON FUEL UTILIZATION IN HUMANS: PLASMA GLUCOSE, MUSCLE GLYCOGEN AND LIPIDS

1. Even though plasma glucose oxidation is strongly stimulated during low-intensity shivering (+138%), this fuel only plays a minor role in total heat production (10% $\dot{H}_{prod}$).
2. Muscle glycogen oxidation doubles during mild cold exposure, providing 75% of total CHO oxidized.
3. Lipids are the most important fuel, showing close to a 4-fold increase in oxidation rate and accounting for the production of as much heat as all other metabolic substrates combined.

CHAPTER 3: EFFECTS OF CARBOHYDRATE AVAILABILITY ON SUSTAINED SHIVERING: I. OXIDATION OF PLASMA GLUCOSE, MUSCLE GLYCOGEN AND PROTEINS

1. Plasma glucose oxidation remains a minor fuel under all conditions (<13% $\dot{H}_{prod}$), falling to 7 % $\dot{H}_{prod}$ in glycogen-depleted for LO subjects.

2. Heat production in glycogen-depleted individuals is not compromised, because protein (19% $\dot{H}_{prod}$) and lipid oxidation (53% $\dot{H}_{prod}$) are both stimulated to compensate for the reduced contribution from CHO.
3. Humans can show remarkable flexibility in oxidative fuel selection to ensure that thermogenic rate is not compromised during sustained cold exposure.

CHAPTER 4: EFFECTS OF CARBOHYDRATE AVAILABILITY ON SUSTAINED SHIVERING: II. RELATING MUSCLE RECRUITMENT TO FUEL SELECTION

1. Drastic changes in oxidative fuel selection are achieved without modifying the EMG signature of shivering muscles.
2. Results provide support for the notion that the same population of muscle fibres is responsible for oxidizing widely different fuel mixtures, but they are not compatible with the hypothesis that changing fuel selection is realized by recruiting fuel-specific motor units.

CHAPTER 5: OXIDATIVE FUEL SELECTION DURING HIGH INTENSITY SHIVERING: EARLY RELIANCE ON CARBOHYDRATES

During high-intensity shivering:

1. Plasma glucose oxidation is increased by 50% over values measured during low-intensity shivering but still plays a minor role for thermogenesis (< 15% $\dot{H}_{prod}$).
2. Muscle glycogen remains the main source of CHO to sustain thermogenic rate, providing 75% of all the glucose oxidized in the cold.

3. Contrary to expectations, CHO were the most important fuel during high intensity shivering showing a 403% increase in oxidation rate and accounting for more heat than all the other metabolic fuels combined (55 % $\dot{H}_{prod}$ vs 37% $\dot{H}_{prod}$ for lipids and 8% $\dot{H}_{prod}$ for proteins).

CHAPTER 6: FUEL SELECTION DURING INTENSE SHIVERING: EMG PATTERN REFLECTS CARBOHYDRATE OXIDATION

1. EMG signature of active muscles can provide important quantitative information on metabolic fuel utilization.
2. During intense cold exposure, differences in burst shivering rate are directly linked to differences in CHO oxidation.
3. The recruitment of type II fibers is linked to an increase in CHO use and plays a key role in orchestrating fuel selection.

GENERAL DISCUSSION

I. Partitioning energy utilization from carbohydrates

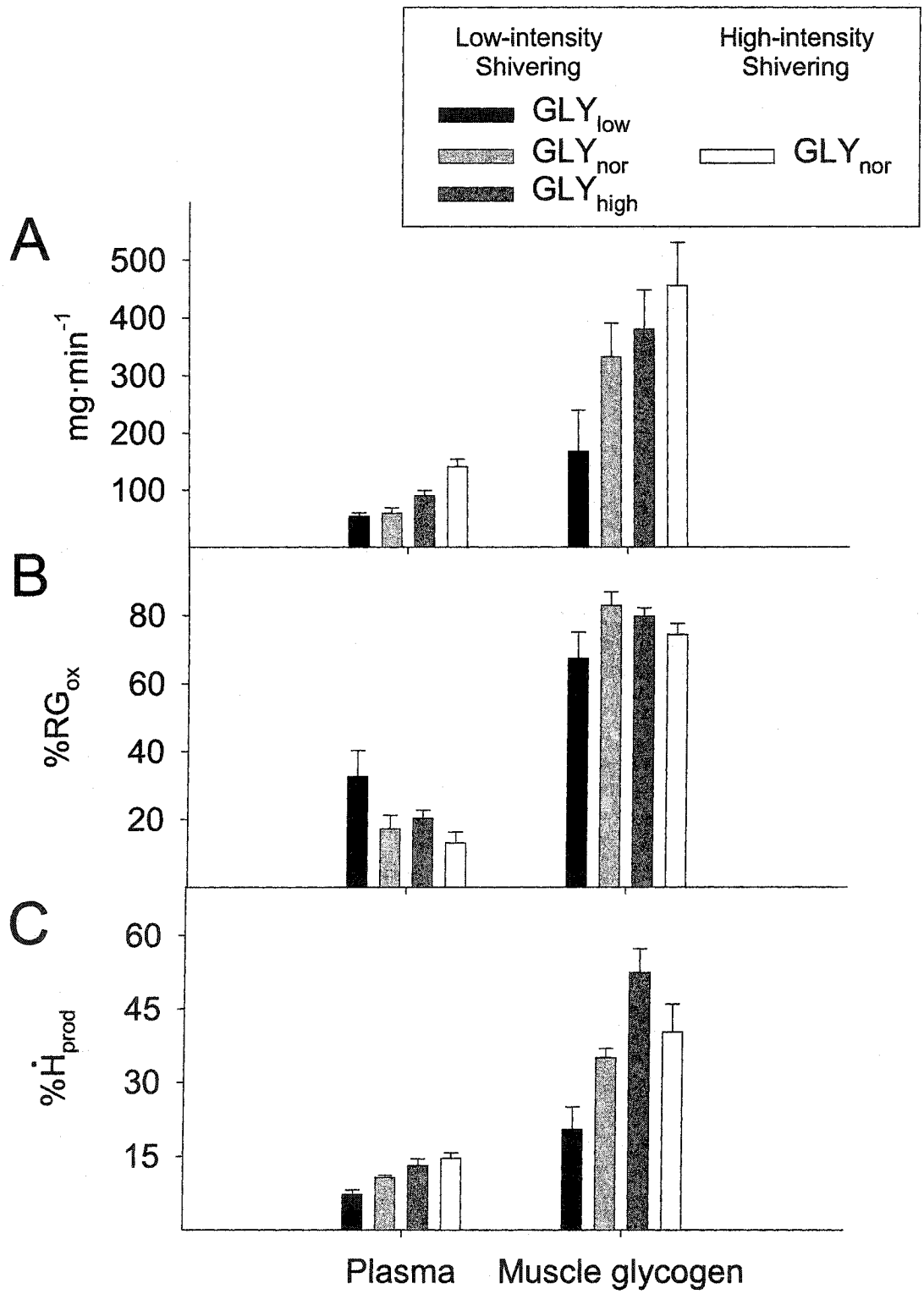
The ^{13}C-glucose tracer technique selected for this study allowed us to quantify the role of circulatory glucose and muscle glycogen as oxidative fuels to support shivering (CHAPTERS 2, 3 and 5). It provides the first direct, whole-body estimates of plasma glucose ($RG_{ox\text{-}plasma}$) and muscle glycogen ($RG_{ox\text{-}mus}$) oxidation rates during shivering. Results show that, even though plasma glucose oxidation is stimulated with the increase

in metabolic rate in the cold, its relative contribution to total heat production always remains minor (< 15% $\dot{H}_{prod}$). In contrast, muscle glycogen stores play a more prominent role, constantly providing between 70 and 80% of all the glucose oxidized in the cold. Figure 7.1 summarizes absolute oxidation rates as well as the relative contributions of plasma glucose and muscle glycogen to total CHO and total heat production under all conditions found in this thesis.

The minor role of circulating glucose

During sustained, low-intensity shivering, $RG_{ox\text{-}plasma}$ is stimulated in direct proportion to metabolic rate (2.3-fold) in individuals with normal (N; CHAPTER 2) and high-CHO availability (HI; CHAPTER 3); whereas a smaller increase is observed when glycogen reserves are low (1.8-fold). These results suggest that hepatic glucose production is lower in glycogen-depleted subjects, since gluconeogenesis does not make up for decreased liver glycogenolysis in this group.

Figure 7.1. Plasma glucose vs. muscle glycogen : A. Absolute oxidation rate, B. relative contribution to total carbohydrate oxidation ($\%RG_{ox}$) and, C. relative contribution to total heat production ($\dot{H}_{prod}$) for all conditions measured in this thesis.


Low-intensity Shivering
High-intensity Shivering
GLY low
GLY nor
GLY high
GLY nor
A
mg·min-1
500
400
300
200
100
B
%RGox
80
60
40
20
C
%Ḣprod
60
45
30
15
Plasma
Muscle glycogen

In all conditions, the relative use of circulatory glucose to total heat production is always minor (10% $\dot{H}_{prod}$ for N, 13% $\dot{H}_{prod}$ for HI and 7% $\dot{H}_{prod}$ for LO). Consequently, plasma glucose oxidation does not compensate for the large decrease in muscle glycogen oxidation of LO, and other fuels (lipids and proteins) must be used to sustain heat production. However, plasma glucose oxidation rates of subjects were clearly not limited by maximal hepatic glucose production because much higher $RG_{ox\text{-}plasma}$ have been reported during exercise (6 to 8 $mg \cdot kg^{-1} \cdot min^{-1}$ vs 0.7 to 1.3 $mg \cdot kg^{-1} \cdot min^{-1}$ in the cold) (Bosch, 1993, Bosch *et al.*, 1994, Bosch *et al.*, 1996, Coggan *et al.*, 1990, Friedlander *et al.*, 1997, Jeukendrup *et al.*, 1999, Péronnet *et al.*, 1998). Friedlander *et al.* (1997) showed that $RG_{ox\text{-}plasma}$ is stimulated by as much as 5-fold during exercise at 45% $\dot{V}O_{2max}$, the highest metabolic rate observed during maximal shivering (Eyolfson et al., 2001). Considering the low metabolic rates reached during mild shivering (2.3 times resting metabolic rate or 20% $\dot{V}O_{2max}$), individuals with normal CHO availability were exposed to higher shivering intensities to establish whether plasma glucose could play a greater role (CHAPTER 5).

During high-intensity shivering (HIGH), results show that plasma glucose oxidation is increased by 120% over values found during low-intensity shivering (LOW) (129 ± 9 $mg \cdot kg^{-1} \cdot hr^{-1}$ vs 58 ± 3 $mg \cdot kg^{-1} \cdot hr^{-1}$). However, this substantial stimulation of $RG_{ox\text{-}plasma}$ for HIGH had little effect on the relative contribution of plasma glucose to total H_{prod}, which only accounted for an additional 5% of heat (10% $\dot{H}_{prod}$ for LOW vs 15% $\dot{H}_{prod}$ for HIGH). Together, these results clearly show that, independently of

changes in CHO availability or shivering intensity, plasma glucose is always a minor fuel source in the cold (<15% $\dot{H}_{prod}$).

Muscle glycogen: the major CHO source

The major source of CHO is muscle glycogen, providing 70 to 80% of all the glucose oxidized in the cold (Chapters 2, 3 and 5). In addition, this fuel always plays an important thermogenic role accounting for 20 to 50% of all the heat produced in the cold. During sustained, low-intensity shivering, $RG_{ox\text{-}mus}$ is stimulated similarly for N (2.1-fold) and HI (1.8-fold) whereas, the greatest relative change caused by cold exposure was observed in LO (5.5-fold). These results show that muscle glycogen reserves are always strongly mobilized for thermogenesis, even when they have been reduced before cold exposure. When shivering intensity is increased (CHAPTER 5), $RG_{ox\text{-}mus}$ is more than twice as high for HIGH than for LOW (374 ± 67 mg·kg^{-1}·hr^{-1} vs 175 ± 32 mg·kg^{-1}·hr^{-1}) and contributes even more heat than lipids (40% vs 37% for lipids). This increase in intracellular glycogen reserve utilization (and/or lactate through the lactate shuttle) with increasing exercise intensity is generally expected in exercising humans (Friedlander et al., 1997). However, in the case of shivering, results in this thesis show that the relative contribution of muscle glycogen to total CHO oxidation is not affected by changes in shivering intensity (75% RG_{ox} for LOW and for HIGH). At these relatively low metabolic rates, utilization of fuel reserves may be more interchangeable than during high intensity exercise (>65% $\dot{V}O_{2max}$).

Oxidizing lipids in the cold

The dual role of lipids as a heat insulation layer and as a large, energy-dense, metabolic fuel (>95% of total energy stored) has been recognized for a long time (Schmidt-Nielsen, 1990). However, the quantitative importance of lipids as a substrate to support shivering is controversial, especially because most studies over the last decade have focused on CHO-dependent heat production (Jacobs *et al.*, 1994). Results herein show that during sustained low-intensity shivering lipids are the most important fuel; this fuel shows close to a 4-fold increase in oxidation rate and produces as much heat as all other metabolic substrates combined (CHAPTER 2). This large increase in lipid utilization is an important strategy to spare limited CHO reserves. Any increase in the relative use of lipids allows maintaining heat production for longer and, therefore, improves chances of survival in the cold. However, such a conclusion also depends on the further assumption that CHO oxidation is essential and that low-intensity shivering cannot be sustained exclusively on lipids and proteins.

In glycogen-depleted and glycogen–loaded individuals (CHAPTER 3), it was also found that lipids play a significant role in maintaining shivering (23% $\dot{H}_{prod}$ in HI and 53% $\dot{H}_{prod}$ in LO). A strong stimulation of lipid oxidation was observed in HI where cold exposure caused lipid oxidation to increase from 0 to 68 $mg \cdot min^{-1}$. For LO, a large 2.8-fold increase was found and, as previously observed in N (CHAPTER 2), lipids provided as much heat as all other metabolic fuels combined.

Interestingly, during high intensity shivering (CHAPTER 5), lipid oxidation rate was stimulated to the same extent as during low-intensity shivering (3.8- vs 3.3-fold),

resulting in the same absolute rates by the end of 90 min in the cold (133±22 mg·kg^{-1}·min^{-1} vs 138±25 mg·kg^{-1}·min^{-1}). Consequently, the 50% increase in $\dot{H}_{prod}$ from LOW to HIGH was supported entirely by a 2.2-fold increase in CHO oxidation (233±32 mg·kg^{-1}·min^{-1} vs 503±71 mg·kg^{-1}·min^{-1}). As a result, the relative contribution of lipids to total heat production decreased by close to 30% from low- to high-intensity shivering (52% vs 37% $\dot{H}_{prod}$) while that of CHO increased by 33% (37% vs 55% $\dot{H}_{prod}$). This finding is unexpected because all other studies reporting rates of oxidation during high intensity shivering show a preference for lipid oxidation (~60% of $\dot{H}_{prod}$, (Jacobs, 1997, Martineau and Jacobs, 1988, Martineau and Jacobs, 1989a, Martineau and Jacobs, 1989b, Tikuisis *et al.*, 2002, Tikuisis *et al.*, 2000b, Weller *et al.*, 1998)). Physiological reasons for such a difference are unclear but may be related to differences in cooling protocol (water immersion in all other studies vs LCS in the present study) and/or nutritional state (i.e. condition of glycogen stores) (Haman *et al.*, 2002). For example, during high-intensity shivering, modifications in the size of glycogen stores through dietary and exercise manipulations was shown to have a profound effect on fuel selection to the extent that a complete shift from lipid to CHO dominance could be elicited (Martineau and Jacobs, 1989b, Young *et al.*, 1989).

The overlooked importance of proteins

The contribution of protein oxidation to shivering thermogenesis has generally been assumed to be minor (~10% $\dot{H}_{prod}$) and, therefore, rarely measured directly (Haman *et al.*, 2002, Vallerand *et al.*, 1995, Vallerand *et al.*, 1999b). This thesis shows that while

this assumption may still be true when glycogen reserves are normal or artificially elevated (CHAPTERS 2, 3 and 5), the relative importance of proteins increases substantially when CHO reserves are reduced (up to 19% $\dot{H}_{prod}$) (CHAPTER 3). Altering glycogen reserves through dietary and exercise manipulations had a major effect on the relative use of proteins before cold exposure and this increased contribution of proteins plays a key role in compensating for the decrease in CHO use after glycogen depletion. Under this condition, protein oxidation accounted for 40% $\dot{H}_{prod}$ before cold exposure. Because absolute rates of protein oxidation were not affected by cold exposure, the 2.3-fold increase in metabolic rate observed in the cold was simply translated as a proportional decrease in the relative role of proteins (down to 19% $\dot{H}_{prod}$). Failing to take protein oxidation into account in fuel oxidation budgets during shivering leads to a significant overestimation of CHO and lipid oxidation rates, particularly when glycogen reserves are depleted.

Shivering intensity and fuel selection

The effect of shivering intensity on metabolic fuel selection remains unclear. Considering the relatively low metabolic rates reached during shivering (up to 45% $\dot{V}O_{2max}$), one would expect that lipids may still play a significant role if fuel selection patterns are identical between exercise and shivering. Exercise studies reveal that lipid oxidation predominates for prolonged work at all intensities below 50% $\dot{V}O_{2max}$ (Bergman and Brooks, 1999, Brooks *et al.*, 1999, Roberts *et al.*, 1996, van Loon *et al.*,

2001), whereas CHO oxidation increases progressively thereafter as intensity increases. However, here results clearly show that an important stimulation in CHO metabolism occurs much earlier during shivering (between 20 and 30% of $\dot{V}O_{2max}$ or 40 and 60% of $Shiv_{peak}$) than during exercise (between 60 and 65% of $\dot{V}O_{2max}$). This important difference in the pattern of fuel selection suggests that the mechanisms responsible for fuel selection in the cold may be different from those found during exercise.

Fuel selection and muscle recruitment

Shivering is essential for human survival in cold environments and this thermogenic response depends critically on coordinating muscle fiber recruitment and fuel metabolism. Changing fuel selection in active muscles can be achieved by mobilizing different metabolic pathways within the same fibers or by recruiting distinct fiber populations specialized for different fuels. During low-intensity shivering, contrary to expectation, drastic changes in oxidative fuel selection (CHAPTER 3) are achieved without modifying the EMG signature of shivering muscles (CHAPTER 4). Since no change in fiber recruitment was observed between LO and HI, this result supports the notion that the same population of muscle fibers is responsible for oxidizing widely different fuel mixtures, but it is not compatible with the hypothesis that changing fuel selection is realized by recruiting fuel-specific motor units. It also appears that most of the heat is generated within type I fibers, because burst shivering only represents <10% of total shivering activity. Unfortunately, mechanisms responsible for regulating such a

large change in fuel mixture within the same fibers have not been investigated in shivering muscle.

In order to verify whether this same strategy (oxidizing widely different fuel mixtures within the same muscle fibers) could also be used during high intensity shivering (rather than recruiting different populations of fuel-specific fibers), experiments were conducted at a shivering intensity of 60%$Shiv_{peak}$ (3.5 times RMR) (CHAPTER 6). Results show that shivering pattern plays an important role in determining fuel selection. For example, low (~4 burst·min^{-1}) to high (~8 burst·min^{-1}) changes in burst rate were sufficient to elicit a complete shift from lipid (60% $\dot{H}_{prod}$) to CHO dominance (78% $\dot{H}_{prod}$). This observation supports the concept, proposed during exercise, that the recruitment of fast-glycolytic fibers is key in increasing CHO utilization rate in working muscles. It proposes that individual shivering patterns may play a key role in determining the oxidative fuel selection in the cold.

GENERAL CONCLUSION

This thesis investigates metabolic and electrophysiological aspects of shivering thermogenesis. Experimental evidence demonstrates that plasma glucose is always a minor fuel source while muscle glycogen consistently provides most of the glucose oxidized in the cold. Therefore, adjusting plasma glucose oxidation to compensate for changes in muscle glycogen oxidation is not a mechanism used for maintaining heat production. Instead, proteins and lipids share responsibility for this compensation. These observations indicate that during low-intensity shivering, humans are able to sustain thermogenic rate by oxidizing widely different fuel mixtures within the same muscle fibers. However, this fuel selection strategy is not used during high-intensity shivering. Evidence presented herein also suggests that an increase in the recruitment of fast-glycolytic muscle fibers (Type II) is directly linked to the increase in CHO utilization rate in shivering muscles. Consequently, changing fuel selection in the cold can be achieved through the recruitement of distinct fiber populations specialized for different fuels. This increased recruitment of fast-glycolytic fibers may also help explain the early "crossover" in fuel selection (compared to exercise). Still, very little is known on the relative importance of *continuous, low-intensity shivering* and of *burst shivering* to total heat generation. How they each contribute to total shivering activity may have important consequences on cold endurance and additional research is needed to determine the physiological significance of this dual pattern.

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