FOOD INTAKE DURING COLD EXPOSURE: EFFECTS OF THE QUANTITY OF FOOD INGESTED ON SHIVERING AND NONSHIVERING THERMOGENESIS

by

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SUMMARY

Humans are known as homeothermic endotherms. To ensure thermic balance at rest when exposed to cold, they dispose of two main thermogenic processes: shivering thermogenesis (ST) and non-shivering thermogenesis (NST). ST consists of involuntary muscle contractions and NST represents the component of H_{prod} that is not ST. While ST is difficult to tolerate, it is not yet known which nutrients and how much are required to stimulate NST and lower ST in the cold. Whether or not food caloric intake has an impact on the relative contribution of ST and NST to total H_{prod} remains to be determined. Therefore, the purpose of this thesis was 1) to quantify the effects of ingesting two quantities (1507 vs 3015 kJ) of same relative compositions on cold-induced whole-body H_{prod} and 2) to establish the effects of these two quantities of food on the relative contribution of ST and NST to total H_{prod}. Five healthy male participants were exposed to a 3h mild cold, using a liquid conditioned suit with water flowing at 15°C (COLD) or 33 °C (CON) for a total of 4 trials. Thermal, metabolic and shivering responses were measured at baseline, before and after shake ingestion. Results demonstrated that H_{prod} and ST intensity increased in the cold, while no significant differences were found between the ingested shakes at two different caloric equivalents. In addition, ST intensity did not change, which confirmed that NST remained the same between the two conditions. Thus, knowing that the caloric intake will not maximize the thermogenic effects in the cold (i.e. improve the comfort of the individual), is it more advantageous to bring food or additional clothing, for any activity? Clearly, more research on the exact pathways of each processes in the cold with food consumption needs to be made. To that extent, the investigation of the effect of food quality on changes in the thermogenic processes during cold exposure strikes us as a fascinating area for future research.

RÉSUMÉ

L'être humain est connu comme étant homéotherme et endotherme. Pour assurer son équilibre thermique au repos lors d'une exposition au froid, il dispose de deux mécanismes de thermorégulation: la thermogenèse avec frisson (TF) et la thermogenèse sans-frisson (TSF). TF consiste de contraction musculaire involontaire tandis que TSF est l'augmentation de H_{prod} sans augmentation de TF. Alors que TF peut être inconfortable, on ne sait pas quels types et de combien de nutriments le corps a besoin pour stimuler TSF et réduire TF au froid. Il reste donc à déterminer si la quantité de nourriture à un impact sur la contribution relative de TF et TSF par rapport à la H_{prod} total. Ainsi, le but de cette étude était de : 1) quantifier les effets de l'ingestion de deux breuvages différents (1507 vs 3015 kJ) mais de même composition énergétique et 2) établir les effets de ces deux quantités sur la contribution relative de TF et TSF par rapport à la H_{prod} totale. Cinq hommes en santé étaient exposés à un froid modéré d'une durée de 3h, avec un habit de refroidissement où l'eau circulait soit à 15°C (COLD) ou 33 °C (CON) pour un total de 4 sessions. Les réponses thermiques, métaboliques et de frisson ont été mesurés au repos, avant et après l'ingestion des breuvages. Les résultats ont démontré que H_{prod} et TF ont augmentés dans le froid. Néanmoins, aucune différence significative entre les deux équivalents caloriques fut démontrée. De plus, l'intensité de TF n'a pas changé, ce qui confirme que TSF est demeurée identique entre les deux conditions. Ainsi, sachant que la quantité ne maximisera pas les effets thermogéniques dans le froid (c.-à-d. améliorer le confort de l'individu), est-il plus avantageux d'apporter de la nourriture ou un vêtement supplémentaire pour une activité quelconque? De toute évidence, des recherches supplémentaires sur les voies exactes des processus du froid avec une consommation alimentaire doivent être faites. Dans cette mesure, l'étude de l'effet de la qualité des aliments lors d'une exposition au froid nous apparaît comme un domaine fascinant pour de futures recherches.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations

ATP: adenosine triphosphate

AUC: Area under the curve

BAT: Brown adipose tissue

CNS: Central nervous system

CHO: Carbohydrates

DIT: Diet induced thermogenesis

EMG: Electromyography

g: gram

H_{prod}: Whole body heat production

H_{loss}: Whole body heat loss

kJ: Kilojoules

l: liter

LCS: Liquid conditioned suit

LIP: Lipids

min: minute

MVC: Maximal voluntary contractions

NST: Non-shivering thermogenesis

PEC: Pectoralis

PRO: Protein

RMS: Root mean squares

RMR: Resting metabolic rate

SCM: Sternocleidomastoid

SD: Standard deviation

SEM: Standard error of the mean

SNS: Sympathetic nervous system

ST: Shivering thermogenesis

T_{core}: Core temperature

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

TRA: Trapezius

UCP1 : Uncoupling protein 1

VAS : Vastus lateralis

VCO_2: Carbon dioxide consumption

VO_2: Oxygen consumption

Symbols

°C: degree Celsius

%: percentage

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*Significantly different from COLD, $p < 0.05$.
†Significantly different from baseline values before cold exposure, $p < 0.005$
‡Significantly different from control or cold, $p < 0.005$

CHAPTER 1: GENERAL INTRODUCTION

INTRODUCTION

The human body has developed several key physiological and metabolic mechanisms to resist decreases in ambient temperatures. These include increased peripheral vasoconstriction as well as the stimulation for heat production via non-shivering thermogenesis (NST) and shivering thermogenesis (ST) (Haman, 2010). ST uses various metabolic fuels based on the nutrition or reserves of the individual. For example, with normal glycogen reserves and when exposed to mild cold (2.3 xRMR), the relative contribution of lipids (LIP) is of greater importance compare to carbohydrates (CHO) and proteins (PRO). As shivering intensifies (3.5 xRMR) CHO becomes dominant (Haman et al., 2002, 2005). However, it is not yet known which nutrients and how much the body needs to stimulate NST and lower ST in the cold. The modulation of these two parameters are of interest particularly because ST can be quite uncomfortable (Haman et al., 2004a). This thesis focuses on the effects of ingesting food with different energy equivalent on changes in the contribution of ST and NST to total heat production (H_{prod}). In this context, this review will be aimed at providing current knowledge as it relates to 1) cold-induced thermogenesis, 2) fueling of shivering thermogenesis and 3) the changes in metabolic responses in the cold following the ingestion foods of varying energy equivalents.

Cold-induced Thermogenesis

When exposed to cold, the body needs to increase H_{prod} to maintain a stable core temperature (T_{core}). Pathways to reduce H_{loss} are activated by mechanisms of efferent signals by the central nervous system (CNS) (Muzik et al., 2018). As an initial response to cold temperatures, vasoconstriction of peripheral blood vessels minimizes heat transfer from the skin to the surrounding environment (Castellani & Young, 2016; Nishimura et al., 2015). As H_{loss} continues, H_{prod} increases to counteract the loss of additional heat and maintain T_{core} around 37°C. This is of

particular importance to avoid T_{core} below 35°C where brain function is affected from reduced rates of chemical reactions (Shephard, 1985). Even when ambient temperature drops, T_{core} remains unchanged under compensable cold conditions (Haman et al., 2007). In contrast, under non-compensable conditions such as extreme cold air temperatures or cold-water immersion, T_{core} drops continuously if behavior is not modified or until the individual removes himself from the environment (Gordon et al., 2019).

ST consists of involuntary muscle contractions of the skeletal muscles aimed specifically at producing heat and not external work (Blondin et al., 2010a, 2010b; Blondin & Haman, 2018; Haman et al., 2002; Shephard, 1985). Muscle contraction patterns (continuous vs bursts) during ST can be measured by electromyography (EMG). Continuous ST has been attributed to low intensity ST and recruits type I muscle fibers (slow oxidative, fatigue resistant), whereas bursts occur sporadically throughout cold exposure and are associated with type II fiber recruitment (fast glycolytic, more fatigable) (Haman et al., 2005). However, ST is not the only process responsible for H_{prod} in the cold.

NST was originally described as a metabolic process responsible for the increase of H_{prod} induced by the cold (Himms-Hagen, 1984), with no subsequent increase in metabolic rate by ST (Cannon & Nedergaard, 2004). It is stimulated through the release of noradrenalin by the sympathetic nervous system (SNS) (Glick et al., 1981; Muzik et al., 2018) and can originate from various tissues and organs such as brown adipose tissue (BAT), the liver, and the heart (Blondin & Haman, 2018; JANSKý, 1973). NST may also take various forms, including the stimulation of futile cycles (e.g. TAG/NEFA cycle) (Levine, 2003; Rowland et al., 2015; Vallerand et al., 1999). The activation of spontaneous physical activity like fidgeting and postural changes, which are components of non-exercise activity thermogenesis (NEAT) (Levine, 2003), and/or the heat

dissipation through food assimilation (i.e. diet-induced thermogenesis) (Tappy, 1996) are also forms of NST. Similar to cold-induced NST, DIT activates the chain reaction of the uncoupling protein 1 (UCP1) in BAT (Cannon & Nedergaard, 2004; Himms-Hagen, 1984). The relationship between NST and DIT during cold exposure still needs to be investigated.

For decades, the existence of BAT in humans was quite controversial as stated in the recent review by Blondin and Haman (2018). It was thought that H_{prod}, primarily in cold exposure, was attributed to ST. In 2009, the presence of BAT in adults was confirmed by non-invasive imaging techniques such as position emission thermography (PET), single photon emission computed tomography (CT), with magnetic resonance spectroscopy and near infrared spectroscopy (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). The stimulation of BAT by the secretion of noradrenaline occurs when exposed to cold conditions and when food is ingested (Fig. 1.1). It is the activation of the SNS that stimulate the release of noradrenalin which activates the ß-adrenergic receptors of BAT that promote the cyclic adenosine monophosphate production (AMPc). This cycle activates the degradation of triglycerides in the form of free fatty acid in the brown cells. This leads to the metabolism of the free fatty acid that then triggers the activity of the UCP1 and the release of the proton (H^+) gradient formed in the respiratory chain (Cannon & Nedergaard, 2004). This H^+ release allows UCP1 to generate heat through the mitochondrial combustion of substrate, without being coupled from adenosine triphosphate (ATP) (van der Lans et al., 2014). In the brown adipocyte, LIP are be the main contributor to most of the energy during cold exposure as lipolysis is the predominate action (Fedorenko et al., 2012). Indeed, Blondin et al. (2017a) suppressed the action of intracellular lipolysis with the ingestion of nicotinic acid.

NST can be modified by ingesting polyphenols (i.e. green tea) (Mele et al., 2017; Gosselin & Haman, 2013) and by cold acclimation (Blondin et al., 2017b; Gordon et al., 2019). Gosselin et al. (2013) showed that the co-ingestion of green tea extracts (1600 mg of epigallocatechin-3-gallate and 600 mg of caffeine) in the cold has thermogenic effects. It was observed that green tea increased the total H_{prod} by 10% and decreased ST intensity by 20% suggesting that NST was stimulated under these conditions. Moreover, recent cold acclimation studies have found that in acute mild cold exposure, the activity of NST significantly increases for the same H_{prod} in individuals (Blondin et al., 2017b; Gordon et al., 2019). Such decreases in ST with an increased or a stable H_{prod} are indicative of the increased contribution of NST to total H_{prod} during a cold exposure. The studies demonstrated that the contribution of NST and ST can be modified in such conditions. In addition, the quantification of BAT by Blondin et al., (2017b) reported that BAT increased in volume and activity after cold acclimation. While this effect can be seen following the ingestion of polyphenols or cold acclimation, it is unclear if the ingestion of food with different energy equivalents could also modulate the contribution of NST and ST to total H_{prod}.

Fueling thermogenesis in the cold

Fuel selection during cold exposure has been well investigated over the last few decades, which lead to better knowledge and understanding of the metabolic pathways involved and pattern of fuel use. Fuel selection of contracting muscles can be modified by one of three ways: 1) by mobilizing different metabolic pathways within the same fibers, 2) by recruiting distinct fibers subpopulation specialized for different fuels, and 3) by recruiting muscles varying in fiber composition (Haman, 2006). At low metabolic rates, such as during low-intensity ST or at rest, the non-oxidative disposal represents a significant fraction of the rate of appearance of glucose,

hence the importance in quantifying directly the role of plasma glucose as an oxidative fuel. The addition of indirect calorimetry helps delineate the contribution of both CHO and LIP to total H_{prod} during ST. While a portion of H_{prod} can be attributed to fuel oxidation to produce energy in skeletal muscles, the remainder of H_{prod} is associated with the muscle contraction itself (Rolfe & Brand, 1996).

During a fasted state mild cold exposure (1.5 xRMR), plasma glucose and muscle glycogen plays a significant role as they represent ~20-25% of total H_{prod} (Haman et al., 2002, 2005; Haman, Péronnet, et al., 2004a). Plasma glucose has been shown to be stimulated in direct proportion to metabolic rate whereas muscle glycogen is more solicited, at ~75-80% of total CHO oxidized in the cold (Haman et al., 2002, 2005). When compared to CHO loading, CHO depletion decreases the relative use of total CHO by shivering muscles, while total H_{prod} remains unaffected (Haman et al., 2004a). This could be explained by a shift in the contribution of the macronutrients where LIP and PRO compensate for the lack of CHO availability (CHO: 28%, LIP: 53% and PRO: 19% of H_{prod}) as compared to glycogen loading (65% CHO: 50% muscle glycogen, 15% plasma glucose, LIP: 23% and PRO: 12% of H_{prod}) (Haman et al., 2004a). For both glycogen loading and depletion, LIP plays a significant role during mild cold exposure and provides as much heat as all other fuels combined, which helps spare the CHO reserves to potentially prolong survival.

LIP and PRO are also an important part of fuel oxidation in the cold. It was demonstrated that during mild cold exposure, LIP are responsible for as much as 50% of total H_{prod} and able to support prolonged low-intensity ST (Haman et al., 2002). Similarly, not only are LIP an important contributor in the cold, lipolysis is known to be predominant in thermogenesis of BAT in the cold (Blondin et al., 2017a; Juravlyova et al., 2018). Additionally, PRO is often assumed to not have a

contribution in the cold. Even if PRO contribution is low, it still consistently contributes to H_{prod} as well as compensating when glycogen reserves are low (Haman, Péronnet, et al., 2004b).

Nutrition in the cold

There remain important literature gaps on the metabolic requirements and associated dietary needs to sustain ST in the cold. While it is known that ingested food requires energy for its digestion, absorption and storage, the amount of energy required will vary depending on the quantity and the types of food consumed (i.e. CHO, LIP and PRO) (Soucy & Leblanc, 1998). CHO represent only ~1% of our total energy stores (Brooks et al., c2005). Their oxidation is the source of energy for normal cellular function found in all major tissues (Lowell & Spiegelman, 2000). There are two metabolic pathways for disposal of glucose: 1) oxidation and 2) storage (non-oxidative disposal). The process of storage occurs when there is not an immediate need for energy by oxidizing CHO. Glucose can be stored as glycogen in the liver and in muscles. The glycogen reserves can also be reconverted into glucose through the action of glycogenolysis (Brooks et al., c2005). Glucose can also be stored via *de novo lipogenesis*, where in excess it will be converted in fatty acids to triglycerides for later use as energy (Ameer et al., 2014). Since the body has a limited amount of hepatic and muscle storage, glycogen reserves are rapidly exhausted if the food supply of CHO are too low (Hole, 1978).

In the case of CHO oxidation, the plasma glucose will be converted into ATP through glycolysis, the citric acid cycle, and the respiratory chain in mitochondria, where the oxidation of major product such as glucose, pyruvate, NADH are greatly dependent of oxygen (Brooks et al., c2005).

Few studies investigated the effect of food intake during, or before cold exposure (Blondin et al., 2010a; Glickman-Weiss et al., 1994; Glickman-Weiss et al., 1993; Vallerand, 1992; Vallerand et al., 1988; Vallerand et al., 1993). In fact, most of the dietary cold studies were done specifically with CHO ingestion in the purpose of identifying their thermoregulatory response (Glickman-Weiss et al., 1993; Vallerand, 1992; Vallerand et al., 1993). It was discovered that CHO ingestion does not increase total H_{prod} during ST, while only the studies from Blondin et al., (2010a), Vallerand, (1992) and Vallerand et al., (1993) were able to report estimated changes in macronutrients oxidation. Interestingly, in the cold, exogenous glucose oxidation is maximized at ~200mg/min for ingestions rates of 400mg/min and 800mg/min during moderate shivering intensity (3 xRMR) (Blondin et al., 2010a). This represents about one-third of what have been reported during exercise (Jeukendrup, 2004).

In addition, it was concluded by Blondin et al., (2010b) that ingesting glucose at the onset of cold is better rather than later (i.e. after a 60 min steady state cold), to ensure optimization of the sparing of muscle glycogen. In other words, the endogenous reserves are maintained at the expense of exogeneous glucose utilization, when ingestion is done at the beginning of the cold exposure. On the other hand, when glucose is administrated during the cold, muscle glycogen stores are reduced substantially in the first 60 min, which is not ideal for maximizing the energy stores of the body for prolonged cold periods.

With most of the cold studied done specifically with CHO ingestion, there are limited amount of information on the thermoregulatory response of LIP and PRO in the cold. The body stores greater levels of LIP than CHO or PRO, as LIP represent ~97% of our total energy reserves (Haman, 2006). They also provide more energy (i.e. 9 kcal/g) for the same mass of CHO and PRO (i.e. 4 kcal/g) (Wilmore et al., 2009). PRO on their end, are composed of amino acids that can

provide up to ~5-10% of energy during prolonged exercise, however their use is greatly affected by glucose availability (Haman et al., 2004a).

Diet-induced thermogenesis

When in energy balance, the food assimilation process can contribute ~10-15% of basal H_{prod} (Westerterp, 2004). This increased activity required by the body to absorb and convert macronutrients needed for ATP production (obligatory thermogenesis) (Jequier, 1983; Sims & Danforth Jr, 1987; Tappy, 1996) and the regulation of energy balance (regulatory thermogenesis) (Himms-Hagen, 1984) is known as diet-induced thermogenesis (DIT). By definition DIT is an increase in metabolic rate following ingestion of food that is not related to the immediate thermogenic processing of the components of the food itself, and which are activated by the sympathetic nervous system (Himms-Hagen, 1984). Once macronutrients are absorbed, a series of metabolic pathways are activated to enable their biochemical conversion to ATP which is then utilized to generate heat, also known as energy expenditure (Jequier, 1983). The obligatory component of DIT comprises ~60-70% of total DIT (Sims & Danforth Jr, 1987) while the regulatory thermogenesis varies with the stimulation of sympathetic activity (Jequier, 1986). By manipulating either the quality of each macronutrient or the total caloric content during available nourishment, the contribution of each substrate goes as such: ~0-3% for LIP, ~5-10% for CHO and ~20-30% for PRO (Tappy, 1996). Whether or not DIT can contribute to total H_{prod} during prolonged cold exposure remains unclear at best (Haman, 2010). In addition, it is unknown whether the relative contribution of ST and NST to total H_{prod} during mild shivering can be modified following the ingestion of food and associated increase in DIT.

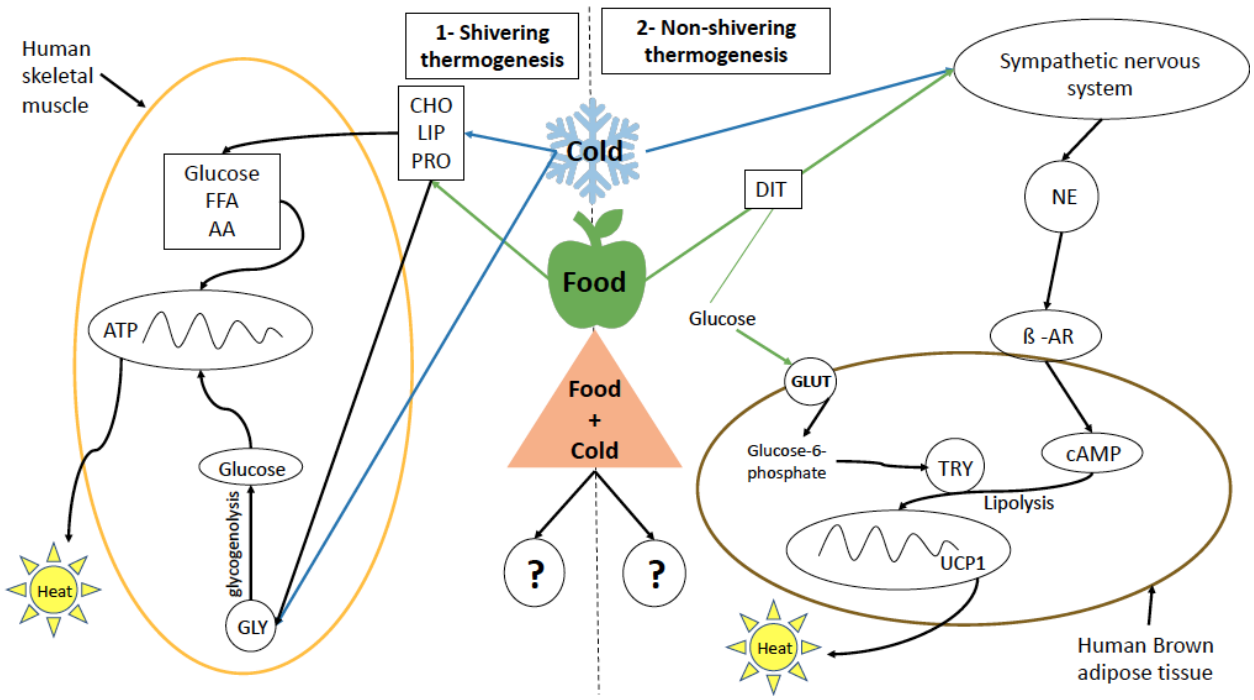

Figure 1.1. Cold-induced shivering (ST) and non-shivering (NST) thermogenesis: ingestion of food during cold exposure. Upon cold exposure, ST and NST are activated through two distinct processes. 1- There are two ways shivering can be fueled, either by endogenous or exogenous sources of energy. When no external source of food is available, fueling will depend in part on muscle and liver glycogen (GLY) availability to be transformed in glucose by glycogenolysis. This glucose is then broken down into ATP to generate heat in the skeletal muscle. When food is available, it is broken down in three macronutrients, glucose, fatty acid and amino acids to then be transformed in ATP in the mitochondria. If the macronutrients are not needed for immediate energy they will be stored in the liver or skeletal muscles. There is a need for more investigation on the exact pathways of mixed diets during cold exposure for the exact metabolic cost of shivering. 2- Cold-induced NST and diet-induced thermogenesis (DIT), both activates the sympathetic nervous system with the release of noradrenaline (NE). The ß-adrenergic receptors are then stimulated to increase the cyclic adenosine monophosphate (cAMP) production. The cycle then activates lipolysis and the release of FFA will generate heat through the uncoupling protein-1 (UCP1) from the brown adipose tissue. When food is ingested, glucose can also activate the release of heat through UCP1 with the help of a glucose translocator (GLUT) into the BAT. The effect of the combination of both, cold exposure and food intake, on NST is yet to be determined.

Objectives and hypotheses

This thesis will focus on the effects ingesting same quantity of food at two energy equivalents on the thermogenic responses in the cold. More specifically, during three hours of mild cold exposure at ~1.5 xRMR, the aims of my thesis are two-fold: 1) to quantify the effects of ingesting 1507 kJ and 3015 kJ on cold-induced whole-body H_{prod} and thermal responses and 2) to establish the effects of these two different energy equivalents on the relative contribution of ST and NST to total heat production. I hypothesized that: 1) based on preliminary data at rest, at the same given thermal stress, H_{prod} will remain the same at an ingestion level of 1507 kJ and will double at the ingestion level of 3015 kJ and 2) the ST will decrease to a greater extent at the ingestion level of 3015 kJ than at 1507 kJ, due greater stimulation of NST.

Significance

Over the years, the effects of the cold on the thermoregulatory responses have been of great interest. However, because of the enormous variability amongst the protocols, interpretations should be done carefully. Its only in the last few decades that cold exposure studies have been redefined using rigorous and systematic protocols. From a metabolic point of view, this thesis will provide new insights on the thermogenic responses of caloric intake, which could eventually be transferred in recommendations and nutritional strategies for cold exposures. The results and methods of this study are presented in the article format found in Chapter 2.

CHAPTER 2: EFFECTS OF INGESTING 1507 KJ AND 3015 KJ ON COLD-INDUCED THERMOGENESIS PROCESSES IN MEN

INTRODUCTION

Throughout evolution, humans have developed several key physiological and metabolic mechanisms to resist lowering ambient temperatures (Haman, 2010). As the first response to cold exposure in humans, vasoconstriction of peripheral blood vessels is followed by an increase in H_{prod}, an important strategy to reduce the H_{loss} into the environment. This response is essential in delaying hypothermia, where chemical reactions in the body are slowed (Shephard, 1985). As the body starts to lose heat, non-voluntary activity of skeletal muscles will begin as a preventative mechanism to delay the decrease of T_{core} below 37°C. In compensable cold exposure, T_{core} remains unchanged despite a decrease in mean skin temperature ($\overline{T}_{skin}$). These involuntary muscle contractions, known as ST are accompanied by NST, both part of the thermogenic response in the cold. ST will account for most of the whole-body thermogenic response while NST also is an important contributor in the cold as it corresponds to a metabolic rise which does not include a rise in ST (Cannon & Nedergaard, 2004).

BAT is an important contributor to total NST in mammals and is stimulated through the release of noradrenaline (Aydin et al., 2008; Blackburn, 2011; Cannon & Nedergaard, 2004; Himms-Hagen, 1984; Muzik et al., 2018). NST may also take various forms, including the stimulation of futile cycles (e.g. TAG/NEFA cycle, SERCA by sarcolipin) (Levine, 2003; Rowland et al., 2015; Vallerand et al., 1999), the activation of spontaneous physical activity like fidgeting and postural changes, which are components of non-exercise activity thermogenesis, (NEAT) (Levine, 2003) and/or the heat dissipation through food assimilation (i.e. diet-induced thermogenesis) (Tappy, 1996). DIT, similar to cold-induced NST, activates the chain reaction of UCP1 in BAT (Cannon & Nedergaard, 2004; Himms-Hagen, 1984).

Recently, shivering studies have been done in respect to specific macronutrient ingestions and have been able to demonstrate the fuel selection pathways in response to ST. More specifically muscle glycogen availability and the contribution of LIP is of great importance in the contribution of ST and cold-induced NST, respectively (Blondin, Frisch, et al., 2017a; Haman et al., 2002; Haman, Péronnet, et al., 2004a). LIP are important in cold exposure as lipolysis is the main contributor in the fueling of BAT for generating heat. It is known that the maximal absorption rate of glucose during a mild cold exposure is of 200 mg/min with a max ingestion rate of 400 mg/min (Blondin et al., 2010a). As well, for optimal absorption and sparing of endogenous reserves of glycogen, it is better to consume glucose at onset of cold exposure (Blondin et al., 2010b). However, in a survival setting, managing these parameters can be challenging. The metabolic responses of these studies where specific to glucose ingestion, while the effects of mixed diets of different quantities have never been studied in shivering studies.

Therefore, the purpose of this study is to quantify the effects of ingesting 1507 kJ and 3015 kJ on cold-induced whole-body H_{prod} and thermal responses as well as to establish the effects of these 2 different caloric equivalents on the relative contribution of NST and ST to total H_{prod}. It is hypothesized that based on preliminary data at rest, at the same given thermal stress, H_{prod} will remain the same for the 1507 kJ and will double the effect for the 3015 kJ. There will also be a decrease in ST of greater extent for the 3015 kJ than the 1507 kJ, due greater stimulation of NST. The stimulation of NST will be assumed, if H_{prod} increases after the shake ingestions despite of a similar shivering intensity or if shivering intensity decreases after the shake ingestions for a similar H_{prod}.

METHODS AND MATERIAL

Subjects

A total of eight healthy non-cold acclimatized men between the ages of 18 and 35 years old volunteered for these trials but only five of them could be analysed for reason of non-completion of the trials. Written informed consent have been obtained from all subjects in accordance with the *Declaration of Helsinki.* All procedures involving human subjects have been approved by the Faculty of Health Sciences ethics committee at the University of Ottawa. Exclusion criteria included the following: participants with any history of cardiovascular, respiratory or metabolic diseases and those taking dietary supplements or stimulants. Also, participants with intolerances/allergies related to the food given in the study were excluded for safety reasons. Anthropometric measurements such as weight, height and body composition measurements were taken before the first session (Table 2.1).

Shake Selection and Composition

Initially a store bought pre-made well balanced beverage of 1507kJ was chosen for the study. The idea was to use one beverage as the first quantity (1507 kJ) and to double it as the second quantity (3015 kJ), as they would represent a small and large meal, respectively. However, for the optimal control of the meal given to the participants, it was of great importance to customize our own shake. The trials being randomized and blinded, it was of also of interest to make the two shakes of equal viscosity and volume to eliminate the possible effects on the digestion. The viscosity and the volume of the shakes can have an effect on the gastric emptying and the absorption (Jeukendrup & Jentjens, 2000). We normalized the shakes by measuring the viscosity of the larger quantity (3015 kJ) and matching it to the smaller quantity (1507 kJ) by adding a

thickener (Resource - Thickenup®). The two shakes were composed of the same volume of a base of whole milk to control for the effect of vitamins and minerals. Then equal proportions of fat (sunflower oil), proteins (whey protein) and carbohydrates (maltodextrin and lactose) were added to the milk base. These ingredients were chosen for their neutral taste, and simple composition. For example, maltodextrin is a glucose polymer with a similar oxidation rate as glucose and is easily hydrolysed. See *experimental protocol* section below for the detailed proportions of the shakes.

Experimental protocol

Participants were asked to attend a preliminary information session lasting approximately 1 h to provide them with a detailed description of the project and to determine their eligibility for these trials. They also had an opportunity to tour the facility as well as view all the related equipment and ask questions. When ready, participants who were interested in participating have been provided a *Background and Informed Consent package* and were asked to sign the informed *consent page*. Once this was complete and they were prepared, they had their height and weight measured. An assessment of body composition using Dual Energy X-Ray Absorptiometry (DXA) was then performed. Body composition was estimated using DEXA, a device used to determine body composition (fat and lean tissue) and bone density (full body composition assessment exposes participants to low radioactivity levels equivalent to 1/20th of the radioactivity received over an 8-hour period in the sunlight).

Then participants were asked to make themselves available for 4 experimental sessions (2 cold trials and 2 control ambient trials). The order of the experimental trials was randomly assigned and counterbalanced between participants to avoid any carry-over effects. The experimental

sessions lasted approximately 5h (1 hour to equip participant with experimental equipment, 1 hour of baseline measurements, 3h of cold or ambient exposure, depending on the session). Participants were asked to arrive to the laboratory in a 12h post absorptive state and refrained from consuming caffeine or alcohol for the same duration prior to the experimental session. Participants were also asked to show up to the lab at 07:00, where they were asked to go to the bathroom to void their bladder prior to placing the experimental equipment. Next, for the experimental cold trials, four EMG electrodes were placed to measure shivering intensity. Maximal voluntary contraction (MVC) measurements was done using EMG. For that matter, participants were asked to contract a specific muscle group for 5s. MVC was repeated for same muscle 3 times with one-minute interval between each set. The best of the 3 MVC were used. MVC is done for normalization of the shivering measurements. Skin for EMG placement was prepared using 3M Red Dot Trace Prep (3M Canada, London, ON, Canada) and ethanol swabs. Electrodes were secured using medical transpore tape (3M Canada, London, ON, CANADA). Then, they were instrumented with 12 skin thermocouples (Concept Engineering, Old Saybrook, CT, USA) located on the head, hand, upper back, chest, lower back, abdomen, bicep, forearm, quadriceps, hamstring, front calf, and back calf and placed on the surface of the skin of the right side of the body. This measured $\bar{T}_{skin}$. Participants were then asked to put on the liquid condition suit (LCS) (CORETEC, Delta Temax, Inc., Pembroke, ON, Canada) wearing only their underwear. The LCS fits tightly to the body and contains a high-density of water-perfused tubes. Following the instrumentation, participants were asked to lie in a hospital bed, for 45 min with the bath temperature set to maintain the $\bar{T}_{skin}$ at 33 degrees Celsius. The rest period was followed by a 15 min baseline data collection for EMG (only for experimental cold), $\bar{T}_{skin,}$ and H_{prod}. The cold exposure started following the completion of baseline data collection. The same measurements as in baseline (EMG, $\bar{T}_{skin}$, and H_{prod}) was taken

during total cold exposure (180 min). By circulating a cooling liquid through the LCS suit, participant's $\bar{T}_{skin}$ was lowered to about 26 degrees Celsius for the experimental cold trial and was kept at 33 degrees Celsius for the control ambient trial, based on the measured baseline $\bar{T}_{skin}$. The water of the bath was brought to 15 degrees Celsius for the whole duration of the cold trial to obtain a stable $\bar{T}_{skin}$. This temperature has been previously used in our laboratory in order to stimulate H_{prod} without or with minimal shivering, also define as compensable cold exposure. The cold exposures lasted 180 min and 60 min into the cold exposure participants were given an experimental meal though a feeding bottle and tube system (normal composition meal of relative contribution 61%, 19% and 19% for CHO, LIP and PRO, respectively). The participant was able to maintain the same lying position while drinking the shake to avoid any disturbance in the EMG recording. The experimental meal #1 was of 1507 kJ (45g CHO, 14g LIP, 14g PRO) and the experimental meal #2 was of 3015 kJ (90g CHO, 28g LIP, 28g PRO) experimental shake made with whole milk, sunflower oil, unflavored protein powder, maltodextrin powder, vanilla extract, lactose powder for the 3015 kJ and a commercial thickener for the 1507 kJ (Resource - Thickenup®). For the baseline period and throughout the experiment, air expired by the subject was captured from a canopy placed over the participant's head by pulling air using a calibrated flow kit (Field Metabolic System (FMS), Sable Systems International Inc., Las Vegas, NV, USA). Gases were analyzed using O_2, CO_2 and H_2O analyzers (FMS, Sable System International Inc., Las Vegas, NV, USA). The participant was asked to show how they felt on a thermal comfort scale every 5 min (Haman, Péronnet, et al., 2004a).

Thermal response

Thermocouples (Concept Engineering, Old Saybrook, CT, USA) were used to estimate skin temperature from 12 sites weighted in the following proportions: head 7%, hand 4%, upper back 9.5%, chest 9.5%, lower back 9.5%, abdomen 9.5%, bicep 9%, forearm 7%, quadriceps 9.5%, hamstring 9.5%, front calf 8.5%, and back calf 7.5%. Mean ($\bar{T}_{skin}$) and mean heat flux were calculated using an area-weighted equation (Du Bois & Du Bois, 1916).

Metabolic measurements

H_{prod} was determined using indirect calorimetry (Field Metabolic System, Sable Systems International Inc., NV, USA). $\dot{V}CO_2$ ($l.min^{-1}$) and $\dot{V}O_2$ ($l.min^{-1}$) was calculated based on energy expenditure equations (Elia, 1991; Péronnet & Massicotte, 1991)

(1) CHO_{ox} ($g.min^{-1}$) = $4.59x\dot{V}CO_2$ ($l.min^{-1}$) $-3.23\dot{V}O_2$ ($l.min^{-1}$)
(2) LIP_{ox} ($g.min^{-1}$) = $-1.70x\dot{V}CO_2$ ($l.min^{-1}$) + $1.70\ \dot{V}O_2$ ($l.min^{-1}$)

where $\dot{V}CO_2$ ($l.min^{-1}$) and $\dot{V}O_2$ ($l.min^{-1}$) was corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1.010 and 0.843 $l.g^{-1}$, respectively). Protein oxidation rate was estimated at 66 mg/min based on previously published urinary urea excretion measurements made on 12-h post-absorptive men with normal CHO reserves (Haman et al., 2002; Haman, Legault, & Weber, 2004c). Energy potentials of 16.3, 40.8, and 19.7 $kJ.g^{-1}$ were used to calculate the relative contributions of CHO (%CHO) lipid (%LIP) and (%PRO) oxidation to total H_{prod}, respectively (Elia, 1991; Péronnet & Massicotte, 1991).

Determination of shivering intensity

Muscle activity and shivering intensity were measured using electromyography (EMG). EMG collection sites are located on the left side of the body on the following muscle: pectoralis

major (PEC), trapezius (TRA), vastus lateralis (VAS), sternocleidomastoid (SCM). Raw EMG signals were analyzed with the use of custom-designed MATLAB algorithms (Mathworks, Natick, MA). EMG signals were filtered to remove spectral components below 20 Hz and above 500 Hz, as well as 60-Hz contamination (and associated harmonics). Shivering intensity of individual muscles (EMG_{shiv}) were determined from root-mean-square values (RMS) rectified from EMG signals using a 50-ms overlapping window (50%). Baseline RMS values ($RMS_{baseline}$: 15 min RMS average measured before cold exposure) were subtracted from RMS shivering (RMS_{shiv}) as well as RMS_{mvc} values. EMG_{shiv} was normalized to RMS_{mvc}.

$$(4)\ EMG_{shiv}\ (\%MVC) = \frac{RMS_{shiv} - RMS_{baseline}}{RMS_{mvc} - RMS_{baseline}}$$

Thermal comfort

Thermal comfort was measured using a thermal comfort scale that ranged from +5 (hottest ever) to -5 (coldest ever) (Haman, Péronnet, et al., 2004a). The participants were asked to give the number on the hand of the side of the body that did not have EMG electrodes to avoid creating noise by voluntary movements.

Statistical Analysis

Differences in $\bar{T}_{skin}$, H_{prod}, EMG_{shiv} were assessed by a 2-way within-subject ANOVA for repeated measures to determine the main effect as well of the interaction between the shakes (1507 or 3015 kJ) given over the course of 3h in a set temperature, either ambient or cold (SPSS for PC version 26.0, Chicago, IL). Significant differences found at a given time, were determined using a

Bonferroni *Post-Hoc* test. Statistical differences were considered significant when $p < 0.05$. All values are means ± standard error of the mean (SEM).

RESULTS

Thermal response

Changes in $\bar{T}_{skin}$ at baseline, during control, cold and following shake ingestion are presented in *Fig. 2.1*. $\bar{T}_{skin}$ remained constant at 32.7 ± 0.01°C and 32.9 ± 0.02°C during the whole 180 min for 1507 kJ – CON and 3015 kJ – CON, respectively. In contrast, $\bar{T}_{skin}$ decreased by 23.5% from 32.9 ± 0.2°C at baseline to 25.1 ± 0.3°C by the end of cold exposure in 1507 kJ – COLD and decreased by 22.5% from 32.6 ± 0.2°C to 25.2 ± 0.3° in 3015 kJ – COLD ($p < 0.0001$). $\bar{T}_{skin}$ of the COLD conditions (27.1 ± 0.3°C) was significantly lower than the CON (32.8 ± 0.2°C; $p < 0.0001$). No significant difference was found between the 2 shakes $p = 0.473$.

Heat production

Changes in H_{prod} at baseline, during control, cold and following shake ingestion are presented in *Fig 2.2*. At baseline, H_{prod} was the same for all conditions. In the control conditions, H_{prod} was stable during baseline 6.0 ± 0.1 kJ·min^{-1} and increased to 6.4 ± 0.1 kJ·min^{-1} in 1507 kJ – CON after the ingestion of the shake as well from 6.0 ± 0.3 kJ·min^{-1} to 6.7 ± 0.2 kJ·min^{-1} in 3015 kJ – CON, $p = 0.388$. However, these responses were not different between the 2 shakes ($p = 0.403$). Furthermore, in response to cold exposure, H_{prod} increased by 14% following the shake ingestion from 8.1 ± 0.5 kJ·min^{-1} to 9.2 ± 0.4 kJ·min^{-1} in 1507 kJ – COLD and by 18% from 7.4 ± 0.3 kJ·min^{-1} to 8.7 ± 0.3 kJ·min^{-1} in 3015 kJ – COLD. Interestingly, the peaks in H_{prod} for both shakes occurred at 120 min reaching 10.0 ± 0.7 kJ·min^{-1} for 1507 kJ and 9.6 ± 0.7 kJ·min^{-1} for 3015 kJ while no significant difference was found between the shakes ($p = 0.116$). Again, H_{prod} was higher in COLD (8.4 ± 0.2 kJ·min^{-1}) compared to CON (6.4 ± 0.4 kJ·min^{-1}; $p = 0.019$).

Figure 2.5a,b presents differences in AUC for each condition at baseline, before and following shake ingestion. In the control conditions, H_{prod} was 28% higher following shake ingestion compared with baseline (from 287 ± 10 kJ·h^{-1} to 368 ± 12 kJ·h^{-1}; p = 0.001), while there was no significant main effect of shake, p = 0.436 (*Fig. 2.5a).* On the other hand, during cold exposure, H_{prod}, following ingestion of shake, increased by 71% from 299 ± 11.0 kJ·h^{-1} at baseline to 510 ± 25 kJ·h^{-1} by the end of the cold exposure, p = 0.002 (*Fig. 2.5b).* Finally, the AUC of H_{prod} at ingestion of shake, was significantly higher for the COLD conditions (503 ± 30 kJ·h^{-1}) compared to the CON conditions (368 ± 12 kJ·h^{-1}; p = 0.027), however, no significant difference was found between the interaction of shake and time, p = 0.664.

Fuel selection response

Changes in RG_{ox} and RF_{ox} at baseline, during control/cold and following shake ingestion are presented in *Fig. 2.3.* Under control conditions, RG_{ox} remained constant at 0.14 ± 0.01 g·min^{-1} for 1507 kJ – CON and at 0.13 ± 0.03 g·min^{-1} for 3015 kJ – CON before the shake ingestion. Following shake ingestion, RG_{ox} increased by 67% from 0.13 ± 0.01 g·min^{-1} to 0.21 ± 0.01 g·min^{-1}at 140 min for both shakes, p = 0.375. Again, these changes in RG_{ox} were not different between the shakes, p = 0.125. Now looking at the RF_{ox}, at baseline, remained constant at 0.09 ± 0.002 g·min^{-1} for 1507 kJ – CON and 0.09 ± 0.001 g·min^{-1} for 3015 kJ – CON. When shakes were ingested, RF_{ox} increased slightly by 19% from 0.01 ± 0.01 g·min^{-1} to 0.12 ± 0.01 g·min^{-1} at 100 min for both quantities. These changes were not different between shakes p = 0.436.

During cold exposure, RG_{ox} increased over time and following the shake ingestion RG_{ox} increased by 115% from 0.14 ± 0.02 g·min^{-1} to 0.3 ± 0.01 g·min^{-1} at 140 min for both shakes, p = 0.041. However, no significant main effect of the shake was found, p = 0.106. As for RF_{ox}, in

response to the shakes in the cold, it reached its peak 20 min after the ingestion from 0.14 ± 0.02 $g \cdot min^{-1}$ to 0.17 ± 0.01 $g \cdot min^{-1}$ ($p = 0.264$) with a total increase of 21% while, no significant difference between the shakes was found, $p = 0.063$. Overall, RG_{ox} was greater in the COLD conditions (0.19 ± 0.01 $g \cdot min^{-1}$) than in the CON conditions (0.16 ± 0.01 $g \cdot min^{-1}$) as well, RF_{ox} was greater in the COLD conditions (0.14 ± 0.01 $g \cdot min^{-1}$) than in the CON conditions (0.01 ± 0.01 $g \cdot min^{-1}$), without any significant difference, $p > 0.05$

Shivering intensity

Changes in mean muscle shivering intensity at baseline, during cold and following shake ingestion are presented in *Fig.2.4.* In response to cold, shivering intensity increased and remained constant at 1.8 ± 0.2 $\%MVC \cdot min^{-1}$ for 1507 kJ and 1.7 ± 0.2 $\%MVC \cdot min^{-1}$ for 3015 kJ just before the ingestion of the shake. However, following ingestion, mean muscle shivering intensity continued to increase over time, peaking at 120 min at 4.6 ± 1.2 $\%MVC \cdot min^{-1}$ for 1507 kJ and 4.4 ± 1.6 $\%MVC \cdot min^{-1}$ for 3015 kJ. From there, EMG_{shiv} decreased by 26% for 1507 kJ (3.4 ± 0.6 $\%MVC \cdot min^{-}1$) and by 22% for 3015 kJ (3.4 ± 0.9 $\%MVC \cdot min^{-1}$; $p = 1.0$) until the end of the trial, while no differences were found between the two quantities ($p = 0.960$).

The AUC of mean shivering intensity, presented in *fig. 2.5c,* shows that regardless of the quantity of the shake ($p = 0.937$), total shivering activity is higher during the cold+shake period at 189 ± 46 % $MVC \cdot h^{-1}$ for 1507 kJ and 134 ± 70 % $MVC \cdot h^{-1}$ for 3015 kJ than just in cold at 85 ± 26 $\%MVC \cdot h^{-1}$ for 1507 kJ and 67 ± 27 $\%MVC \cdot h^{-1}$ for 3015 kJ, $p = 0.049$ (*fig. 2.5c*). In contrast, when looking at muscles individually, the AUC of total shivering intensity of TRA was significantly higher in the cold+shake period (229 ± 59 $\%MVC \cdot h^{-1}$) than in the cold (87 ± 29 $\%MVC \cdot h^{-1}$; $p = 0.045$) for both quantities.

Thermal comfort

Mean thermal comfort after the ingestion of both shakes was the same for the control trials at -0.1 ± 0.02 for 1507 kJ – CON and -0.1 ± 0.02 for 3015 kJ – CON. Not to mention, the thermal comfort was also similar within the cold trials at -3.0 ± 0.3 for 1507 kJ – COLD and -3.1 ± 0.3 for 3015 kJ – COLD, without any significant difference between the shake ($p = 0.937$). However, a significant difference was found in the main effect of temperature ($p < 0.0001$).

Table 2.1 Characteristics of participants. Mean ± SD; n = 5

Age (years)	19.2 ± 0.7
Mass (kg)	71.8 ± 7.4
Height (cm)	175 ± 6.4
Percent body fat (%) *	23.0 ± 6.8
Lean mass (kg) *	52.2 ± 3.1

*Dual energy X-Ray Absorptiometry (DXA)

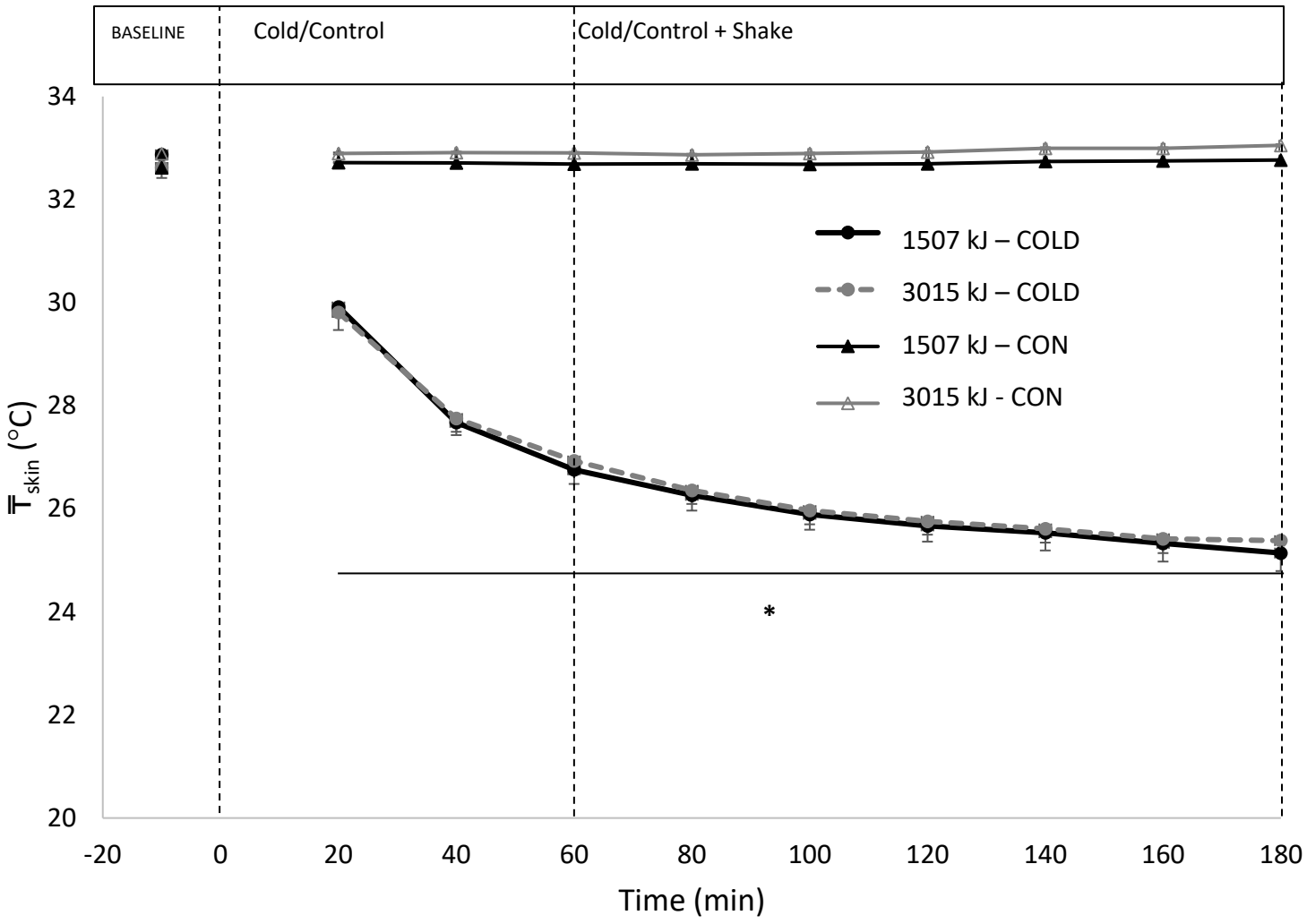

Figure 2.1. Change in mean skin temperature ($\bar{T}_{skin}$) at baseline as well as before and following the ingestion of a shake at 1507 kJ or a shake at 3015 kJ in men under control condition (CON) and mild cold exposure (COLD) using a liquid condition suit.

*Significantly different from baseline, $p < 0.001$

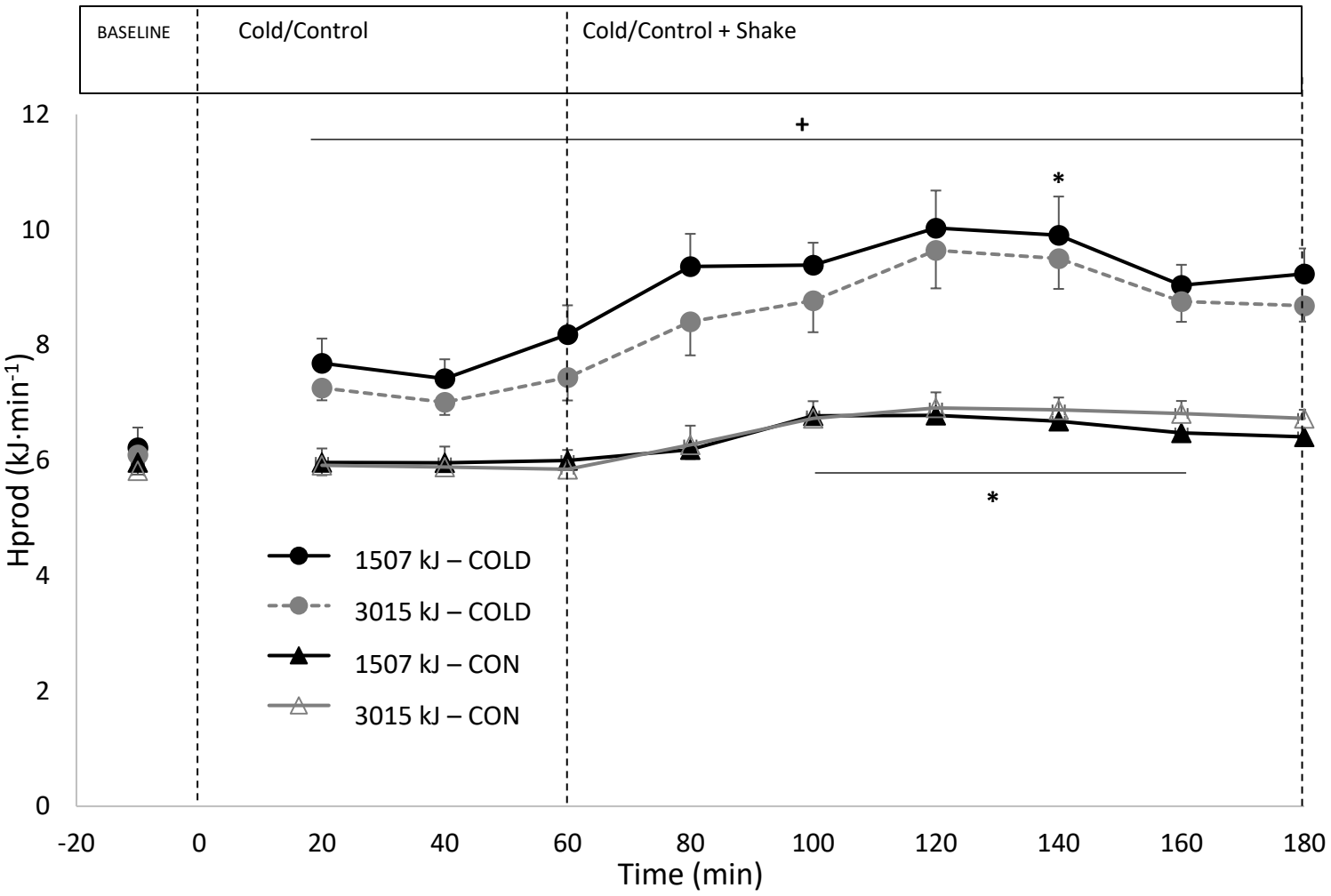

Figure 2.2. Absolute heat production (H_{prod}) at baseline as well as before and following the ingestion of a shake at 1507 kJ or a shake at 3015 kJ in men under control condition (CON) and mild cold exposure (COLD) using a liquid condition suit.

*Significantly different from 60 min, $p < 0.05$

+Significantly different than CON, $p < 0.05$

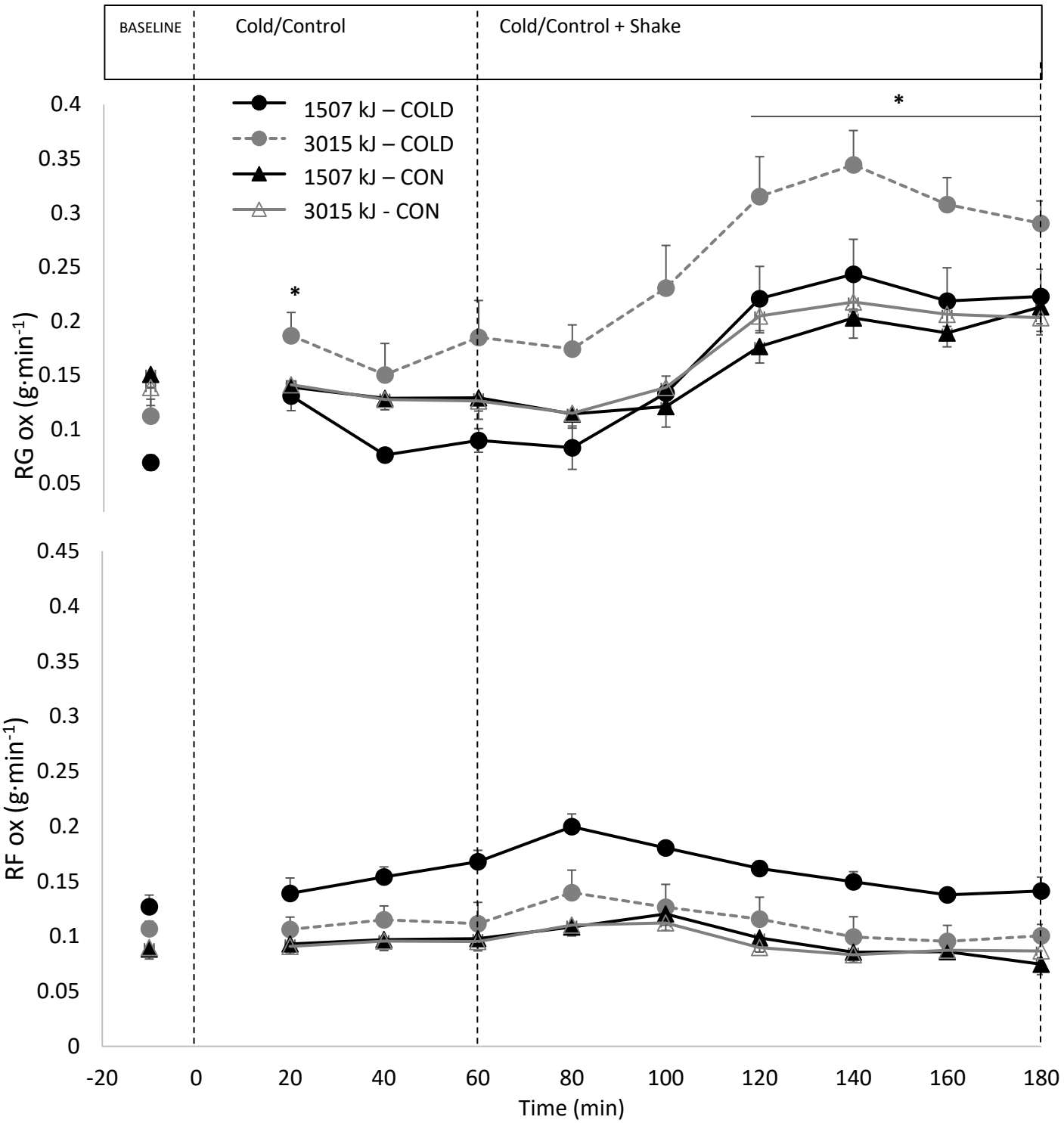

Figure 2.3. Absolute oxidation rate of carbohydrates (RG_{ox}) and lipids (RF_{ox}), at baseline as well as before and following the ingestion of a shake at 1507 kJ or a shake at 3015 kJ in men under control condition (CON) and mild cold exposure (COLD) using a liquid condition suit.
*Significantly different from baseline, $p < 0.05$

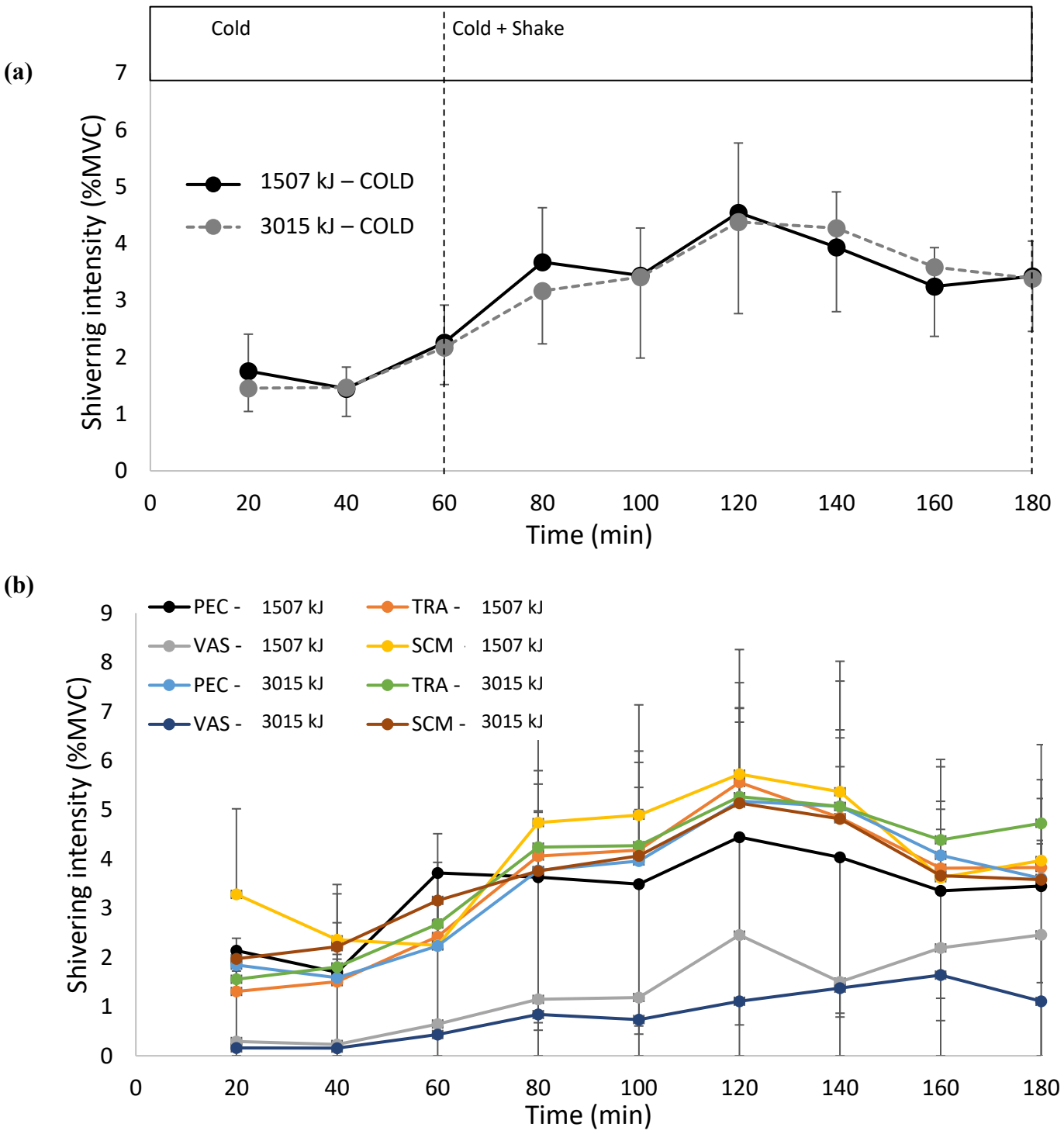

Figure 2.4. Mean shivering intensity in percent maximal voluntary contraction (%MVC) of all muscles (PEC, TRA, VAS, SCM) **(a)** and mean shivering intensity by muscles **(b)** at baseline as well as before and following the ingestion of a shake at 1507 kJ or a shake at 3015 kJ in men under control condition (CON) and mild cold exposure (COLD) using a liquid condition suit.

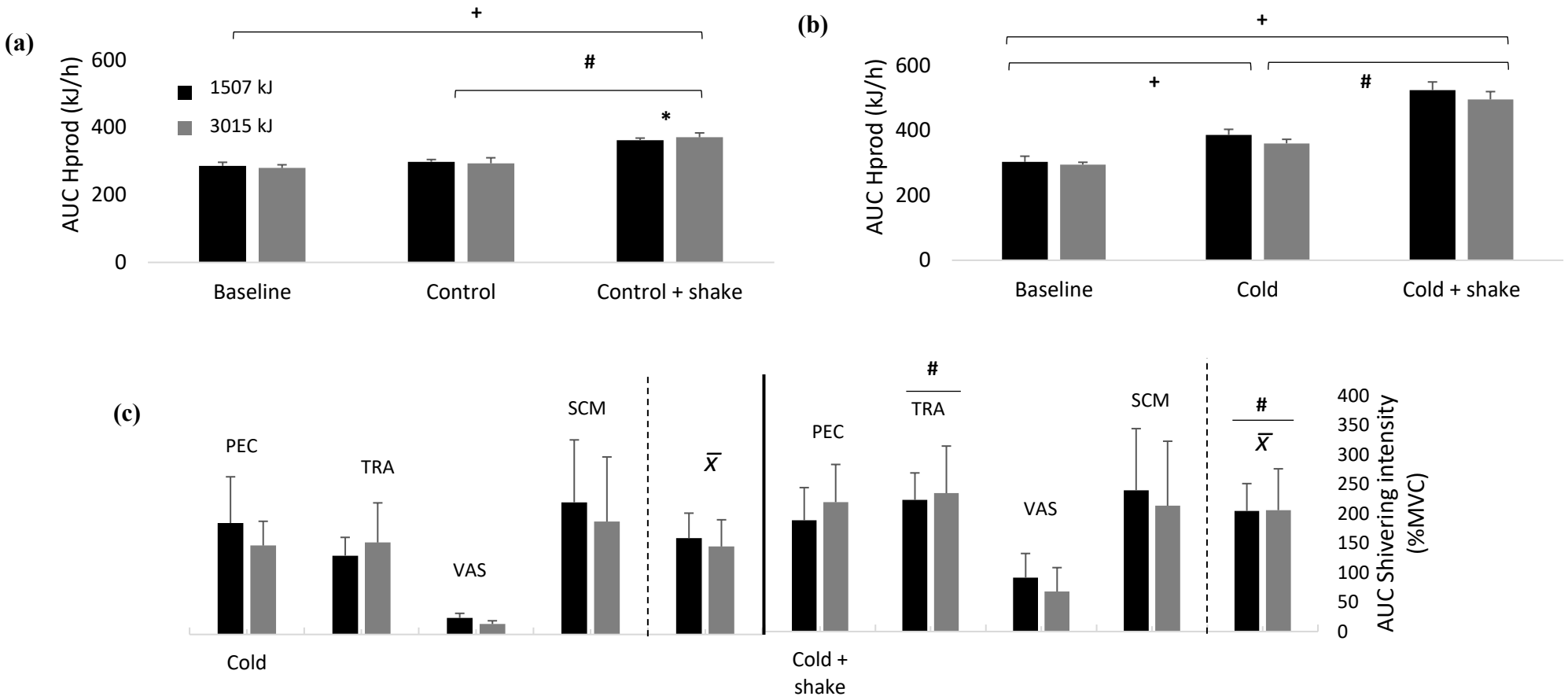

Figure 2.5. Area under the curve (AUC) of whole-body H_{prod} of control condition (CON) **(a)**, of whole-body H_{prod} in cold (COLD) **(b)**, and of shivering intensity of each muscles (PEC, TRA, VAS, SCM) individually as well as their mean $(\bar{x})$ **(c)** at baseline as well as before and following the ingestion of a shake at 1507 kJ or a shake at 3015 kJ in men under control condition (CON) and mild cold exposure (COLD) using a liquid condition suit.

*Significantly different from COLD, $p < 0.05$
+Significantly different from baseline values before cold exposure, $p < 0.005$
#Significantly different from control or cold, $p < 0.005$

DISCUSSION

For more than a decade, studies have shown that NST is an important contributor in human cold-induced thermogenesis. However, few studies investigated the thermoregulatory effects and overall energy balance on food consumption before or during cold exposure (Glickman-Weiss et al., 1994; Glickman-Weiss et al., 1993; Haman, Péronnet, et al., 2004a; Vallerand, 1992; Vallerand et al., 1988; Vallerand et al., 1993). The aim of this study was to determine if the caloric intake of two different energy equivalents can change the contribution of NST relative to H_{prod} with the measurements of whole-body calorimetry and EMG_{shiv}. Thus, this study focused on quantifying the thermoregulatory responses of 1507 kJ and 3015 kJ shakes in the cold, on ST and NST relative to H_{prod}.

The combination of cold exposure with food intake supports that the thermogenic rate of both caloric intakes is increased and that the contribution of ST is also increased. However, in contrast to what was hypothesized, our results did not demonstrate significant differences in H_{prod} following the two ingested shakes for the same thermal stress (+14% and +18% for 1507kJ and 3015kJ, respectively; Figure 2.2). In addition, no significant differences were found between the two shakes in EMG_{shiv} intensity (Fig. 2.4a). This study has also demonstrated that in unacclimated men, the ingestion of the shakes during mild cold exposure generated a significant increase in total $H_{prod.}$ from baseline of 71% compared to 28% in control conditions (Fig. 2.5a,b). This is also comparable to Ouellet et al., (2012) findings where H_{prod} increased by 80% after continuous glucose infusion and 3h mild cold exposure. It also appears that after the two ingested quantities, ST increased in most muscles (Fig. 2.5c).

Whole body heat production

Measurements of indirect calorimetry and EMG were used to determine changes in H_{prod} and shivering intensity in non-acclimatized young men. They were exposed to a 3h mild cold stress at 15°C and ingested a meal after a 60 min period into the cold (Blondin et al., 2010b). In these circumstances, the comparison of H_{prod} and shivering intensity could be made, and any differences found in these variables between the two shakes could have been attributed to the stimulation of NST by the quantity of food ingested.

In both CON and COLD, the caloric intakes elicited a predictable increase in H_{prod}. In part, the energy intake is the one responsible for the elevation in H_{prod} when compared to their respective RMR (Welle et al., 1980). Additionally, the greater increase in energy expenditure in the cold, in comparison to the ambient conditions, is likely due from the higher thermogenic stress on the body (Castellani & Young, 2016). When combined, the thermogenic effects of the cold and the shakes have shown to be significantly greater compared to CON (Fig. 2.2). However, there is no change in H_{prod} following the ingestion of both shakes, which speculates that the quantity does not have an effect on H_{prod} in the cold. Total H_{prod} following the shake ingestions was 43% greater in the COLD than in CON (Fig. 2.5a,b). Clearly, metabolic responses are more apparent in the cold compared to the control condition (Fig. 2.2). The overlap of CON is indicative that the individual response was relatively similar, however, it was not the case for COLD (Fig. 2.2). Experimental factors and variability can be an explanation for that observation.

Furthermore, an increase in H_{prod} was found in all conditions. They reached their maximal H_{prod} at 120 min or 60 min after food ingestion, regardless of the quantity given or exposed temperature (Fig. 2.2). This is comparatively similar to the findings of Rochelle & Horvath (1969) where the maximal increase for the ingestion of a CHO or steak meal (894 kJ) appeared at 75 min

into cold air (7.5°C). They also found that the whole-body H_{prod} increased in parallel for the 3 distinct ingested meals of 894 kJ each (CHO, glycine or steak). In contrast to our study, they focused their attention on meals with the same caloric intake but with different compositions. The glycine meal elicited the highest increase in H_{prod}, supporting that the effect of DIT is known to depend mainly on nutrient composition (Jequier, 1986). In fact, PRO have been shown to increase DIT to a larger extent than regular mixed diets (Westerterp-Plantenga, 2008), where they represent ~20-30% of energy content (Haman, 2010). These observations were specific to quality intake, while our findings demonstrated that the quantity has no effect on the short response in the cold. Perhaps the meal with the highest caloric intake would have presented a longer postprandial response (Barr & Wright, 2010; Westerterp, 2004) as DIT can last for a few hours after food ingestion. However, not only was it not the purpose of this study, but additionally prolonged cold exposures can provide challenges in regards of study logistics (i.e. recruitment and motivation of participants).

Oxidation rate of macronutrients

A significant difference can be found in RG_{ox} and RF_{ox} between the CON and COLD (Fig. 2.3), regardless of the quantity, which is highly due to the higher thermic stress of the cold exposure (Haman et al., 2002). Also, a slight increase in RG_{ox} can be observed after 20 min of ingestion of the shakes for all conditions, CON and COLD (Fig. 2.3). This could correspond to the onset of DIT where it normally appears 20-30 min after ingestion of a meal no matter its composition (Scott et al., 2007). Even if there could be presence of DIT observed, it is still not clear if it contributes to the cold-induced NST in this study.

Moreover, the oxidation rates of both macronutrients between the 1507 kJ and 3015 kJ shakes where not significantly different but did increase after their ingestions. Despite the cold already generating heat due to the thermic stress, the ingestion stimulated the action of the SNS and the release of noradrenaline activated the DIT (Himms-Hagen, 1984; Scott Johnson et al., 1982; Welle et al., 1980). A significant increase of RG_{ox} did arise at 120 min which could be caused by an increase of glucose uptake by BAT (Ouellet et al., 2012).

It is well known that absorption rate of glucose will never exceed 1g/min during exercise (for ingestion rate of 1.2g/min) (Jeukendrup & Jentjens, 2000) and will be maximized at around 200mg/min in the cold (for ingestion rates of 400mg/min). This could be an explanation of the absence of significance found in H_{prod}, RG_{ox} and RF_{ox} between the two quantities ingested (Fig. 2.2, 2.3). The optimization of the absorption rate of glucose is obtained with an ingestion of about 50g of glucose (Blondin et al., 2010a). In our study, the highest quantity given (3015 kJ) contained 90g of CHO and the lowest quantity had 45g. It clearly exceeds the max ingestion rate for optimization of absorption. Essentially, even if a high caloric meal is given in the cold, the absorption rate will remain the same. Moreover, even in different content meals, it does not seem to have an effect on thermogenic rates. Vallerand et al. (1993), reported that the thermoregulatory response was not altered compared to a placebo with the ingestion of 710 kJ CHO (100% CHO jellies vs high CHO bar) at onset of cold exposure. They also reported that similar results were obtained when 2970 kJ was given in the cold, meaning that different caloric intakes are not the limiting factor for changes in cold-induced thermogenesis.

Fuel selection patterns during ST are known to vary by CHO availability prior to cold, the intensity of bursts, muscle fibers (Haman, 2010), and inter-individual variability (Haman, Legault, et al., 2004c). While energy potential differs widely by substrates (16 kJ/g for CHO, 41 kJ/g for

LIP and 20 kJ/g for PRO), fuel selection in the cold is dependent of the shivering intensity. Our results showed that H_{prod} increased in parallel to shivering intensity when the shakes were ingested. The absence of significant differences in the thermal responses could be caused by a masking effect of DIT by ST (Vallerand, 1992; Vallerand et al., 1993). However, it is still not clear whether it's the cold exposure over time or the food ingestion that causes the increases in H_{prod} and ST intensity.

EMG shivering activity

ST intensities were measured throughout the 3h of cold exposure using EMG on 4 distinct muscles (pectoralis, trapezius, vastus lateralis and sternocleidomastoid). The increases in H_{prod} for the COLD conditions were accompanied by an increase in EMG_{shiv} activity. However, contrary to what was predicted, no significant differences were found in the shivering intensities between the two different energy equivalents. This demonstrates that H_{prod} and shivering activity increased in parallel, but the caloric equivalent of the two shakes had no effect on the thermoregulatory responses in the cold. It could be hypothesized that the liquid meal created a raise in H_{prod} while increasing contractions in the meantime (Vallerand, 1992; Vallerand et al., 1993).

As previously mentioned, few cold studies have measured quantitatively shivering activity, which makes it difficult for comparison among other studies. For example, in the study of Rochelle & Horvath (1969), there is no mention of ST measurements, however, they reported a pronounced shivering activity over time in combination of the meals ingested. The only exception was for the glycine meal, where ST was almost non-existent. The metabolic increase and the reduced ST linked with the glycine meal was explained by a contribution of NST. However, the validity of the observations is unclear since these were simply observational self-reporting shivering.

Most of cold and energy intake studies such as Vallerand (1992) and Vallerand et al. (1993), did not measure muscle shivering activity, meaning we are not able to determine if the increase in H_{prod} is due to greater muscle contraction or not. On the other hand, Ouellet et al. (2012) did measure EMG_{shiv} and found an inverse relationship between BAT volume activity and shivering, but these observations were made regardless of food consumption.

Since there is a significant increase in total H_{prod} after the ingestion of the shake in the cold (Fig. 2.5b), it could be argued that there may be a possible supplementary effect of the DIT in the cold. However, since no differences were found between the quantities, we cannot make that assumption. This is consistent with Rochelle & Horvath (1969) findings where DIT did not seem to be easily noticed when shivering. They thought that the increased ST associated with the CHO and glycine meals was the main cause for the increase in H_{prod}.

Furthermore, cold-induced NST and nutrient-induced thermogenesis increases the sympathetic system and stimulates the release of noradrenaline leading in the activation of BAT (Cannon & Nedergaard, 2004; Welle et al., 1980). In cold exposure, overfeeding in rats seems to contribute to hypertrophy of BAT, compared to a restricted feeding (Scott Johnson et al., 1982). Even if the cold and the feeding activates the SNS, the overfeeding in the cold had a larger significant impact. This can be explained by a smaller fuel intake caused by the restricted diet limiting lipolysis, hence the growth of brown tissue (Scott Johnson et al., 1982). Indeed, lipolysis is the predominant action in BAT (Blondin et al., 2017a). Repeated cold exposure in humans, such as cold acclimation, also has hypertrophy on BAT effect and increases its activation level (Blondin et al., 2017b, 2014a; Gordon et al., 2019). It is yet to be validated that food and cold combined have the same effect in humans. Even if it was previously confirmed that the contribution of BAT represents only about 0.5% of whole body H_{prod}, (u Din et al., 2016), cold exposure increases BAT

oxidative metabolism as well as the uptake of glucose and non-esterified fatty acids (NEFA) (Ouellet et al., 2012). As a result, mild cold exposure induces NST through BAT in humans.

Finally, bursts rates were not included in this study but could have been useful to determine which fibers where prioritized during shivering with food consumption. The type of fiber plays an important role in the fuelling of shivering, where type I are known to be associated with continuous shivering and type II with bursts (Meigal, 2002). More specifically, type I would be known to be supported by LIP oxidation (Tipton et al., 1997). We can speculate that the high increase in RG_{ox} (Fig. 2.3) can be supported by type II fibers after the ingestion of the shake compared to the steady state cold, but again it is difficult to make such conclusion without bursts rate.

Conclusion

In conclusion, this thesis showed that ingesting shakes at two different caloric equivalents does not affect the contribution of H_{prod} and ST. In addition, ST intensity did not change, which confirmed that NST remained the same between the two conditions. This study was designed and controlled in order to determine the fundamental metabolic effects of cold exposure on the human body. It is worthy to note that in reality, individuals exposed to cold would have access to clothing. Hence, the cold responses could have been much different if the study was aimed in that direction. However, our results can be applied on possible cold scenarios such as the conception of survival rations. For example, since quantity intake does not have an effect on the thermic response in the cold, the choice of the survival rations or the food brought on military missions is not of great importance. For a given mission, if the choice is between a set amount of extra clothing or supplementary food, perhaps the clothing would be the better choice. Clearly, more research on the exact pathways of each processes in the cold with food consumption needs to be made. To that

extent, the investigation of the effects of food quality on the thermogenic processes during cold exposure strikes us as a fascinating area for future research.

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CHAPTER 3: GENERAL CONCLUSION

The general objective of this thesis was to determine whether food quantity has an effect on thermogenic properties in cold conditions. More precisely, that the ingestion of a shake of 1507kJ and 3015 kJ would stimulate cold-induced NST in men exposed to a mild cold.

CHAPTER 1 provides current knowledge related to cold-induced thermogenesis, fueling of shivering thermogenesis and the changes in metabolic responses in the cold following the ingestion of different foods. It also explains in which ways NST can be a contributor in the increase of total thermoregulatory responses with food consumption.

The experiment in CHAPTER 2 refuted the hypothesis that a greater caloric intake (3015 kJ) would have a greater effect on total heat production compared to a lower caloric intake (1507 kJ). In addition, in the present study the contribution of NST to total H_{prod} was not different when ingesting shakes at two different caloric equivalents because changes in ST and H_{prod} where not different in the cold. While, cold-induced NST and DIT are stimulated by the same neurotransmitter in the activation chain of UCP1, the exact mechanisms are still unclear.

In view of these findings, it is impossible to say that if food consumption has an effect on the thermoregulatory response in the cold. There is evidence from past literature that cold-induced NST and DIT share the same motor command, but it cannot be assumed that there is a contribution of NST linked with the quantity intake in men exposed to cold. However, the quantity of heat produced when the shakes were ingested could have been an added effect of muscle contraction during shivering and/or the energy required for fueling shivering.

Finally, the need of this kind of study has been established for years. Understanding the living choices of northern communities in relation to their metabolism has been quite interesting in relation to populations living in non-extreme cold conditions. It has been useful to apply this knowledge to training practices in relation to food rationing in the military forces while on mission

in cold environments. This type of analysis needs constant data, research and observation for delaying the onset of life-threatening hypothermia (Committee on Military Nutrition Research et al., 1996). More investigation needs to be done on the effects of food consumption during cold exposure, as shivering can alter manual dexterity and be particularly uncomfortable. It would be interesting to investigate the effects of different composition meals in prolonged cold exposure (more than 3h) and in extreme cold conditions. However, some challenges can be associated with cold studies, such as finding participants that meet the requirements and that remain motivated throughout the experimental trials as it can be unbearable for participant to be put in such cold conditions for a long period of time. While ST can be quite uncomfortable, ST intensity does not change with caloric intake. Consequently, the amount of food ingested in mild cold exposure is not a strategy to lower ST and increase NST. The next step for future research could examine the effects of food quality intake on the thermogenic response.

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