MEETING THE METABOLIC DEMAND DURING 24H OF COLD SURVIVAL SIMULATION: A NEW PERSPECTIVE ON ENERGY NEEDS, FUEL SELECTION AND MUSCLE RECRUITMENT

par / by

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SUMMARY

Cold environments have been an ongoing challenge for humans over the ages. To date, however, knowledge on the capacity to sustain the metabolic demand of shivering is mostly limited to modeling efforts. Actual models of shivering endurance, by a presumed analogy with exercise, rely on the assumptions that carbohydrate stores are used at a constant rate and essential to maintain heat production. Therefore, my thesis firstly aimed to characterize a hypothesized capacity to adjust the fuel mixture oxidized in order to maintain heat production during 24h of cold exposure. An accidental cold exposure with food scarcity was simulated. Following a 5 km walk, lightly clothed individuals were exposed to an environmental temperature of 7.5 °C for 24h. Results show that thermogenesis increased to 8 kJ per minute, a metabolic demand that was sustainable for 24h since lipid oxidation substituted a decreased carbohydrate oxidation. Secondly, this thesis aimed at characterizing the roles of muscle glycogen and plasma glucose in these changes in carbohydrate metabolism. It was found, as hypothesized, that such changes result from a reduction in muscle glycogen utilization. Electromyographic activity of shivering muscles was also measured to address the third objective of the thesis: determining whether shivering contributes similarly to heat production during 24h and whether muscles contribute in similar proportions. Results show that total shivering intensity was constant during 24h. However, different muscles contributed differently over time, enlightening a potential mechanism for changes in fuel selection. Questions remain concerning the origin of this shift in fuel use towards lipid dominance during this prolonged cold exposure. Blood metabolites and glucose tracer data suggest that a high availability of circulating fatty acids is more likely in cause than a depletion of glycogen reserves while the implication of inadequate feeding needs to be clarified.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND SYMBOLS

ATP: Adenosine triphosphate

ß-HB: Beta-hydroxybutyrate

BAT: Brown adipose tissue

F_eCO_2: Fraction of expired carbon dioxide

F_eH_2O: Fraction of expired water

F_eO_2: Fraction of expired oxygen

F_iCO_2: Fraction of inspired carbon dioxide

F_iH_2O: Fraction of inspired water

F_iO_2: Fraction of inspired oxygen

F_{ox}: Lipids oxidation

FR_e: Expired flow rate

FR_i: Inspired flow rate

G_{ox}: Glucose oxidation

GP_{ox}: Plasma glucose oxidation

GM_{ox}: Muscle glycogen oxidation

$\dot{H}_{prod}$: Heat production

NEFA: Non-esterified fatty acids

NST: Non-shivering thermogenesis

P_{ox}: Protein oxidation

R_{exp}: Enrichment of expired CO_2

R_{glu}: Enrichment of plasma glucose

R_{ref}: Background enrichment

RMR: Resting metabolic rate

sEMG: Surface electromyography

ST: Shivering thermogenesis

STP: Standard conditions for temperature and pressure

T_{core}: Core temperature

$\bar{T}_{skin}$: Mean skin temperature

$\dot{V}CO_2$: Volume of expired carbon dioxide

$\dot{V}H_2O$: Volume of expired water

$\dot{V}O_2$: Volume of inspired oxygen

$‰\delta^{13}C$ **VPDB** : Enrichment in delta per mill referenced against Vienna Pee Dee Belemnite

°**C**: Degree Celsius

h: Hour

l: Litter

g: Gram

kg: Kilogram

min: Minute

mg: Milligram

ml: Millilitre

mmol: Millimol

pmol: Picomol

µmol: Micromol

LIST OF TABLES AND FIGURES

Tables

Figures

CHAPTER 1 INTRODUCTION

Humans are well known to dissipate heat efficiently in warm climates, but are particularly bad at conserving it in cold environments without proper gear. In this context, preventing core temperature from significantly decreasing is extremely difficult without proper behavioral strategies to reduce heat loss (e.g. building shelters, insulative clothing, external heat source)(Wells & Stock, 2007). When cold exposure is inevitable, humans must rely on a concerted activation of physiological processes that reduce heat loss and increase heat production in order to prevent a decrease in core temperature. Heat loss is reduced by peripheral vasoconstriction and heat production is increased by activating multiple metabolic pathways in various tissues. More specifically, heat originating from involuntary muscle contractions is referred to as shivering thermogenesis (ST) and the rest of the heat produced as non-shivering thermogenesis (NST). Despite the fact that the contribution of NST can no longer be disregarded (discussed in *Muscle recruitment and other thermogenic pathways*), ST is by far the most thermogenic involuntary response in adults. ST is maintained by supplying adequate fuels from the circulation and intracellular reserves at various times and rates. A failure to fuel shivering muscles alters their capacity to compensate for increases in heat loss, which is likely to result in hypothermia. Even in a terrestrial environment where heat dissipation is lower than in cold water, finite endogenous fuel supplies will progressively be reduced by shivering, especially in times of food scarcity. Such an energy deficit could limit the maintenance of ST, perhaps even for less than a day (Haman, 2006). Over the last decades, different shivering endurance models have been developed (Wissler, 1985; Haman, 2006), but unfortunately, very little empirical information is available to validate their long-term predictions and respective premises which may very well be inadequate (Tikuisis *et al.*, 2002). This chapter summarizes current knowledge that evidence whether the metabolic demand of cold exposure can be sustained for a long time by relying solely on ST and NST. The following first focuses on metabolic fuel selection and the depletion of energy stores. It then describes the detrimental effects of energy deficit during cold exposure and explores the thermic and metabolic effects of different macronutrients as potential strategies to compensate. Insights from models of shivering endurance will also be provided. Finally, this chapter

presents current knowledge on muscles recruitment and the stimulation of other thermogenic pathways for cold defense.

Coping with the energy demand during prolonged cold exposure

Heat production ($\dot{H}_{prod}$) or thermogenesis results from the oxidation of substrates and other exothermic biochemical reactions that maintain body functions. In a thermoneutral environment, metabolic activities in the different tissues all together release about 5 kJ of heat per kg of body mass per hour. Skeletal muscles account for about 40% of total body mass in a lean individual (Schmidt-Nielsen, 1984; Rolfe & Brown, 1997). For this reason, they are able to increase whole body thermogenesis well beyond any other tissue in humans. For instance, during exercises, thermogenesis can be voluntarily increased by as much as 15 to 20 times. In contrast, for physiological reasons that are not well understood, thermogenesis during shivering can only increase by up to 5 times (Eyolfson *et al.*, 2001). When prolonged exposure to cold is inevitable and food is limited or unavailable, exercise, despite its high thermogenic capacity, is probably not the most strategic way to offset heat dissipation on the long term. Exercise is under voluntary control and humans do not seem good at estimating exercise thermogenesis (Willbond *et al.*, 2010), suggesting a poor regulation of thermal balance and a misuse of available energy. Additionally, exercise increases blood flow to muscles and thus heat loss. For example, $\dot{H}_{prod}$ is normally larger than heat loss during cross-country skiing in a cold environment (Shephard & Astrand, 2008) making this activity a poor way to thermoregulate if locomotion is not mandatory. In such a context, combined activation of ST and of NST becomes the main way of maintaining $\dot{H}_{prod}$ if it can entirely compensate for heat loss.

$\dot{H}_{prod}$ is sustained by the combined oxidation of carbohydrates (CHO), lipids and proteins (see Weber & Haman, 2005 for review; Haman, 2006). These metabolic fuels differ in their energy potential and are stored in widely different amounts. In order to sustain ATP production during ST, they are supplied to muscles at appropriate times and rates from both intramuscular reserves and other tissues via

the circulation. Current knowledge on the importance of CHO, lipids and proteins to cope with the metabolic demand of cold exposure will be described in the following sections.

Carbohydrates

CHO represent only ~1% of total energy reserves (~95% for lipids and ~4% for available amino acids) and with 17.3 kJ g^{-1} they have less than half the energy potential of lipids (Weber & Haman, 2004). This fuel is well known to limit prolonged endurance exercise, however, its implication in shivering endurance is less clear. During ST, in men and women with normal glycogen reserves, CHO are a substantial fuel source, providing ~20-80% of all the heat produced with rates of oxidation ranging from ~130 to 500 mg kg^{-1} h^{-1} from mild to moderate cold exposure (Haman *et al.*, 2005; Blondin *et al.*, 2011).

The glucose required for maintaining ATP production in shivering muscles is supplied from liver glycogen via the circulation and from *in situ* utilization of glycogen within muscle fibers. Both fuel sources were reported to play significant roles for sustaining $\dot{H}_{prod}$ during cold exposure (Haman *et al.*, 2002a; Haman *et al.*, 2004c; Haman *et al.*, 2005). Research has indicated that muscle glycogen supplied at least ~75-80% of the glucose required to sustain thermogenesis whereas the contribution of plasma glucose remained constant at ~20-25% (Haman *et al.*, 2002a; Haman *et al.*, 2004c; Haman *et al.*, 2005). Therefore, it appears that as for exercise**,** muscle glycogen is the greatest source of glucose for sustaining whole body CHO oxidation and the metabolic demand in the cold.

Lipids

Lipids are by far the largest and most energy dense of all metabolic fuel stores (Weber & Haman, 2004). From one individual to another, sizes of reserves differ as a function of adiposity. During exercise, lipids are the main fuel below ~50-60% of maximal power. In contrast, Haman *et al.* (2005) showed that, in the cold, their relative contribution to $\dot{H}_{prod}$ dominates below ~20% of $\dot{V}O_{2\ max}$. The role of this fuel is reduced progressively as shivering intensifies and CHO oxidation increases due to the absence of a concomitant increase rather than a reduction in its oxidation rate. Whether at low to moderate intensity shivering during cold exposure (Haman *et al.*, 2005) or up to maximal shivering intensity during

rewarming (Haman *et al.*, 2007), absolute rates of lipid oxidation always remain constant at an average of ~140 $mg \cdot kg^{-1} \cdot h^{-1}$. It suggests that maximal fat oxidation rate is already reached at low shivering intensities of ~2.5XRMR, but the reason why lipid oxidation would plateau at a value ~3 times lower than what would be found during exercise at a similar metabolic rate (Achten *et al.*, 2002) remains unclear. The recruitment of different muscle fibers could explain this discrepancy.

Lipids are oxidized as fatty acids, which are obtained from the lipolysis of triacylglycerol stores located in adipose tissue in the liver or in muscles. In the cold, the relative contribution of each compartment is still unknown. Using stable isotope tracer methods, Vallerand *et al.* (1999) and Ouellet *et al.* (2012) indicated that both lipolysis rate and turnover rate of circulating fatty acids increase proportionally with the increase in metabolic rate during mild cold exposure. This indicates that circulating fatty acids are likely to play a significant role in the cold.

Proteins

Evidence clearly shows that proteins can contribute as much as plasma glucose in the cold (~10%Hprod, Haman *et al.*, 2002a) depending on the shivering intensity (Haman *et al.*, 2005) and the nutritional status of individuals (Haman *et al.*, 2004c)(see *Depleting energy sources*). Most importantly, no research to date has indicated that absolute rates of protein oxidation are affected by acute cold exposure. It remains to be determined whether protein utilization is affected as energy stores are reduced during a cold exposure sustained for longer than a few hours, if indeed $\dot{H}_{prod}$ can be sustained in such conditions.

Depleting energy sources

Over the last decades, metabolic research in the cold has focused on understanding whether, like exercise, the depletion of CHO reserves could limit ST endurance (Wissler, 1985). However, to date, evidence from short-term studies indicates that adult humans are able to sustain ST using a wide variety of metabolic fuels. When the small CHO stores are depleted or reduced prior to cold exposure, other more available fuel sources compensate in order to maintain $\dot{H}_{prod}$.

In three independent studies, CHO availability was artificially altered through dietary and exercise manipulations before exposing men to either mild (2.5X RMR; Haman *et al.*, 2004b) or moderate cold exposure (3.5X RMR; Martineau & Jacobs, 1989; Young *et al.*, 1989). Results showed that at both shivering intensities modifying the size of glycogen reserves caused a large shift in fuel use from CHO dominance (up to ~80% $\dot{H}_{prod}$) to lipid dominance (up to ~80% $\dot{H}_{prod}$). In addition, Haman *et al.* (2004c) demonstrated that protein oxidation can play a more significant role (~25% $\dot{H}_{prod}$) and that lipid use is at its apparent maximum for shivering (~140 $mg \cdot kg^{-1} \cdot h^{-1}$) when glycogen reserves are low. However, it decreases by more than half (~60 $mg \cdot kg^{-1} \cdot h^{-1}$) when CHO are highly available. This large shift in substrate utilization allowed core and skin temperatures as well as $\dot{H}_{prod}$ to remain the same when glycogen reserves are low. Such a metabolic flexibility may not be surprising considering the relatively small metabolic rates achieved in the cold. Regarding the mechanisms involved, Haman *et al.* (2004a; 2004c) showed that, during mild shivering, humans are able to sustain $\dot{H}_{prod}$ without modifying surface electromyography (sEMG) signal intensity and characteristics. Therefore, the drastic switch in fuel metabolism found as a result of glycogen depletion and loading prior to cold exposure (CHO, ~28 vs 65%; lipids, ~53 vs 23%; proteins, ~19 vs 12% $\dot{H}_{prod}$, respectively) appears to occur within the same muscle fibers. Research during exercise has shown that large changes in fuel selection within the same fibers can be regulated by modulations in intracellular metabolites (acetyl-CoA, malonyl-CoA, Ca^{2+}, ADP, AMP, P_i and AMPK l)(Jeukendrup, 2002; Spriet, 2002). The same mechanisms are likely involved during shivering. Another potential mechanism would be to recruit in different proportions muscles varying in fibers composition to adjust the fuel mixture oxidized, but presently no study has showed such a response. Results summarized in this section emphasize the importance of proteins and lipids in compensating for large decreases in CHO availability. However, it is has not been demonstrated whether the metabolic flexibility occurs during prolonged cold exposure and whether it allows a complete compensation of heat loss.

Energy deficit and macronutrients in the cold

Little is known on the effects of energy deficits on ST. Following a 12h and 48h fast, MacDonald *et al.* (1984) showed that core temperature, during five progressively reduced levels of cold stress, was lowest in the prolonged unfed state. This response was associated with an increased heat loss linked to a greater forearm blood flow. Later, Young *et al.*(1998) and Castellani *et al.* (2003) reported that chronic energy deficits from underfeeding and strenuous military training lasting longer than 48-h (84-h to 61 days) reduce $\dot{H}_{prod}$ in the cold and decrease lean and fat mass. It has been speculated that the decreased in $\dot{H}_{prod}$ could result from lowered fuels supply or hypoglycemia generally observed in prolonged fasted individuals [from 5.2 mmol/L in 12-h fast to 3.8 mmol/L in 60h fast; (Carlson *et al.*, 1994)]. Collectively, these findings supports the premise that shivering endurance is affected by chronic energy deficit and its effects on CHO availability (Tikuisis *et al.*, 2002) and blood glucose (Haight & Keatinge, 1973; Passias *et al.*, 1996). Models of shivering endurance similarly assume that CHO are essential to maintain shivering, which will be described later. They clearly suggest that providing supplemental exogenous substrates through feeding may prove beneficial to sustain prolonged ST.

Despite the premise that survival in the cold is limited by the depletion of muscle glycogen (Tikuisis *et al.*, 2002), or hypoglycaemia (glucose concentration <2.8 mM) (Haight & Keatinge, 1973; Gale *et al.*, 1981; Passias *et al.*, 1996), to date, no studies have successfully elicited these responses during prolonged cold exposure. As mentioned above, it is well documented that when muscle glycogen reserves are reduced, lipid and protein oxidation compensate to maintain a constant $\dot{H}_{prod}$ (Haman *et al.*, 2004c). However, It is unclear whether this change in fuel use is sufficient to maintain $\dot{H}_{prod}$ for a prolonged period, particularly if muscle glycogen, this limited resource, is still strongly mobilized. This has lead researchers to suggest that sparing CHO reserves through CHO supplementation may be an important strategy in prolonging thermogenesis (Haman *et al.*, 2002a; Tikuisis *et al.*, 2002).

Few studies have investigated the effects of food consumption before or during cold exposure on thermoregulatory responses and the overall energy balance (Vallerand *et al.*, 1988; Vallerand *et al.*, 1992;

Glickman-Weiss *et al.*, 1993; Vallerand *et al.*, 1993; Glickman-Weiss *et al.*, 1994; Blondin *et al.*, 2010). Of these, only Vallerand, *et al*. (1992; 1993) and Blondin *et al*. (2010) reported estimates of changes in CHO, lipid and protein oxidation in men during low to moderate intensity shivering (2.0 – 3.0 X RMR) following the ingestion of CHO. Vallerand *et al.* (1993) showed that when cold-exposed men ingested starch jellies (712 kJ or ~45g CHO) or a high-CHO bar (712 kJ; ~30g CHO, ~4g fat and ~4 g protein), at the beginning and after 90 min of cold exposure, noticeable changes in CHO and lipid utilization were only observed when ingesting the jellies. A more recent study, using a combination of stable isotopes and indirect calorimetry methodologies, showed that the relative contribution of CHO to total heat production increased by ~14% and that of lipids tended to decrease to a similar extent when glucose was ingested at a rate of 400 or 800 mg/min over 2h (Blondin *et al.*, 2010). The utilization of liver-derived glucose decreased in a dose-dependent manner, thus sparing valuable endogenous CHO reserves, while muscle glycogen utilization did not differ from the control condition. Interestingly, the rate of exogenous glucose oxidation reached a peak and plateau of 195 mg/min at the lower ingestion rate; an oxidation rate that was one-third less than what has been reported during exercise eliciting a similar metabolic rate (Pirnay *et al.*, 1982). This study also showed that 55-77% of the ingested glucose was not oxidized and thus was unaccounted for. Presumably the remaining portion was directed towards non-oxidative disposal. Ingesting multiple transportable CHO (glucose with fructose) increased the rate of exogenous glucose oxidation even further (Blondin *et al.*, 2012) while also potentially increasing non-oxidative disposal as a result of the gluconeogenic properties of fructose metabolism.

While CHO ingestion has received a lot of attention, far less is known on the effects of lipid and protein feeding on cold endurance. The practice of ingesting protein and/or fat during cold exposure is one that has been employed around the world for centuries by Northern inhabitants (Sinclair, 1953; Scott, 1956). These substrates have higher thermal effects than glucose when ingested (Tappy, 1996), but there is contradictory evidence showing that the heat released could supplement (Rochelle & Horvath, 1969) or be additive to shivering (Beavers & Covino, 1959; Vallerand *et al.*, 1992; Vallerand *et al.*, 1993). In this context, many questions remain concerning the ideal substrates mix to ingest, especially during periods of energy deficit, to improve shivering endurance.

Given that neither ST nor fuel utilization has been well documented past ~2h of cold exposure, it is unknown whether an energy deficit rapidly impairs thermogenesis during extended periods of cold exposure or whether the metabolic flexibility described in the previous section occurs (Fig. 1.2). Therefore, estimating the capacity to maintain thermogenesis during an accidental exposure to cold air of 24h is for now a difficult task.

Insights from models of shivering endurance

The stimulation of shivering is closely related to changes in both skin and core temperatures which are the ultimate driving force of ST (Tikuisis & Giesbrecht, 1999; Eyolfson *et al.*, 2001; Xu *et al.*, 2005). It is important to note that even without significant changes in core temperature, shivering intensity increases progressively as average skin temperature decreases. Under compensable cold conditions, where $\dot{H}_{prod}$ match rates of heat dissipation and core temperature do not decrease, the stimulation of shivering and total thermogenic rate can occur rapidly with quick alterations in skin temperature (Imbeault *et al.*, 2013). In such conditions, survival time depends on how long the metabolic demand can be sustained to offset heat loss. Models of shivering endurance have been developed in order to predict the onset of a shivering fatigue where, presumably, $\dot{H}_{prod}$ can no longer be maintained (Wissler, 1985; Haman, 2006). Based on a model of endurance time during exercise and its relation with muscle glycogen depletion, Wissler (1985), developed a model of shivering endurance. Later, Tikuisis *et al.* (2002) tested its accuracy during cold water immersions. Their results showed that shivering continued well beyond estimates of fatigue. They also observed, concurrently with an increase use of lipids, habituation, or a lower drive to shiver given a reduced core temperature and no change in $\dot{H}_{prod}$. In contrasts, during a milder cold exposure, first measurements with an isotopic tracer of muscle glycogen oxidation rates in shivering muscles revealed a higher than anticipated use of the substrate and no habituation and shift towards lipids (Haman *et al.*, 2005). It further confirmed that there is little analogy to be made between fuel utilization during shivering and exercise (Tipton *et al.*, 1997). Consequently, a new model emerged (Haman, 2006), suggesting that shivering endurance was little affected by the fuel

mixture oxidized. Taken together, experimental results thus far available show that muscle glycogen levels likely decrease faster at the onset of shivering than previously expected, but lipids may be able, at least partly, to compensate for reduced CHO reserves. In addition, thermal habituation rather than shivering fatigue is perhaps the ultimate reason for a failure to maintain core temperature.

Unfortunately, for cold exposures long enough to characterize such responses, little empirical information is available. Castellani *et al*. (1998) performed measurements over 10h using three serial cold water immersions (20°C) of 2h. Between cold exposures, participants were warmed to return their rectal temperature to pre-immersion values of control trials (occurring at the same times during different days). Compared to control trials, rectal temperature was lower and heat debt higher for the second consecutive cold exposure. Most importantly, $\dot{H}_{prod}$ was lower during all the repeated cold exposures. These findings do not clearly indicate whether shivering muscles underwent fatigue or habituation lowered the drive to shiver. To date, however, there is no clear documentation of shivering fatigue, but as mentioned by the authors, a depletion of energy reserves could affect skeletal muscles metabolism directly or the central nervous system that activates muscle contractions.

Muscle recruitment and other thermogenic pathways

During shivering, relative intensity of contractions and other electrophysiological characteristics of muscles have been quantified with sEMG (Bell *et al.*, 1992; Haman *et al.*, 2004a; Haman *et al.*, 2004b; Ouellet *et al.*, 2012). Studies showed that the trunk and upper leg muscles are more active than those of the limbs (Bell *et al.*, 1992). They also showed that individuals rely differently on various muscles and bursts of higher intensity contractions to maintain $\dot{H}_{prod}$ (Bell *et al.*, 1992; Haman, 2006). Continuous muscle tone and bursts of higher intensities are respectively associated with the recruitment of low threshold (type I, specialized for lipid use) and high-threshold fibers (type II, specialized for CHO use) (Meigal, 2002). In this context, during moderate intensity shivering, inter-individual differences in CHO use seems to coincide with shivering burst rate. However, even when lipid oxidation is increased and CHO oxidation decreased following CHO depletion (Haman *et al.*, 2004a), total shivering intensity and

burst rate remained consistent. It has been proposed that fuel selection can be adjusted by the selective recruitment of muscles that differ in their fiber composition (Haman, 2006), but so far, no demonstration of this mechanism has been made. Similarly, it can be wondered whether brown adipose tissue (BAT) could also be selectively stimulated and affect fuel utilization at the whole-body level.

Despite the fact that human BAT has been identified in the 16th century (Gessner, 1551), its metabolic relevance in adults has, until recently, remained uncertain. This tissue, now with little doubt, could be involved in sustaining $\dot{H}_{prod}$ in the cold since its volume of activity has been quantified by ^{18}FDG uptake using PET imaging. Pioneer evaluations have reported highly variable quantities (from ~60-170g in three studies (Virtanen *et al.*, 2009; Ouellet *et al.*, 2012; Blondin *et al.*, 2014) and up to ~1 kg in another (van der Lans *et al.*, 2013)). Whether it significantly influences whole-body $\dot{H}_{prod}$, shivering intensity and fuel selection remained to be determined. It may be expected that BAT contribute the most when ST is at its minimum. As cold stress intensifies, $\dot{H}_{prod}$ from an increasingly greater recruitment of skeletal muscles should exceed BAT thermogenic capacity. Ouellet *et al*. (2012) estimated that BAT accounts for ~20% of $\dot{H}_{prod}$ when, overall, it increased by ~1.8 fold during three hours of cold exposure (Ouellet *et al.*, 2012; Blondin *et al.*, 2014). The authors also reported that BAT volume differs greatly in individuals of similar adiposity, morphology and age, and showed that these differences were related to shivering intensity. This finding that more active BAT leads lo less shivering is consistent with changes in volume of BAT in rodents (Nedergaard *et al.*, 2007). Collectively, these studies suggest BAT metabolism may play a key role in young, healthy adults exposed to cold (Ouellet *et al.*, 2012). In this line of thought, advances in our understanding of BAT metabolism in humans bring forth many questions concerning its role in survival in the cold. For instance, it has never been investigated whether NST can be increased to supplement fatiguing shivering muscles. In fact, changes in ST have rarely been assessed with sEMG. It was more often assumed that $\dot{H}_{prod}$ was related only to ST rather than to a combination of ST and NST. Stimulating NST could be a strategy to prolong the compensation of heat dissipation during sustained

cold exposure. Current models would be greatly challenged following a demonstration of this potential mechanism.

Rationale for the study

During cold exposure, physiological processes that increase $\dot{H}_{prod}$ are activated to compensate heat loss and prevent a decrease in core temperature. In attempts to further understand the capacity to sustain this metabolic demand, models of shivering endurance have been developed (Wissler, 1985; Tikuisis *et al.*, 2002; Haman, 2006). The premise was that ST endurance can not be sustained for extended periods of time. As such, in initially compensable cold conditions, estimates of ST capacity forecast when the thermogenic rate can no longer offset heat dissipation and maintain core temperature. Models focus on a potential dependence of ST on muscle glycogen and the most recent one is the only based on actual rates of muscle glycogen utilization measured during shivering (Haman, 2006). The predictions of this model are depicted in figure 1.1A. Accordingly, muscle glycogen is depleted in roughly 20h no matter what are the initial levels of the substrate. Of course, the biggest limitation lies on the assumptions that the relative contribution of the various energy sources and the thermogenic rate remain constant over time. This is highlighted by an unlikely faster depletion of muscle glycogen predicted when initial CHO reserves are high due to a greater initial reliance on this fuel. The second panel shows the theoretical effects of different thermogenic rates. But again, the observed linearity, which results from a constant reliance on each fuel, is uncertain. The bottom panel represents the theoretical effects of food availability. The muscle glycogen model assumes that food is unavailable. In theory, however, access to sufficient amount of food containing enough CHO should prevent any important reduction of glycogen reserves. If food is ingested insufficiently, the depletion of muscle glycogen should be delayed depending on the severity of the energy deficit.

Despite a lack of experimental data for prolonged cold exposure that likely limits their reliability to predict survival, models of shivering endurance have provided important first estimates of the capacity to sustain the metabolic demand of cold. However, the premise that muscle glycogen is essential to maintain ST justifies the need to better understand whether the thermogenic rate can be sustained for

24h under cold conditions. Maintaining shivering likely depends on whether the contribution of each fuel is constant over time. Simulating an accidental 24h exposure to cold that could be seen, for instance, following ship or airliner accidents in cold and remote areas is expected to reveal the extent to which assumptions previously described are adequate (Fig. 1.2). It could enlighten responses that have not been accounted for in previous models based on short-term measurements.

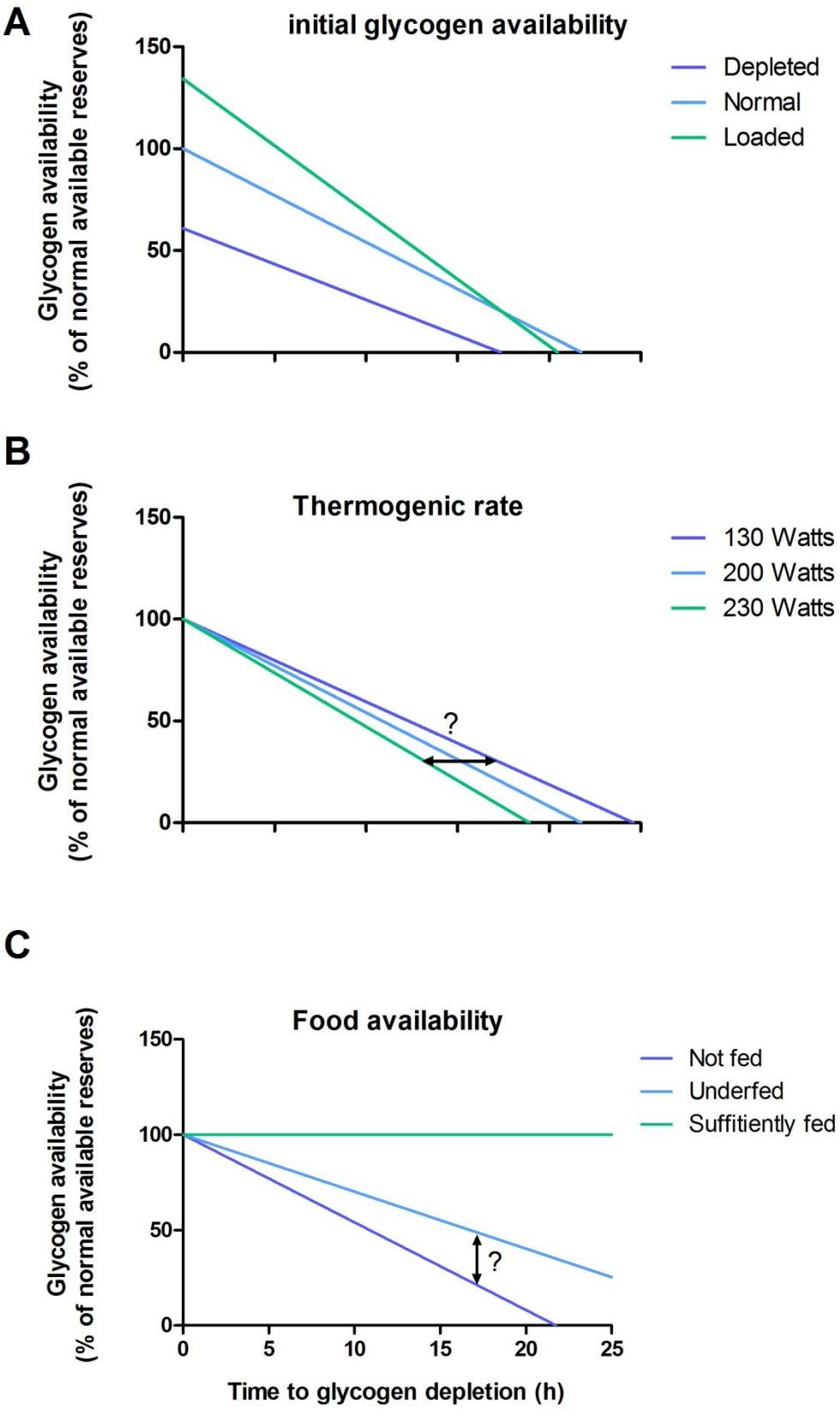
A
initial glycogen availability
Glycogen availability
(% of normal available reserves)
150
100
50
0
Depleted
Normal
Loaded
B
Thermogenic rate
Glycogen availability
(% of normal available reserves)
150
100
50
0
?
130 Watts
200 Watts
230 Watts
C
Food availability
Glycogen availability
(% of normal available reserves)
150
100
50
0
?
0
5
10
15
20
25
Time to glycogen depletion (h)
Not fed
Underfed
Suffitiently fed

Figure 1.1. Estimates of time until muscle glycogen depletion during a sustained cold exposure as a function of different inital levels of muscle glycogen (A). In this model, each fuels contribute similarly to $\dot{H}_{prod}$ over time (53, 50, and 23% $\dot{H}_{prod}$ for lipids; 28, 40, and 65% $\dot{H}_{prod}$ for CHO; and 19, 10, 12% $\dot{H}_{prod}$ for proteins with depleted, normal and loaded muscle glycogen reserves, repectively (Haman *et al.*, 2002b; Haman *et al.*, 2004c)). It is also assumed that 80% of muscle glycogen is available for oxidation with repective concentrations of 62, 102, and 137 mmol kg^{-1} of wet mass with depleated, normal and loaded glycogen reserves (Young *et al.*, 1989). Active muscle mass is considered 70% of 36 kg. Muscle glycogen oxidation is at 16, 21, and 30 µmol kg of body $mass^{-1}$ min^{-1} with depleted, normal, and loaded glycogen reserves, respectively (Haman *et al.*, 2002b; Haman *et al.*, 2004c). Furthermore, theoritical representations of the effects of different thermogenic rates (B) and feeding regimens (C) are presented in subsequent panels. Arrows indicate uncertainity in the effects of changes in these parameters.

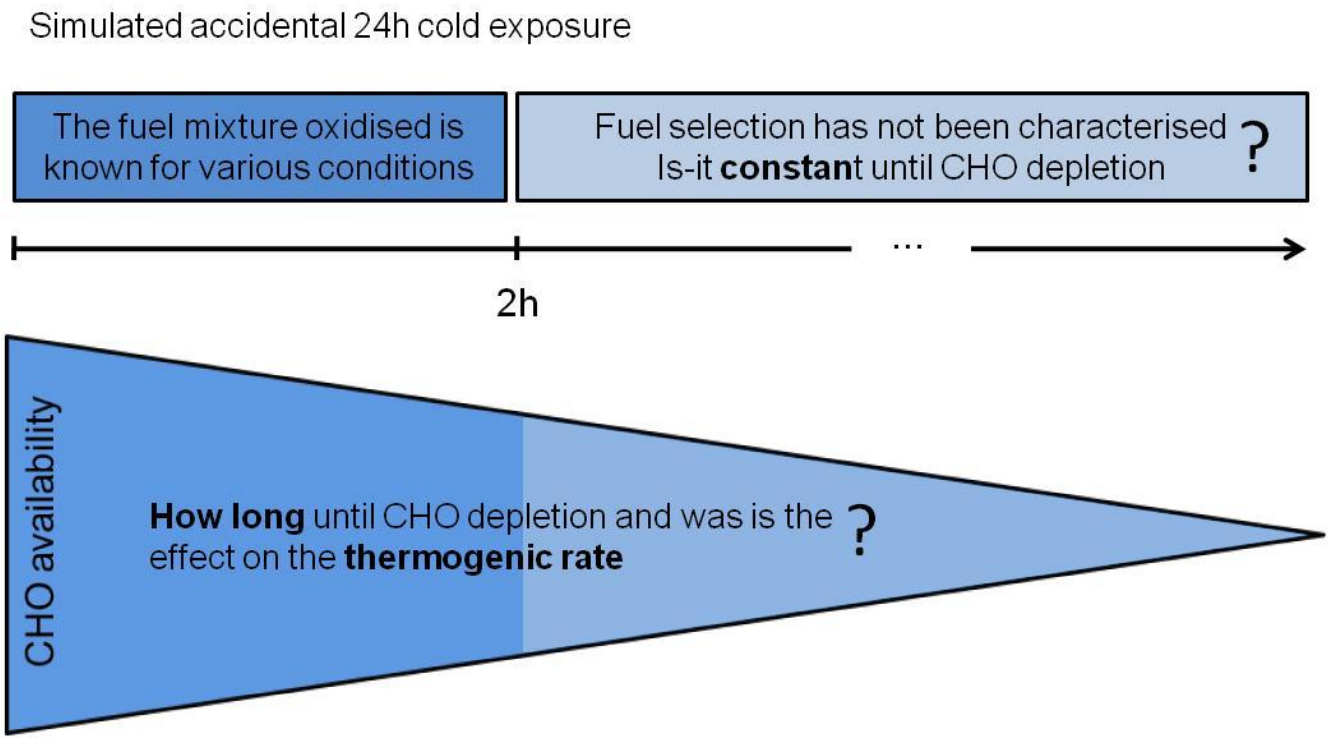

Figure 1.2. Predictions on shivering endurance capacity during 24h of accidental cold exposure are currently based on a combination of empirical evidences from exercise and from the first few hours of cold exposure. Fuel selection during shivering has not been well characterized for longer periods of time. It is therefore unclear whether a depletion of CHO stores occurs and whether it blunts thermogenesis when such a metabolic demand has to be sustained for a long time.

Goals of the investigation

The main purpose of this thesis was to quantify the metabolic demand of thermoregulation by characterizing fuel utilization and muscle recruitment during a simulated accidental cold exposure lasting 24h in underfed conditions. More specifically, over 24h in the cold, the objectives were:

1. To determine whether the thermogenic rate is constant and fuelled by a similar mixture of CHO, lipids and proteins.
2. To determine whether muscle glycogen is utilised at a constant rate until reserves are depleted.
3. To determine whether shivering muscles remain active and contribute to the same level to total thermogenesis.

Before the 24h accidental cold exposure was simulated, a one hour walk (50% $\dot{V}O_{2\ max}$) was performed to mimic hiking into the area where the accident occurred. Following this exercise, non-cold acclimatized men were exposed to 7.5°C for 24h in a thermal chamber. Survival bars, totaling 6886 kJ (1641 kcal), were given at known intervals in order to induce a daily energy deficit similar to the one of a previous study reporting an impeded thermogenesis during cold exposure (Young *et al.*, 1998). Changes in thermogenesis, fuel selection and shivering activity were monitored using a combination of indirect calorimetry, metabolic tracer and electrophysiological methods.

During the 24h in the cold, we hypothesized that:

1. Thermogenic rate will be sustained for 24h since the oxidation of lipids and proteins will compensate for any reduction in CHO availability.
2. Utilization of glucose obtained from muscle glycogen will be reduced progressively.
3. Shivering intensity and the relative contribution of various muscles will remain constant.

The thesis was organized in a classical manner where Chapter 1 presented the introduction and review of literature. The experimental protocol and the measurements are described in chapter 2. Then,

the results are presented and discussed in chapters 3 and 4, respectively. General conclusions are provided in the fifth and final chapter. The knowledge enlightened by this experiment is important to further understand the response to sustained cold exposure and increase the odds of survival in extreme conditions.

CHAPTER 2 METHODS

Participants

Eight healthy males with regular occupational or recreational experience in cold exposure were recruited for this study. Their physical characteristics are presented in Table 2.1. Maximal oxygen consumption and percentage of body fat were determined two days before the experimental sessions using a progressive treadmill test and skinfold measurements with Jackson and Pollock and Siri equations (7 sites)(Jackson & Pollock, 1978). Height and weight were measured and used to calculate body surface area (Du Bois & Du Bois, 1989). The experimental protocol was approved by the Research Ethics Board of Brock University and conforms to the principles outlined in the Declaration of Helsinki. All participants were examined by a physician and written informed consent was obtained from them prior to the experimental sessions.

Table 2.1. Physical characteristics of the participants (n = 8).

Age (years)	32.5 ± 3.8
Weight (kg)	98.8 ± 6.1
Height (cm)	179.4 ± 1.3
Body fat (%)	15.4 ± 1.8
$\dot{V}O_{2max}$ (ml•kg-1•min-1)	49.4 ± 3.6

Experimental protocol

Participants were matched in pairs for the experimental protocol in an attempt to increase the chance of completing the full 24h in the cold. Both participants reported to the laboratory at 8h in the morning following a 12h fast. Upon arrival, they ingested a telemetric capsule and were fitted with thermal and sEMG sensors (Fig. 2.1). To simulate an accidental cold exposure, participants were lightly clothed (Fig. 2.2). Cotton coveralls (Mark's Work Wearhouse "Big Bill" brand), fleece mitts (9g Glide XC Mitts - Mountain Equipment Coop) and 3 mm neoprene boots (Moque Boot High, Mountain Equipment Coop) were provided to the participants and they brought their own socks and tuques. In addition, a 5 km treadmill walk at 50% of $\dot{V}O_{2\,max}$ was performed just before the cold exposure to simulate a hike into a cold environment. After 3, 6, 9, 12,18 and 24h of cold exposure, metabolic and thermal responses as well as sEMG activity of shivering muscles were measured in the seated position. To simulate food rationing, participants were progressively fed with a total of six survival bars (Seven OceanS®, GC RIEBER COMPACT AS, Bergen, Norway). Nutritional content of their diet is described in table 2.2.

Table 2.2. Nutritional contents of the diet.

Total energy intake (kJ)	6866
Protein (%)	5-8
Fat (%)	33-43
Carbohydrates (%)	50-60
Minerals	
Sodiums (mg)	8.33
Vitamins	
Vitamin C (mg)	16.67
Vitamin B_1 (mg)	0.86
Vitamin B_6 (mg)	0.86

Based on Seven OceanS® Emergency Food Ration Data Sheet, compact for life.

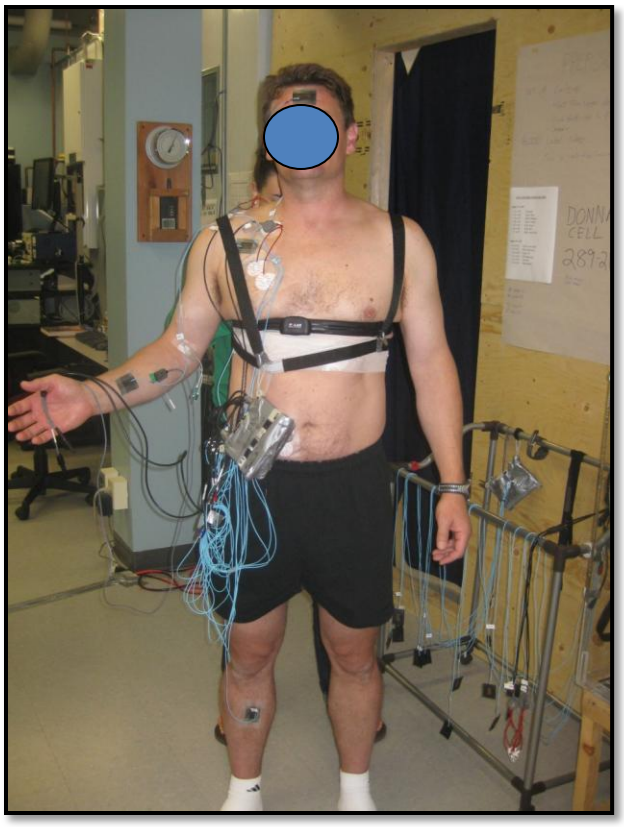

Figure 2.1. A participant fitted with heat flux and sEMG sensors.

Figure 2.2. Clothes provided to the participants. Cotton coveralls (Mark's Work Wearhouse "Big Bill" brand)(A), fleece mitts (9g Glide XC Mitts - Mountain Equipment Coop)(B) and 3 mm neoprene boots (Moque Boot High, Mountain Equipment Coop)(C) were provided. Participants brought their own socks and hats.

Figure 2.3. Thermal blanket (Ascotherm IMO 86 WA – MKIV USCG) used to warm one participant from 16 to 24h of cold exposure since his core temperature had fallen below the ethically approved threshold of 36 °C.

Environmental chamber

The cold exposure was held in a 3x3.5x2.5 m environmental chamber (Can-Trol, Mississauga, Canada) with ambient conditions set at 7.5 ^{o}C and 50 % relative humidity. The chamber contained a bunk bed, two toilets and testing equipment. This left approximately 2.5 m^2 of empty space. Participants were free-living and could rest in the bed for 2h every 6h cycle.

Thermal response and muscle recruitment

Mean skin ($\bar{T}_{skin}$) and core (T_{core}) temperatures were continuously monitored before and during cold exposure using heat flux sensors (SRP8-90961, Concept Engineering, Old Saybrook, CT) and telemetric capsules (CorTemp™, HQ Inc., Palmetto, FL), respectively. $\bar{T}_{skin}$ was calculated with the weightings of Hardy and Dubois (Hardy *et al.*, 1938) using the following sites: forehead, chest, abdomen, forearm, front thigh, back thigh, upper back, lower back, shin and calf. Shivering was measured as previously described (Haman *et al.*, 2004b) with sEMG activity of 4 muscles (*pectoralis major* (PM), *trapezius* (TR), *reactus abdominis* (RA) and *rectus femoris* (RF)). For the five participants who completed the entire study without a thermal blanket, we were able to calculate the relative contribution of various muscles to shivering activity from 12 to 24h of cold exposure.

Heat production and substrates utilization

Metabolic rate and fuel utilization were determined by indirect calorimetry. Air was pulled from a mouthpiece using a mass flow controller (50 l min^{-1},STP)(FlowKit-500, Sable System International, Las Vegas, NV). Oxygen, carbon dioxide and water contents of pulled air were measured with an S-3A oxygen analyzer, a CD-3A carbon dioxide analyzer (Applied Electochemistry, Pittsburgh, PA). volumes of expired carbon dioxide ($\dot{V}CO_2$) and inspired oxygen ($\dot{V}O_2$) were calculated with the following equations (Lighton, 2008):

$$FR_{\mathrm{i}} = FR_{\mathrm{e}} \times (1 - F_{\mathrm{e}}O_2 - F_{\mathrm{e}}CO_2 - F_eH_2O)/(1 - F_{\mathrm{i}}O_2 - F_{\mathrm{i}}CO_2 - F_{\mathrm{i}}H_2O)$$

$$\dot{V}O_2 = FR_{\mathrm{i}} \times F_{\mathrm{i}}O_2 - FR_{\mathrm{e}} \times F_{\mathrm{e}}O_2$$

$$\dot{V}CO_2 = FR_{\mathrm{e}} \times F_{\mathrm{e}}CO_2 - FR_{\mathrm{i}} \times F_{\mathrm{i}}CO_2$$

where FR_i, F_iO_2, F_iCO_2 and F_iH_2O are the flow rate and fractions of the measured gas species entering the mouthpiece and FRe, F_eO_2, F_eCO_2 and FeH_2O are the flow rate and fractions of the measured gas species leaving the mouthpiece. Urea concentration of urine samples was measured every 6h using an assay kit (BioAssay Systems, CA) and used to correct $\dot{V}O_2$ and $\dot{V}CO_2$ for the volumes of O_2 consumed (1.010 l g^{-1}) and CO_2 produced (0.0843 l g^{-1}) during protein oxidation. Rates of carbohydrate (G_{ox}), lipid (F_{ox}) and protein (P_{ox}) oxidation were calculated as follows (Haman *et al.*, 2005):

$$G_{\mathrm{ox}}\ (g\ min^{-1}) = \ 4.59\dot{V}CO_2(l\ min^{-1}) - 3.23\dot{V}O_2(l\ min^{-1})$$

$$F_{\mathrm{ox}}\ (g\ min^{-1}) = \ -1.70\dot{V}CO_2(l\ min^{-1}) + 1.70\dot{V}O_2(l\ min^{-1})$$

$$P_{ox} = \ 2.99 \times UREA_{urine}(g\ min^{-1})$$

Energy potentials of 16.3 kJ g^{-1} (carbohydrates), 40.8 kJ g^{-1} (lipids) and 19.7 kJ g^{-1} (proteins), were used to calculate $\dot{\mathrm{H}}_{\mathrm{prod}}$ and the relative contribution of each fuel (Elia, 1991; Peronnet & Massicotte, 1991).

Plasma glucose and muscle glycogen oxidation

Plasma glucose and muscle glycogen oxidation were measured during 4 blocks of 3h (3-6 h, 9-12h, 15-18h and 21-24h of cold exposure). Each block consisted of seven ingestions of 1.0 g of glucose dissolved 17.9 ml of water. The solutions were composed of corn sugar ($^{13}C/C = 0.01098$) to which a [^{13}C]glucose tracer was added (U-13C-glucose, 13C/C> 99%, Isotec, Miamisburg, OH) to obtain a final enrichment of 200 $‰\delta^{13}\mathrm{C\ VPDB}$, where VPDB is the Vienna-Pee Dee Belemnite standard.

Blood and breath samples were taken at the beginning of each block to assess background enrichment followed by the ingestion of the first solution of [^{13}C] glucose. Every 30 min until the end of each block, breath samples were collected before the ingestion of the other solutions of [^{13}C] glucose. Isotopic composition of blood glucose was determined prior to and every three hours during cold exposure and isotopic composition of expired CO_2 was measured accordingly to breath samples collections. Blood samples were kept cool and spun. Separated plasma was stored at -80 ^{o}C.

To determine the enrichment of plasma glucose (Peronnet *et al.*, 1998), proteins were precipitated from 0.50 ml of plasma by adding 0.75 ml of BaOH (0.3 N) and of $ZnSO_4$ (0.3N) before centrifugating. The supernatant was run on superimposed layers of anionic and cationic resins (AG 50W-X8 H^+, 200–400 mesh, and AG 1-X8 chloride, 200–400 mesh) to isolate glucose. Water was evaporated and glucose was oxidized with CuO. Enrichment of CO_2 produced during plasma glucose oxidation and in breath samples was determined with a Prism mass spectrometer (VG, Manchester, UK) and expressed relative to VPDB standard (Craig, 1953):

$$‰\delta^{13}\mathrm{C\ VPDB} = \left[\left({}^{13}\mathrm{C}/{}^{12}\mathrm{C}_{\mathrm{sample}}\right)/\left({}^{13}\mathrm{C}/{}^{12}\mathrm{C}_{\mathrm{standar}}\right) - 1\right] \times 1.000$$

To calculate the oxidation rate of plasma glucose, it was assumed that the cold exposure elicited a metabolic rate sufficient to limit the influence of the bicarbonate pool (Pallikarakis *et al.*, 1991). Plasma glucose (GP_{ox}) and muscle glycogen (GM_{ox}) oxidation rates were calculated with the following equations (Harvey *et al.*, 2007)(using $\dot{V}CO_2$ not corrected for protein oxidation):

$$GP_{\mathrm{ox}}\ (g\ min^{-1}) = \dot{V}CO_2 \times \left(R_{exp} - R_{\mathrm{ref\,exp}}/R_{\mathrm{glu}} - R_{\mathrm{ref\,glu}}\right)/(k_1/k_2)$$

$$GM_{\mathrm{ox}} = G_{\mathrm{ox}} - GP_{\mathrm{ox}}$$

R_{exp} is the enrichment of expired CO_2, R_{glu} is the enrichment of plasma glucose and R_{ref} is the background enrichment. The volume of CO_2 released per gram of glucose oxidized is k_1 (0.7426 l g^{-1}) and k_2 is the fractional recovery in the breath of labelled CO_2 (1).

Blood analysis

Plasma glucose and lactate concentrations were measured spectrophotometrically at 340 nm on a Beckman DU 640 (Bergmeyer, 1985) and total plasma non-esterified fatty acid (NEFA) and β-hydroxybutyrate (β-HB) concentrations were determined using assay kits (NEFA C, Wako Chemicals, Osaka, Japan and Sigma kit no. 310, Sigma-Aldrich Canada, Oakville, ON). Plasma insulin concentration was measured using a radioimmunoassay (no. KTSP-11001, Medicorp, Montréal, QC).

Statistical analyses

One-way repeated measures ANOVAs were run to determine changes over time in isotopic compositions, substrates metabolisms, $\bar{T}_{skin}$, T_{core}, $\dot{V}O_2$, $\dot{V}CO_2$ and shivering (SPSS for Windows version 21; SPSS, Chicago, IL). Following significant main effects of time, *post hoc* analyses were performed with Bonferonni-Holm corrections for multiple comparisons to detect differences with resting values, pre-ingestion values for isotopic compositions and 12h values for the contribution of muscles to shivering. Due to small sample size and to better evaluate the initial and the prolonged responses to cold, another statistical model was developed with baseline, 6h and 24h values where differences were assessed with Fischer's LSD test following significant main effects of time. Furthermore, paired t-tests were run to detect changes in blood metabolites over cold exposure. Results were considered statistically significant when $p \leq 0.05$, unless indicated otherwise. Data are presented as mean $\pm$ standard error of the mean.

CHAPTER 3 RESULTS

Thermal response

Changes in $\bar{T}_{skin}$, T_{core}, $\dot{V}CO_2$ and $\dot{V}O_2$ are presented in figure 3.1. $\bar{T}_{skin}$ decreased from 32.7 ±0.3 ^{o}C in ambient conditions to 27.6 ±0.2 ^{o}C (p <0.05) after 24 h of exposure to 7.5 ^{o}C. T_{core} remained unchanged for the first 6h and had decreased by 2 % after 24h (from 37.4 ± 0.1 to 36.6 ± 0.1 ^{o}C; p <0.05)(Fig. 3.1A). Reducing environmental temperature resulted in a significant increase in $\dot{V}O_2$ (from 0.273 ±0.043 to 0.388 ±0.063 l min^{-1}; main effect of time; p = 0.056) and in no change in $\dot{V}CO_2$ (p = 0.173). Three participants did not fully adhere to the protocol and were consequently excluded from statistical analysis. Two of them voluntarily withdrew themselves from the study, one after 6.5h in the cold and another after 13h. The other completed the study under a thermal blanket (Ascotherm IMO 86 WA – MKIV USCG)(Fig. 2.3) since his core temperature had fallen below the ethically approved threshold of 36.0 $^{\circ}$C after 16h. Body mass index was 31.0 and body fat 16.0% on average for participants who completed the study and 30.0 and 14.4 % for those who did not. The high body mass index resulted from a higher than average fat free mass since body fat was ranging from 12.6 to 23.6 %.

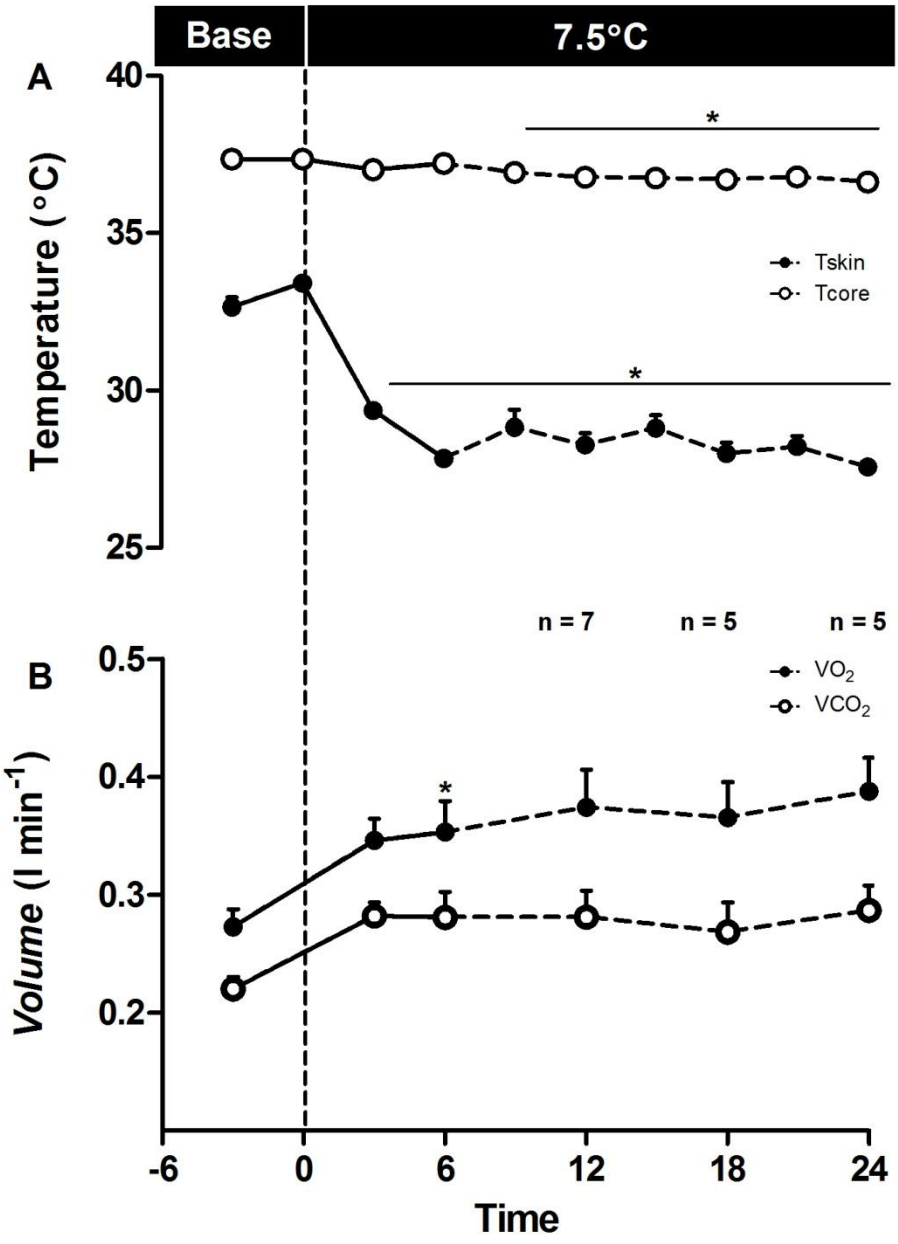

Figure 3.1. Changes in core and mean skin temperatures (A), $\dot{V}CO_2$ and $\dot{V}O_2$ (B) before and during cold exposure. *Significantly different from baseline before cold exposure (p <0.05). An effect of time on $\dot{V}O_2$ was observed at p = 0.056.

Metabolic fuel selection

The oxidation rates of CHO and lipids and their relative contributions to $\dot{H}_{\mathrm{prod}}$ are shown in Figure 3.2. At 22 ^{o}C , CHO and lipid oxidation rates averaged 97.8 ±18.1 and 74.1 ±11.8 mg min^{-1}, respectively. At 7.5 ^{o}C, higher rates of heat production elicited a progressive reduction in CHO oxidation (30.5 ±10.1 mg min^{-1} after 24 h (p <0.05, Fig. 3.2A)). Reduced CHO utilization was compensated by an increased oxidation of lipids, plateauing to 146.5 ± 17.8 mg min^{-1} after 12 h (p <0.06, Fig. 3.2A). Lipids relative contribution reached 82.8 ± 3.0 % of $\dot{\mathrm{H}}_{\mathrm{prod}}$ (p <0.05) after 18h whereas CHO contribution non-significantly decreased to 6.2 ± 1.9 % $\dot{\mathrm{H}}_{\mathrm{prod}}$ by the end of the experiment (Fig. 3.2B).

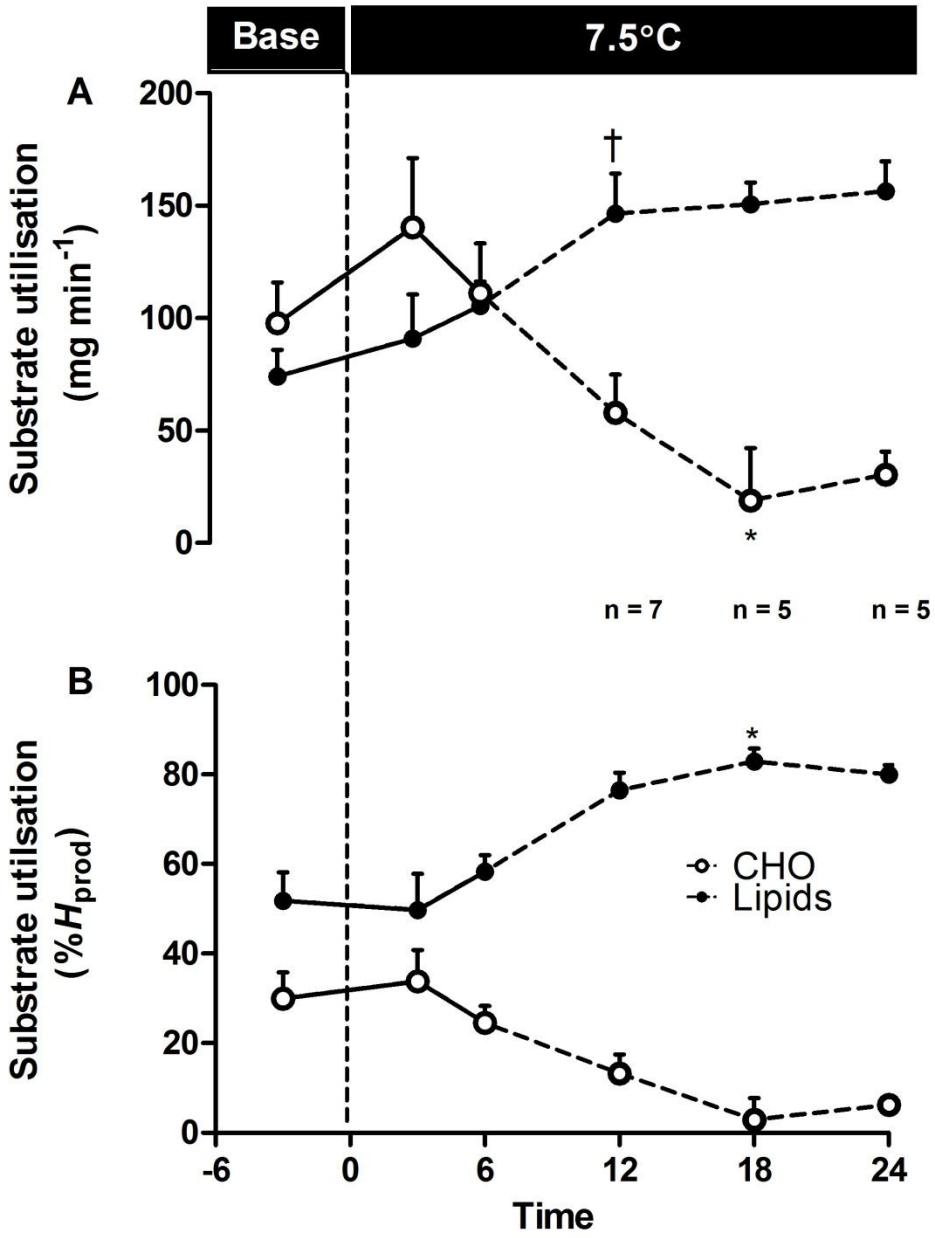

Figure 3.2. The utilization rate (A) and relative contribution to heat production (B) of carbohydrates (CHO) and lipids before and during cold exposure (values are corrected for protein utilization). Significantly different from baseline before cold exposure *(p <0.05), †(p <0.06).

Plasma glucose and muscle glycogen oxidation

Isotopic composition of breath CO_2 and plasma glucose are respectively presented in figure 3.3A and B. Solutions of labelled glucose were repeatedly ingested seven times from 3 to 6, 9 to 12, 15 to 18 and 21 to 24 h of cold exposure. Breath $^{13}CO_2/^{12}CO_2$ ratios rose with the ingestions of the tracer (main effects of time: p <0.05, Fig. 3.3A). Accordingly, plasma glucose ^{13}C enrichment was higher after the ingestions (p <0.05) and the pool had turned over within 3h after the 7th ingestions (Fig. 3.3B). Changes throughout cold exposure in plasma concentrations of glucose, insulin, ß-HB and NEFA are presented in table 3.1. Glucose and insulin concentrations remained unchanged while ß-HB and NEFA levels respectively increased to 682 ± 71 and 718 ± 82 µmol l^{-1} (*ps* <0.05).

Oxidation rates and relative contributions to $\dot{H}_{\mathrm{prod}}$ of the different substrates sources are summarized in table 3.2. From 6 to 24h of cold exposure, plasma glucose oxidation rate consistently increased (from 16.1 ±4.4 mg min^{-1} to 49.8 ±7.0 mg min^{-1}) whereas muscle glycogen oxidation decreased (from 95.0 ±35.8 mg min^{-1} to -19.3 ±10.2 mg min^{-1})(main effects of time: p <0.05). Protein oxidation averaged 53.2 ± 2.2 mg min^{-1} and remained unchanged.

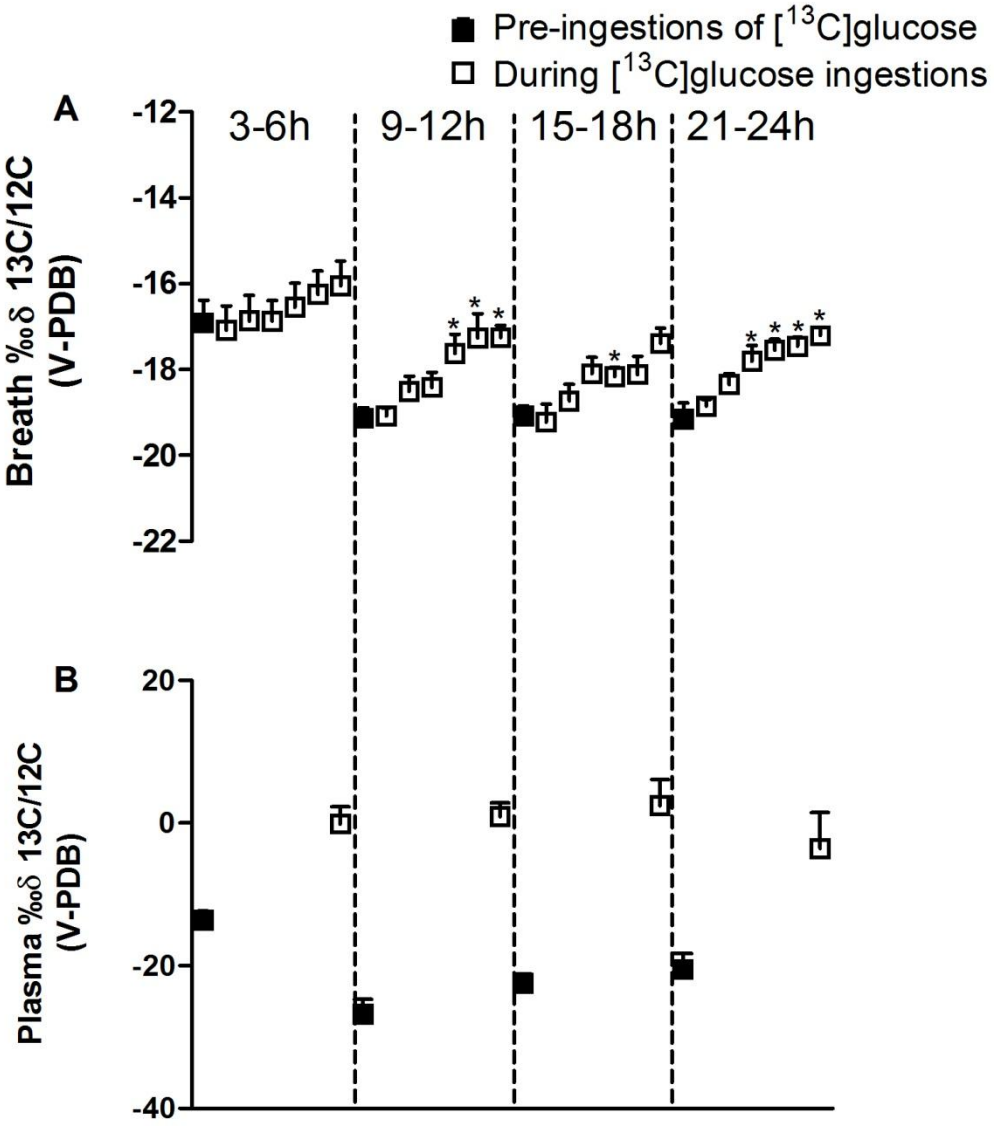

Figure 3.3. Breath CO_2 (A) and plasma glucose (B) isotopic enrichment before and during 4 blocks of 7 repeated ingestions of ^{13}C labelled glucose from 3-6, 9-12, 15-18, and 21-24 h of cold exposure. *Significantly different from pre-ingestion (p <0.05).

Table 3.1. Plasma concentrations of glucose, insulin, beta-hydroxybutyrate (ß-HB), and non-esterified fatty acids (NEFA) before and by the end of a simulated accidental 24h cold exposure.

	Baseline	Cold
Glucose (mmol l^{-1})	4.6 ± 0.2	4.7 ± 0.3
Insulin (pmol l^{-1})	20.7 ± 5.3	17.3 ± 4.7
ß-HB (µmol l^{-1})	457.4 ± 55.9	645.4 ± 68.4*
NEFA (µmol l^{-1})	556.7 ± 81.0	718.2 ± 82.2*

*p <0.05. n = 6 at baseline and 5 after 24 h of cold exposure

Table 3.2. Absolute oxidation rates and relative contribution of substrates to total heat production before and during cold exposure.

		Baseline	3h	6h	12h	18h	24h
Total CHO	mg min^{-1}	97.8 ± 18.1	140.4 ± 30.8	111.0 ± 22.3	57.9 ± 17.1	19.0 ±23.3*	30.5 ± 10.1
	% H_{prod}	30.0 ± 5.8	33.8 ± 7.0	24.5 ± 3.8	13.2 ± 4.3	2.9 ± 4.8	6.2 ± 1.9
Plasma glucose	mg min^{-1}	-	-	16.1 ± 4.4	27.7 ± 3.0	26.6 ± 9.3	49.8 ± 7.0
	% H_{prod}	-	-	4.0 ± 1.2	6.2 ± 0.4	5.6 ± 1.5	10.5 ± 1.3
Muscle glycogen	mg min^{-1}	-	-	95.0 ± 35.8	20.2 ± 18.8	- 7.7 ± 19.8	-19.3 ± 10.2
	% H_{prod}	-	-	21.0 ± 6.5	3.6 ± 4.6	-2.7 ± 4.7*	-4.3 ± 2.1*
Lipids	mg min^{-1}	74.1 ± 11.8	91.0 ± 19.6	105.5 ± 10.9	146.5 ± 17.8†	150.6 ± 9.7	156.4 ± 13.3
	% H_{prod}	51.8 ± 6.3	49.7 ± 8.0	58.3 ± 3.7	76.5 ± 3.9	82.8 ± 3.0*	80.0 ± 2.1
Proteins	mg min^{-1}	53.2 ± 2.2	61.7 ± 4.8	61.7 ± 4.8	40.5 ± 2.6	52.2 ± 4.0	54.55 ± 4.2
	% H_{prod}	18.9 ± 1.4	17.2 ± 1.6	17.7 ± 2.3	10.6 ± 1.0	14.4 ± 1.9	14.0 ± 1.8

*p <0.05; †p <0.06; Comparisons with baseline values. n = 8 at baseline, 3, and 6 h . n = 7 at 12 h. n = 5 at 18 and 24 h

Figure 3.4 shows changes in thermal and metabolic responses occurring from baseline to 6 and 24h of cold exposure. $\dot{H}_{\mathrm{prod}}$ increased in the cold (p <0.05) and did not change from 6 to 24h (p = 0.621, Fig 3.4A), while $\bar{T}_{\mathrm{skin}}$ on average was decreased by 15.5 % (p <0.05) and did not further change after 6h (p = 0.367, Fig 3.4A). Conversely, T_{core} was unchanged after 6h and had decreased by 2.0% after 24h (p <0.05, Fig. 3.4B). No effect of time was observed for CHO oxidation rate (p = 0.065), but a paired t-test suggests that it was lowered by 59.9 mg min^{-1} after 24h. Lipid oxidation was increased by 74.1 mg min^{-1} after 24 h (p <0.05) and protein oxidation remained at a similar level (p = 0.059)(Fig 3.4C). Therefore, CHO and lipids contributions to $\dot{H}_{\mathrm{prod}}$ changed, respectively reaching 6.2 and 80.0 %$\dot{H}_{\mathrm{prod}}$ after 24h (p <0.06 baseline; p <0.05 6h)(Fig. 3.4D).

Muscle recruitment

Shivering reached 4.5 ± 0.9 % of maximal voluntary muscle activity and remained consistent throughout cold exposure. The relative contributions to total shivering intensity from 12 to 24 h of the PM, the TR, the RF, and the RA are presented in figure 3.5. The TR contributed less to shivering activity after 18 (p <0.05) and 24 h (p <0.05) and the RF contributed more after 24h (p <0.05). A significant main effect of time was observed for the PM, however, *post hoc* comparisons did not reveal a significant increase in its contribution. The contribution of RA was low and did not change.

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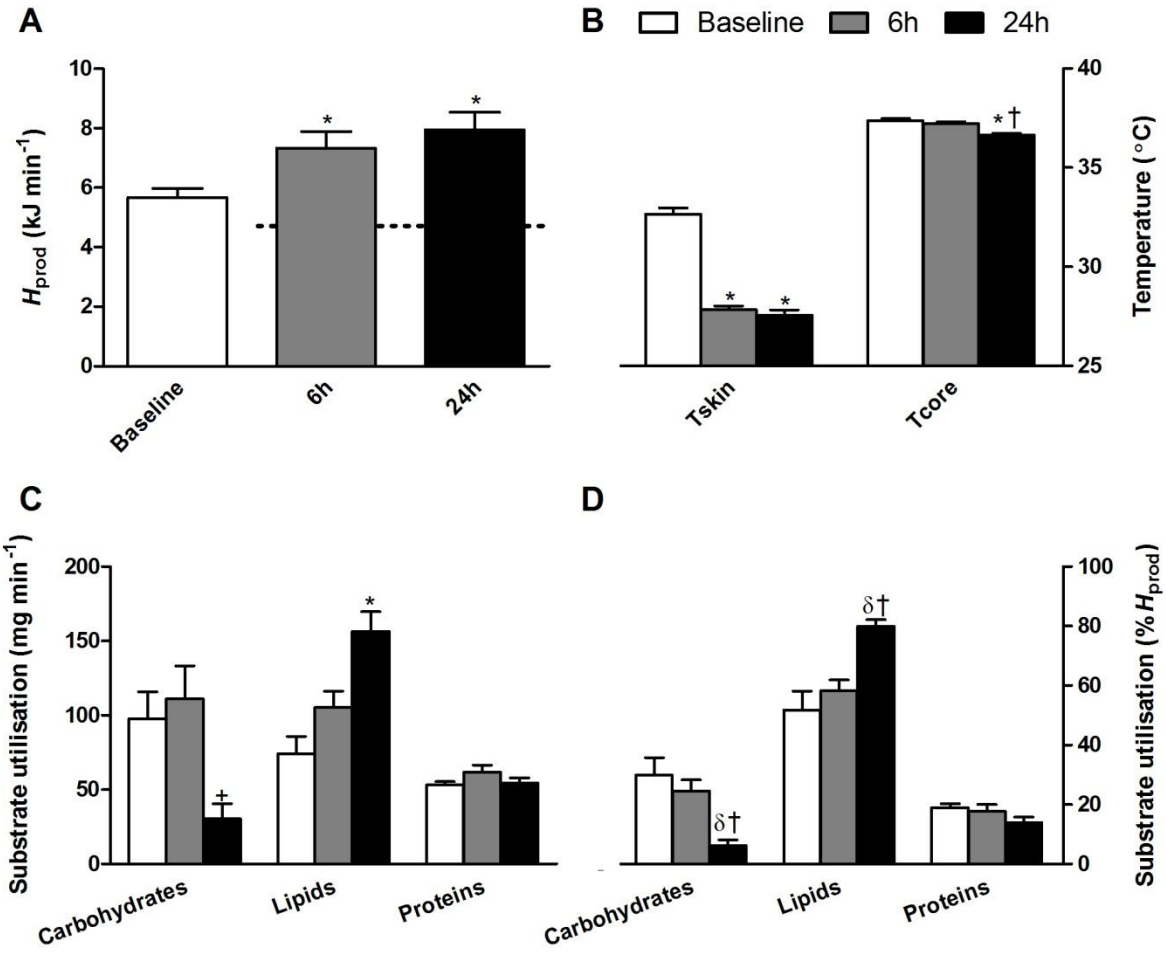

Figure 3.4. Heat production ($\dot{H}_{prod}$: A), Skin ($\overline{T}_{skin}$: B) and core (T_{core}: B) temperatures, absolute oxidation rates (C) and relative contributions to heat production (D) of carbohydrates, lipids and proteins during baseline and after 6 and 24h of cold exposure (n = 8,8, and 5, respectively). The dashed line represents average energy intake. *Significantly different from baseline (p <0.05). †Significantly different from 6 h (p <0.05). δSignificantly different from baseline (p <0.06).+Significantly different from baseline with a paired t-test (p <0.05) in the absence of a significant main effect of time.

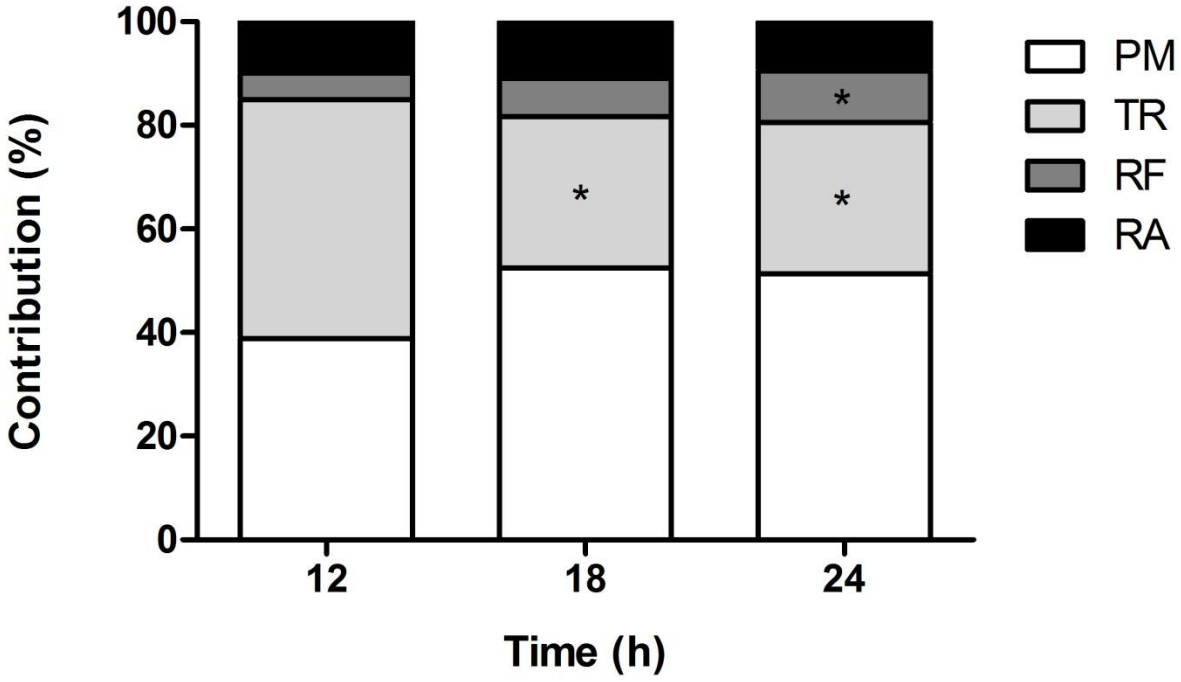

Figure 3.5. Relative contribution (%) of the pectoralis major (PM), the trapezius (TR), the rectus femoris (RF), and the rectus abdominis (RA) to shivering activity (n = 5). *Significantly different from 12h (p <0.05).

CHAPTER 4 DISCUSSION

The goal of this thesis was to quantify the metabolic demand of thermoregulation by characterizing metabolic substrate utilization and muscle recruitment during a simulated accidental cold exposure lasting 24h in underfed conditions. Results showed that while total $\dot{H}_{prod}$ remained constant (Fig. 3.4), fuel selection changed substantially towards lipid utilization (80% $\dot{H}_{prod}$)(Fig. 3.2 and 3.4). This study provides evidence supporting that an increasingly reduced use of muscle glycogen explains a minimal reliance on CHO for thermogenesis (~6% $\dot{H}_{prod}$) during prolonged cold exposure (Table 3.2). Adjustments in fuel selection, surprisingly, coincided with changes in the contribution of different muscles (Fig. 3.5) and were not related to a modification of shivering intensity.

To date, models of shivering endurance assume that metabolic fuels are all use at a constant rate until the limited CHO reserves are depleted and hence impair the maintenance of $\dot{H}_{prod}$ (Wissler, 1985; Haman, 2006). No data was available to clearly support or reject this hypothesis. We therefore simulated this accidental cold exposure of 24h. Collectively, results disprove the hypothesis that metabolic fuels are used at a constant rate and dismiss models of shivering endurance. The metabolic demand can be met for longer than previously estimated because of a shift in fuel use towards lipid dominance. Of all fuel sources monitored in this study, only proteins appeared unaffected.

Simulated cold exposure

Following a 5km exercise mimicking a hike into a cold environment, lightly clothed men were exposed to 7.5 ^{o}C for 24h and underfed with survival rations. We found that the level of $\dot{H}_{prod}$ elicited (8 kJ min^{-1}) was sustainable for the entire simulation (Fig. 3.4), resulting in an average energy deficit of ~3400 kJ day^{-1} (Appendix I). This maintenance of the metabolic demand somewhat contrasts with the findings of Castellani et al. (1998) who reported a reduction of thermogenesis during serial cold water immersions over 10h. They proposed that shivering fatigue or habituation was responsible for this change. In this context, we found that $\dot{H}_{prod}$ in the prolonged phase (24h) of the cold exposure was the same as in the acute phase (6h), despite a fall in T_{core} during this period (Fig. 3.4). This indicates a form of

habituation. Such a response where thermogenesis could be reduced for a given input in the thermoregulatory system has been observed following intermittent cold acclimations in air (Silami-Garcia & Haymes, 1989; Hesslink *et al.*, 1992) and in water (Young *et al.*, 1986; Golden & Tipton, 1988). The current study shows this can occur within a day of exposure to cold air. Tipton *et al.* (1998) gave evidence that the mechanism involved in the habituation of the initial response to cold originates from central pathways of the nervous system rather than peripheral thermoreceptors. A lack of change after two months of cold exposure in static and dynamic behaviours of cold receptors in cats (Hensel, 1981) supports this finding. The advantage of this shift in the thermoregulatory set point remains unclear, but it may appear unattractive as T_{core} did not remain at pre-exposure levels (2% decrease over 24h)(Fig. 3.4). Perhaps it is an adjustment, during times of food scarcity, that spares endogenous fuels in order to sustain an increased metabolic demand for a long time.

Fuel selection

Upon cold exposure, CHO utilization is stimulated in order to fuel shivering (Vallerand *et al.*, 1999; Haman *et al.*, 2002b; Haman *et al.*, 2005) and even in CHO depleted individuals (Haman *et al.*, 2004c). Here, we demonstrate that prolonged shivering can instead be maintained with a down-regulation of CHO oxidation (Fig. 3.4). A minimal reliance on this pathway (~6% $\dot{H}_{prod}$) is compensated by a substantial increase in lipid oxidation (+90%)(Fig. 3.4). These results confirm that even during prolonged cold exposure, shivering can be maintained by supplying very little CHO to oxidative metabolism. It has long been used as a research rationale (Martineau & Jacobs, 1989; Young *et al.*; Haman *et al.*, 2004c) and in modeling efforts (Wissler, 1985; Haman, 2006) that depleted CHO reserves could limit shivering endurance. In contrast, this study demonstrates that a depletion of CHO stores can either be prevented or compensated by adjusting fuel selection.

The effects of depleting glycogen reserves have been investigated using dietary and exercise manipulations prior to 1.5 to 3h of cold exposure (Martineau & Jacobs, 1989; Young *et al.*; Haman *et al.*, 2004c). In all cases, a reduced CHO availability elicited a higher reliance on lipids. However, CHO oxidation rates, according to the only study reporting them (Haman *et al.*, 2004c), still increased at the

onset of shivering when glycogen reserves were low. Could this imply that the low CHO-based thermogenesis (~6 % $\dot{H}_{prod}$)(Fig. 3.4) elicited in the current study may not be related to a severe reduction of CHO reserves? As discussed later, more glucose was ingested during the study than oxidized. We can thus speculate that a high use of lipids is rather a metabolic response that, when shivering is sustained for a long time, spares glycogen reserves. Such a response allows the maintenance of the metabolic demand for a long time since there is no doubt that fat is the most abundant and energy dense form of energy storage (Weber, 2011). Likewise, similar patterns of fuel selection where lipid oxidation substitutes CHO use (Vaillancourt *et al.*, 2009), or at least predominates substantially (Vaillancourt *et al.*, 2005), have been recognized in Wistar rats and Ruff sandpipers exposed to similar temperatures. The underlying mechanism is not well understood but was associated with a diversion of circulating NEFA from re-esterification towards oxidation in the rat (Vaillancourt *et al.*, 2009). Previous work in humans indeed revealed that lipolysis of adipose tissue is stimulated upon cold exposure (Vallerand *et al.*, 1999; Ouellet *et al.*, 2012). Consistently and as others reported (Tipton *et al.*, 1997; Vallerand *et al.*, 1999; Haman *et al.*, 2002b), circulating NEFA increased in this experiment (Table 3.1). In this context, human investigations also indicated that an elevation of plasma NEFA is compensated by insulin secretion in the short-term -but not in the long-term, resulting in an increase in lipid oxidation from 6 to 24h (according to a smaller respiratory quotient)(Paolisso *et al.*, 1995; Carpentier *et al.*, 1999). This effect could explain why CHO oxidation is stimulated at the onset of shivering (Vallerand *et al.*, 1999; Haman *et al.*, 2002b; Haman *et al.*, 2005) but later inhibited during prolonged cold exposure (Fig. 3.4). Together, these findings indicate that circulating NEFA perhaps play a role not only in fueling, but also in stimulating a shift to lipid oxidation. Further work is required to investigate this possibility. Measuring the currently unknown contributions during cold exposure of circulating and intramuscular lipids would provide valuable insights into the role of blood NEFA.

In regards to protein utilization, urea excretion during 24h of cold exposure reveals no change from pre-exposure levels (Table, 3.2). This is in agreement with observations during acute shivering (1.5h) of low and moderate intensities (Haman *et al.*, 2005). However, in glycogen depleted individuals,

protein oxidation is high before cold exposure and remains similarly elevated into the cold (Haman *et al.*, 2004c). A normal rate of proteins utilization could suggest that CHO reserves have been less affected here. Such a difference in protein usage could be linked to discrepancies between protocols: The administration of a low-CHO diet prior to this previous study can have increased protein utilization and the ingestion of substrates during the current study can have spared body proteins.

Plasma glucose and muscle glycogen

Earlier studies have shown that hypoglycemia blunts thermoregulation by lowering thermogenesis (Haight & Keatinge, 1973; Passias *et al.*, 1996), but whether or not it can naturally occur during shivering remains unclear. Here, measurements of ^{13}C enrichment in expired CO_2 and blood glucose imply that the oxidation of plasma glucose increased as total CHO oxidation was reduced (Table 3.2). Despite the observed shift to plasma glucose as a source of energy following half a day of cold exposure (Table 3.2), oxidation rates were relatively low and circulating levels unchanged (Table 3.1). These results demonstrate that hypoglycemia is unlikely during sustained cold exposure in these conditions. The ingestion of survival rations likely favoured the maintenance of blood glucose to adequate levels.

Previously, muscle glycogen have been either depleted or loaded prior to cold exposure, which respectively decrease or increase its utilization (Martineau & Jacobs, 1989; Young *et al.*; Haman *et al.*, 2004c) while plasma glucose remained a minor fuel (Haman *et al.*, 2004c). The isotopic tracer method reveals that a lessen usage of muscle glycogen accounts for a reduced CHO oxidation during sustained cold exposure (Table 3.2). This finding is novel because it shows a regulation of the use of muscle glycogen in the course of a cold exposure. Even though $\dot{H}_{prod}$ was unaffected, it can not be excluded that extreme shivering could be hardly sustainable without relying on muscle glycogen.

Additionally, further analysis reveals that more glucose was ingested with the survival rations than oxidized. It is therefore likely that the [^{13}C]glucose administered was not entirely oxidized without glycogen synthesis. In fact, glycogen reserves are replenish by a direct pathway and indirectly through gluconeogenesis (Kurland & Pilkis, 1989)(Figure 4.1). In the latter case, glycogen is synthesized from

pyruvate and tricarboxylic acid cycle intermediates. When this happens and a glucose tracer is administered at the same time, some labelled CO_2 that is not related to glucose oxidative disposal is released (Folch *et al.*, 2005). Folch and Péronnet (2005) revealed that endogenous glucose oxidation at rest is underestimated for this reason. In the current study, the ingestion of survival rations combined with a down-regulation of CHO utilization (Fig. 3.4), led to a similar phenomenon (Table 3.2). Nonoxidative disposal has already been noticed when glucose was ingested during cold exposure (Blondin *et al.*, 2010). It was found that the contribution of liver-derived glucose decreases in a dose-response manner.

Although CHO were the primary source of ingested energy, the survival rations used in the current study contained a considerable amount of lipids and some proteins (Table 2.2). Unfortunately, the effects of these substrates on glucose disposal during cold exposure are not well understood. It was showed that when CHO were ingested alone (712 kJ or ~45g CHO) or in combination with fat and protein (712 kJ; ~30g CHO, ~4g fat and ~4 g protein) at the onset and after 90 min of cold exposure, discernible changes in the use of CHO and lipids were only observed when CHO alone were ingested (Vallerand *et al.*, 1993). From a practical point of view, glycogen sparing and neoglucogenesis could be of great importance in sustaining the metabolic demand of cold for a long time or to adapt to more severe cooling.

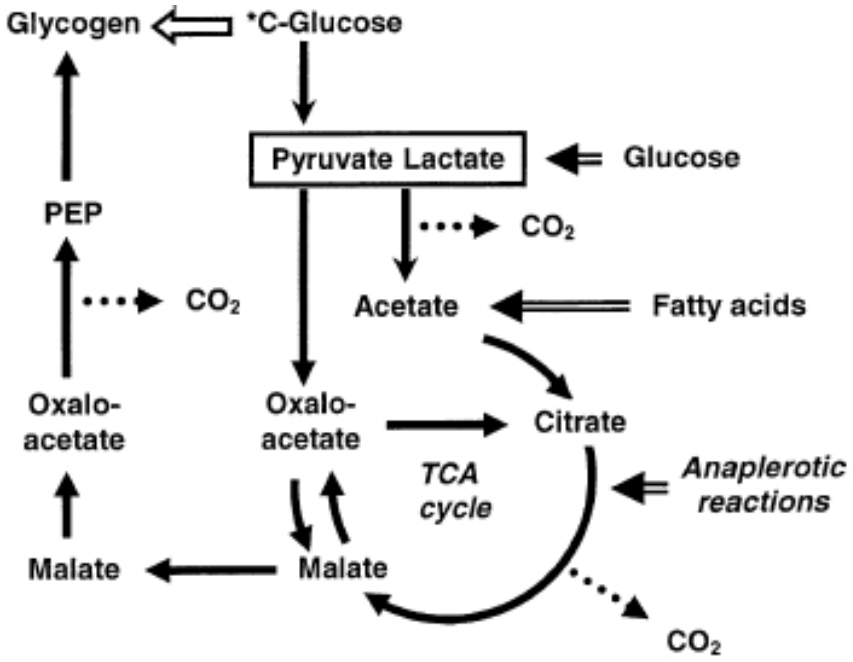

Figure 4.1. Representation of the direct and indirect pathways of glycogen synthesis from labeled glucose (*C-glucose). Glycogen is either synthesized directly or from pyruvate and tricarboxylic acid cycle intermediates resulting in the production of labeled CO_2 that is not related to oxidative disposal. Dotted arrows indicate production of labeled CO_2 and double arrows the supply of unlabeled substrates. PEP: Phosphoenolpyruvate. Adapted from (Folch *et al.*, 2005).

Models of shivering endurance

Taken together, the data presented here has important implications for modeling of shivering endurance. By relying on a model of endurance time during exercise and its known relation with muscle glycogen, a model of shivering endurance has previously been developed (Wissler, 1985). However, since it underestimated shivering endurance during cold water immersions, its accuracy was later questioned by Tikuisis et al. (2002). The authors also made observations consistent with our results of an increase in lipid utilization and a lower drive to shiver as immersions progressed. Furthermore, recent measurements of muscle glycogen oxidation during 1.5h of shivering revealed that metabolic fuels contribute differently during shivering than what is expected from our understanding of exercise (Haman *et al.*, 2005). This further confirmed that there is little analogy to be made between the two processes (Tipton et al., 1997) and led to a new model suggesting that shivering endurance has rather been overestimated (Haman, 2006). Both this model and the previous one rely on the premise that fuel utilization remained constant until the depletion of CHO stores impairs contractions of shivering muscles. Our data does not support this consistency in fuel selection. It demonstrates that shivering endurance could have been underestimated by all previous models due to a metabolic flexibility. Despite an initially high reliance on the limited glycogen reserves, lipids use can be stimulated to almost entirely fuel energy needs. Taken together with the thermal habituation described above (Fig. 3.4), these results add a new perspective towards a more accurate model of the capacity to offset heat dissipation in cold environments.

Muscle recruitment

Previous work has provided evidences that fuel selection in shivering muscles can vary: 1) by changing total shivering intensity or the number of bursts of higher intensity and thus recruit more or less glycolitic fibers (Haman *et al.*, 2004a; Haman *et al.*, 2005), 2), and by activating different pathways within the same muscles fibers (Haman *et al.*, 2004c). Since shivering intensity did not change during this experiment, the latter mechanism may, at least partly, explain adjustments in fuel selection. However, contrary to our hypothesis, the relative contribution of various muscles to shivering activity changed during the cold exposure (Fig. 3.5). It decreased for the TR whereas it increased for the PM and the RF

and remained the same for the RA. Recruiting muscles that could vary in fiber composition represent another potential, yet unproven mechanism for changes in fuel selection (Haman, 2006). To our knowledge, this study is the first to provide evidence suggesting that humans can adjust fuel metabolism by recruiting muscles in different proportions. Further work with muscular biopsies is needed to better explore the relation between the recruitment of individual muscles and fuel selection. Furthermore, results do not support that non-shivering thermogenesis contributed differently in the prolonged phase of the cold exposure when lipid oxidation was high. Nevertheless, they must be interpreted with caution since a recent study showed that sEMG activity is misleading of changes in brown adipose tissue activity (Blondin *et al.*, 2014).

CHAPTER 5 CONCLUSION

The main purpose of this thesis was to quantify the metabolic demand of thermoregulation by characterizing fuel utilization and muscle recruitment during a simulated accidental cold exposure lasting 24h in underfed conditions. In addition, the fate of plasma glucose and muscle glycogen, as well as shivering activity and the contribution of different muscles were characterized. Ultimately, this thesis contributed the following knowledge.

1- Heat production can be sustained for a long time by relying to a great extent on lipids and minimally on carbohydrates during shivering.

Following a 5 km walk, exposure to an environmental temperature of 7.5 ^{o}C for 24h, in lightly clothed men underfed with survival rations, elicited an increase in heat production that remained sustainable for the entire study. Heat production was similar despite a reduction in core temperature from the acute (6) the prolonged (24h) phase of the cold exposure. By the end the experiment, carbohydrate oxidation was minimal, lipids were accounting for 80% of heat production and protein utilization was the same as before the simulation. These results indicate that previous models of shivering endurance probably rely on inadequate premises and an incomplete understanding of the topic.

2- A reduced supply of muscle glycogen to oxidative metabolism explains the minimal reliance on carbohydrates during extended periods of cold exposure.

Results indicate that muscle glycogen oxidation decreased during the cold exposure whereas the utilization of plasma glucose increased. This demonstrates that low rates of carbohydrates oxidation were due to a reduced utilization of muscle glycogen. However, its contribution was underestimated due to non-oxidative disposal of ingested carbohydrates.

3- Total shivering intensity remains constant when shivering is maintained for a long time in given conditions, but the contribution of different muscles can change.

This thesis shows that total shivering intensity remained constant in the simulated conditions. It also demonstrates that the contribution of some muscles changed during the simulation, which yields a useful insight into underlying mechanisms for the high reliance on lipids.

From a practical point of view, the results presented here confirm that shivering endurance is not the limiting factor to the maintenance of core temperature in initially compensable cold conditions. An important role of the central nervous system in orchestrating metabolic responses is put forth. Would adequate food intake prevent a shift in the thermoregulatory set point and ultimately allowed the maintenance of core temperature? Clearly, shivering was far from its maximal intensity and could have been further stimulated. Finding ways of increasing heat production and factors that limit its stimulation should help the maintenance of thermal balance in cold environments.

The novelty of this study is mostly related to the length and the diversity of the measurements. Environmental temperature was lower than in previous long-them studies in environmental chambers (Dauncey, 1980; Warwick & Busby, 1990; van Marken Lichtenbelt *et al.*, 2002; Westerterp-Plantenga *et al.*, 2002; Wijers *et al.*, 2008; Celi *et al.*, 2010; Wijers *et al.*, 2011; Lee *et al.*, 2013). In contrast with these other studies, the measurements of heat production reported here isolate the contribution of shivering and non-shivering thermogenesis but likely underestimate the contribution of physical activity and thus total energy expenditure. Also, underfeeding as simulated in this experiment is likely to occur in a real life scenario due to food rationing. However, such a protocol makes difficult the estimation of the thermic effect of food. Westertep-Plantenga et al. (2002) demonstrated that overeating can prevent core temperature from decreasing during a prolonged cold exposure, suggesting that the thermic effect of food could play an important role in this context. For now, it remains unclear whether the heat released following food ingestion supplements or is additive to shivering thermogenesis. Experiments should be designed to answer this question.

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Appendix I - Supplementary materials

Supplementary results

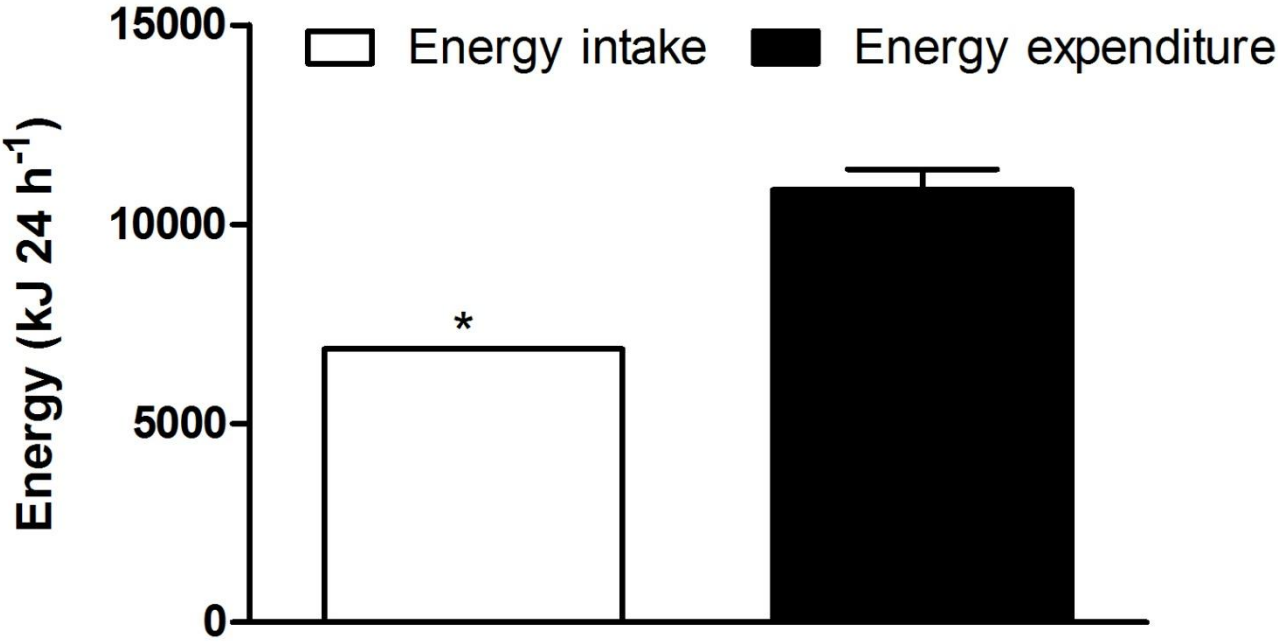

Figure S1. Energy intake and energy expenditure (kJ) for 24 h of exposure to 7.5 ^{o}C. *Significantly different from energy expenditure (p <0.05).

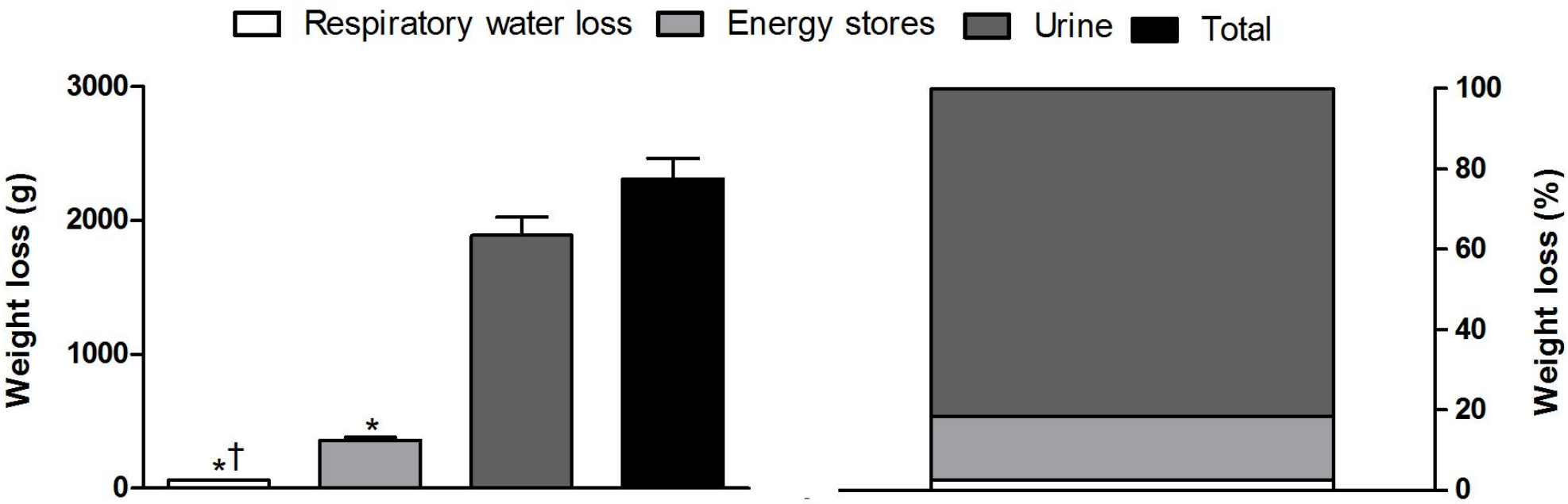

Figure S2. Different components of weight loss (g) and their relative contribution to total weight loss (%) for 24 h of exposure to 7.5 ^{o}C.

*Significantly different from urine (p <0.05). †Significantly different from respiratory water loss (p <0.05).

Energy intake and energy expenditure are presented in figure S1. Energy expenditure for 24h of exposure to 7.5 ^{o}C was 1.6 fold higher than energy intake provided in the form of survival rations (p <0.05). This resulted in an energy deficit of 3390.3 ±521.2 kJ. Weight loss fractionated between its different components is presented in figure S2. Total calculated body weight loss was of 2312.5 ±149.4 g after 24h of cold exposure (Fig. S2). Measurements with a conventional scale, for unclear reasons, gave a slightly lower change in body weight. The mismatch between energy intake and energy expenditure contributed to 15.7 ±0.7 % of weight loss. The loss of body weight was accounted by 81.7 ±0.9 % from the 1908,8 ±136.5 g of urine output and by 2.6 0.2 % from the 60.6 ±4.6 g of respiratory water loss. Accordingly, weight loss was significantly greater though urination than through respiration (p <0.05). Weight loss from energy stores was greater than weight loss through the respiratory tract (p <0.05), but smaller than weight loss by urination (p <0.05). Ingested water was 1.7 times higher than metabolic water (p <0.05). Total fluid loss was of 1969.5 ±137.6 g and significantly higher than fluid gain from metabolic and ingested water (p <0.05). Urine specific gravity was 1.008 ±0.002 and did not change significantly over cold exposure (p = 0.056), but tended to slightly increase.

Supplementary methods

Rates of respiratory water loss ($\dot{V}H_2O$) were calculated as follow (Lighton, 2008) :

$$\dot{V}H_2O = FR_{\mathrm{e}} \times F_{\mathrm{e}}H_2O - FR_{\mathrm{i}} \times F_{\mathrm{i}}H_2O$$

Where FR_i and FR_e are the flow rates of the gas respectively entering or leaving the mouthpiece. F_iH_2O and FeH_2O are the fractions of water respectively entering or leaving the mouthpiece. $\dot{V}H_2O$ was converted with a factor of 0.803 g l^{-1}. Metabolic water production was calculated by adding water yielded during carbohydrates, lipid, and protein oxidation with respective factors of 0.56, 1.07 and 0.39 g H_2O g^{-1} (Schmidt-Nielsen, 1997). The Riemann sum was used to compute total daily values of energy expenditure, respiratory water loss and metabolic water. Independent T-tests with alpha set at 0.05 were run with SPSS (version 21; SPSS, Chicago, IL) to assess differences between energy intake and energy expenditure and between fluid loss and fluid gain. A one-way ANOVA followed by Bonferroni's *post hoc* test was used to assess differences between the components of weight loss. Results are presented as mean ± standard error.