METABOLIC AND THERMAL RESPONSES TO SHORT-TERM, INTENSE COLD WATER ACCLIMATION PROTOCOL

by

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SUMMARY

Non-compensable cold exposure represents a potentially deadly threat to humans, as we lack highly specialized organs and mechanisms necessary to maintain our optimal core temperature of ~37°C. Repeated exposures to cold have been shown to induce protective physiological changes in cold responses through a process known as cold acclimatization (natural) or acclimation (in laboratory). The purpose of this thesis was to determine what physiological changes occur following an intense 7 day, 14°C cold water immersion acclimation protocol, during both non-compensable (Chapter 2) and compensable cold exposures (Chapter 3). This includes identifying changes in the contributions of the shivering (ST) and non-shivering (NST) thermogenic pathways to overall heat production. ST and NST changes were quantified via electromyography and indirect calorimetry, respectively.

This 7 day cold water acclimation protocol resulted in a decrease in cooling rate, a significant increase in mean esophageal core temperature, a decrease in peak heart rate following immersion, and increased thermal comfort from day 1 to day 7 of the 1h 14°C cold water immersions. Further to these findings, changes in ST and NST were measured pre- and post-acclimation with a standardized compensable cold protocol using a liquid conditioned suit (LCS) which lowered T_{skin} to 26°C for 2.5h. The cold acclimation protocol resulted in a ~38% decrease in mean shivering over the 2.5h without any change in thermogenic rate from pre- to post-cold acclimation. In addition, no significant difference in fuel selection was observed. These results indicate that the short, intense cold acclimation protocol did result in a substantial change in the contribution of ST and NST to total heat production which could increase cold tolerance by reducing involuntary muscle contractions during ST.

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LIST OF ABBREVIATIONS

AUC – Area under the curve

ß3AR – Beta-3-Adrenergic Receptor

BAT – Brown adipose tissue

Ca^{2+} - Calcium

CHO – Carbohydrate

CSR – Cold shock response

CT – Computerized tomography

EE – Energy expenditure

EMG – Electromyography

H_{prod} – Whole-body heat production

H_{loss} – Whole body heat loss

kJ – Kilojoules

LCS – Liquid conditioned suit

MVC – Maximum voluntary contraction

NST – Nonshivering thermogenesis

RMS – Root mean squared

SEM – Standard Error of the Mean

SERCA – Sarcoplasmic/Endoplasmic Reticulum Ca^{2+}-ATPase

T_{skin} – Skin temperature

T_{core} – Core temperature

T_{eso} – Esophageal temperature

T_{rec} – Rectal temperature

UCP1 – Uncoupling protein 1

VO_2 – Oxygen consumption

VCO_2 – Carbon dioxide production

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GENERAL INTRODUCTION

INTRODUCTION

As endothermic homeotherms, humans rely on thermoregulatory mechanisms to maintain a physiologically optimal ~37°C core temperature (T_{core}) (Stocks, Taylor, Tipton & Greenleaf, 2004). Humans are particularly maladapted to survive extreme or prolonged cold exposure relative to other mammals (Kaciuba-Uscilko & Greenleaf, 1989). There are many behavioural adaptations humans undertake to thermoregulate and avoid cold exposure, including staying indoors in climate-controlled environments and adding layers while outdoors. Maintaining thermal balance in the cold involves reducing heat lost to the environment and increasing whole-body heat production (H_{prod}) (Castellani & Young, 2016; Kaciuba-Uscilko & Greenleaf, 1989). Intentional repeated cold exposure have been shown to alter physiological responses, known as cold acclimation or acclimatization, and could potentially help improve odds of survival during an extreme, or prolonged cold exposure, by increasing H_{prod} or improving vasoconstrictive response to maintain T_{core} (Castellani & Young, 2016; Kaciuba-Uscilko & Greenleaf, 1989; Young, 1994). The following introduction will describe general concepts of cold response in humans including detecting changes in environmental temperature, reducing whole body heat loss (H_{loss}), increasing thermogenic rate, understanding the cold shock response and how to classify various responses to cold exposure.

1.1. First line of defence: detecting changes in environmental temperature

In the initial stages of temperature regulation, humans detect changes in environmental temperature through strategically situated thermoreceptors, beginning with cutaneous, or skin, thermoreceptors providing advanced warning. These will mediate the signalling required for a rapid thermoregulatory response if necessary, based on environmental temperature fluctuations (Nakamura & Morrison, 2011). When thermoregulatory mechanisms cannot balance heat lost to

the environment with heat produced by the body, T_{core} will begin to decrease, activating core thermoreceptors. This is described as a thermally non-compensable cold exposure. Differentiating between these two types of thermoreceptors is important for understanding cold responses, as evidence has shown that physiological responses to cold exposure can be dependent on whether core thermoreceptors are activated, or a cold stimulus is solely impacting skin thermoreceptors (Imbeault, Mantha, Haman, 2012).

Depending on the intensity and duration of a cold exposure, outcomes will differ, and unprotected exposures will likely lead to hypothermia, cold injury, or death. There is a wide spectrum of physiological distress from minute pain in cold areas and a loss of dexterity, to freezing injury such as frostbite (Stocks, Taylor, Tipton, & Greenleaf, 2004). Cold impacts physiological responses as well, Stocks *et al*. (2004) discuss the negative side effects on muscles cooling from a temperature of 35.2 to 31.3°C, which can occur in just 15 min of resting in cold water at 27°C. These negative effects can include decreased enzyme activity, decreased blood perfusion, decreased acetylcholine and calcium (Ca^{2+}) release, and decreased conduction and action potential repolarization in the muscles. These impairments contribute to a ~3% decrease in muscle power for each ~1°C decrease in muscle temperature (Stocks, Taylor, Tipton & Greenleaf, 2004). Cold exposure of any type presents an immediate threat to humans that are not able to compensate metabolically, physiologically and/or behaviourally. H_{loss} to the environment is exacerbated depending on the medium of exposure, and heat conduction in water is ~25 times greater than in air. What might be considered a very moderate cool water immersion of ~28-30°C can even result in a stress on thermal homeostasis in humans. In extreme scenarios, such as ice water immersion, core H_{loss} can surpass ~6°C/h, and can lead to death in as short as 45 min (Toner & McArdle, 1996).

Human physiological responses to cold exposure are based on maintaining a sensitive homeostasis in the body, balancing heat gained through normal metabolic processes and heat lost to the environment. Shivering, vasoconstriction, and non-shivering thermogenesis are among the mechanisms that are intended to minimize H_{loss} and maximize H_{prod} when cutaneous and T_{core} receptors drop below a critical threshold. This threshold is defined by Toner & McArdle (1996) as the point at which there is a significant increase in oxygen consumption (VO_2) or H_{prod}. The critical temperature for cold exposure in humans is not surprisingly substantially higher in water than in air, ranging from ~30-34°C, depending on the individual (Toner & McArdle, 1996). Two general responses are initiated following a drop below the critical threshold in humans, including initiating cutaneous vasoconstrictive responses to reduce H_{loss}, and upregulating thermogenic processes to increase H_{prod}; all in order to maintain the desired T_{core} of ~37°C (Eyolfson, Tikuisis, Xu, Weseen, & Giesbrecht, 2001; Kaciuba-Uscilko & Greenleaf, 1989; Stocks *et al.*, 2004).

1.2. Reducing rate of H_{loss}

H_{loss} can be reduced through whole-body cutaneous vasoconstriction, stimulated primarily as a reflex due to whole-body cooling which activates a neural signaling pathway through the preoptic area of the hypothalamus, in turn activating the sympathetic nervous system (SNS). Norepinephrine is the neurotransmitter secreted by signaling SNS nerves, responsible for ~60% of the cold-induced vasoconstriction in skin, while neuropeptide Y is the other neurotransmitter, accounting for another ~20 to 30% of vasculature constriction (Castellani & Young, 2016). By constricting peripheral vasculature, blood can be redirected more centrally, to deeper tissues, decreasing conductive H_{loss} from skin to the environment, and increasing the insulative capacity of peripheral tissues. The shift of blood volume from peripheral vessels to the core also results in intrathoracic hypertension and increased cardiac output, which in turn will

lead to a decrease in heart rate (HR) (LeBlanc, 1962; Radomski & Boutelier, 1982; Stocks *et al*., 2004). While vasoconstriction has been shown to definitively reduce H_{loss}, another common belief is that higher body fat individuals have an innate insulative capacity which reduces H_{loss} to the environment. While some polar mammals are built to use fat as an insulative layer (Blix, 2016), humans are not as reliant on insulative organs. There remains some debate in the space, as research over several decades has been conflicting on the topic. Rennie *et al*. (1962) originally supported that fat tissue was correlated strongly to insulative capacity, with women proving to have higher insulative capacity than men. A follow-up study performed from Veicsteinas, Ferretti, & Rennie (1982) elaborated on this, showing that superficial shell insulation from fat was only ~10-15% of maximal shell insulation, implying that other vasoconstriction play a much more significant role, and fat was only a small contributor to insulative capacity. At rest, it has been shown that vasoconstricted muscle provided a more significant insulative capacity than fat tissues in the limbs (Veicsteinas, Ferretti & Rennie, 1982). Finally, research by van Ooijen *et al*. (2006) suggested that despite higher body fat, these individuals do not have an increased insulative capacity, and further elaborated that obesity may impair H_{prod} in humans during a mild cold exposure, creating a net negative effect in relation to surviving a cold exposure.

1.3. Increasing thermogenic rate

H_{prod} in mammals and humans is a term that encompasses a variety of pathways. Generally, H_{prod} is broken down into two primary divisions of thermogenic processes in the body, namely shivering thermogenesis (ST) and non-shivering thermogenesis (NST).

1.3.1. Shivering thermogenesis

In humans, ST represents by far the majority of H_{prod} during cold exposure (Blondin, *et al*., 2014a; Kaciuba-Uscilko & Greenleaf, 1989) and involves sympathetic nervous stimulation of skeletal muscles, which causes asynchronous contractions, accomplishing no physical work, in turn releasing heat as a by-product. ST is immediately initiated when the skin temperature (T_{skin}) receptors detect a decrease in environmental temperature (Imbeault, Mantha, & Haman, 2013), although the intensity of the thermogenic response may be altered depending on T_{core} as well, and a low T_{core} will lead to a multiplicative effect on thermogenesis (Stocks *et al*., 2004). Using electromyography (EMG) on key muscle groups that have been previously identified as key shivering contributors such as: *pectoralis major*, *rectus abdominus*, and *vastus lateralis* (Haman, *et al*., 2004); muscle activation relative to baseline can be quantified, allowing a better understanding of the contribution of ST to H_{prod}. Based on muscle mass, it has been suggested that trunk muscles contribute ~85% of H_{prod} due to shivering (Bell, Tikuisis & Jacobs, 1992) and ST can increase metabolic rate by ~5 times resting levels (Eyolfson *et al*., 2001; Haman *et al*., 2006; Iampietro *et al*, 1960). Researchers have shown that during cold exposure, the bulk of glucose turnover, which is an indirect indicator of metabolic activation, is direct towards skeletal muscle (Blondin *et al*., 2015). ST is sustained primarily at the beginning of cold exposure by intramuscular stores of carbohydrates, which gradually changes to fat oxidation as shivering continues. The relatively low intensity of shivering and reliance on multiple fuel sources allows shivering to be sustained indefinitely (Haman, *et al*., 2004a; Haman, *et al*., 2004b).

1.3.2. Nonshivering thermogenesis

The other main division of thermogenesis is NST, which represents all processes in the human body that generate heat aside from ST. Consider that most every chemical reaction in the human body releases heat as a by-product, and that both the production, as well as the utilization

of ATP are inefficient processes, releasing heat as a by-product. Prior to more recent research into NST, there was little understanding or appreciation of mechanisms outside of ST for H_{prod} in man, illustrated by Iampietro *et al* (1960), which stated: "Input of heat, in the resting man, can only be augmented by shivering". As research into H_{prod} in humans advanced, it became clear that regarding shivering as a sole source for H_{prod} neglected other significant mechanisms of H_{prod}. In particular, brown adipose tissue (BAT) and skeletal muscle Ca^{2+} cycling have been examined more extensively for their contributions to H_{prod}.

The physiological significance of BAT has been well documented in mammals prior to the discovery of active BAT in humans. BAT provided mammals an evolutionary advantage to survive cold stress, during daily and seasonal temperature variation, and at our most vulnerable during infancy (Cannon & Nedergaard, 2004). The histological makeup of brown adipocytes in BAT shows dense packing of mitochondria containing with a specialized protein, uncoupling protein 1 (UCP1) in high prevalence. UCP1 works through a process in which the oxidative process in mitochondria is not coupled to ATP synthesis as it would typically be, but instead this potential energy is released as heat (Cannon & Nedergaard, 2004; J Nedergaard *et al*., 2001; Ouellet *et al*., 2012; Virtanen *et al*., 2009). Before a serendipitous discovery in 2007 and several influential studies released in 2009, it was believed that BAT was inactive in adult humans and not contributing to H_{prod} at rest or during cold exposure (Cypess *et al*., 2009; J Nedergaard, Bengtsson, & Cannon, 2007; Van Marken Lichtenbelt *et al*., 2009; Virtanen *et al*., 2009). In 2007, Nedergaard *et al*. demonstrated that using a standard test for tumours, several areas of unexpected glucose uptake could be detected. This study showed depots of active tissues in adult humans, focused primarily in the supraclavicular and paracervicular areas (J Nedergaard *et*

al., 2007). Nedergaard *et al*. (2007) suggested that these symmetrical depots were likely evidence of active BAT in adult humans.

Even more recent studies surrounding BAT have shown evidence for the concept of beige or brite adipocytes. While classical BAT is made up solely of brown adipocytes, which dissipate energy as heat and are required for NST, these recently identified brite adipose tissue depots are a separate type of thermogenic cell (Wu *et al*., 2012). These depots contain a combination of brown and white, hence the term 'brite'. It is also sometimes referred to as beige adipose tissue, based on its physical appearance (Keipert & Jastroch, 2014). Unique gene expression patterns identify these adipocytes as being a separate entity from classic BAT (Okamatsu-Ogura *et al*., 2013). This tissue is derived from seemingly white adipose tissue sites in a process known as 'browning'. Many browning agents have been identified, but mainly cold stimulation, through activation of the beta-3-adrenergic receptor (ß3AR) by norepinephrine, will elicit a significant increase in UCP1 expression in the mitochondria of this tissue (Nedergaard & Cannon, 2014; Wu *et al*., 2012). Besides the thermogenic properties associated with BAT, one of the hallmarks of brown and brite adipose tissue is its activation by cold exposure. Large variations in levels of UCP1 expression and BAT volume have been identified between winter and summer months in humans (Carey & Kingwell, 2013; Cypess *et al*., 2009). Experiments in mice show that these brite adipose sites can go from minimal UCP1 in normothermic conditions to having comparable amounts of UCP1 to classical BAT sites, the key protein for NST in a cold condition. In fact, these studies have shown that NST is not solely dependent on pure BAT, but also brite adipose sites, although BAT remains the main contributor (Keipert & Jastroch, 2014; Okamatsu-Ogura *et al*., 2013). Many studies examining activation of NST do not go as far as to break down

individual contributions of brite and BAT, but rather both are viewed as one thermogenic organ, even though we are aware there is two uniquely identifiable tissue types.

Considerable research has gone into elucidating the pathways of BAT activation and subsequently how to naturally or artificially stimulate activation. It is well known that thermogenic tissue is innervated and critically controlled by neurons from the SNS, particularly the ventromedial hypothalamus, which plays a role in temperature sensing (Bartness, Vaughan, & Song, 2010; Chechi, Carpentier, & Richard, 2013; Cypess & Kahn, 2010; Nedergaard & Cannon, 2014). The current understanding of intracellular signalling pathways says BAT and beige adipose tissue thermogenesis is triggered by the release of norepinephrine from sympathetic nerve terminals which activate cellular ß3ARs and in turn unleash a cascade of intracellular events which stimulate UCP1 synthesis, activity and overall adipocyte thermogenesis (Bartness *et al*., 2010). It has been suggested that these receptors may be the sole activator of BAT thermogenesis (Cypess *et al*., 2015), but other studies show white or brite adipose tissue may be able to sense temperature in a cell-autonomous fashion for activation of thermogenic response (Ye *et al*., 2013).

Further studies of BAT tissue by Blondin *et al* (2017) show that this tissue relies mostly on fatty acids from intracellular triglyceride to fuel the thermogenic activity of mitochondria. Blondin's study further demonstrated that niacin-induced inhibition of lipolysis of these triglycerides suppresses BAT oxidative metabolism and glucose uptake, decreasing BAT thermogenesis. This decrease in BAT activity resulted in a reciprocal increase in shivering activity (via EMG), which again demonstrates the close physiological relationship of H_{prod} mechanisms, BAT and ST during cold exposure in humans. Standardized quantification of H_{loss} would further inform how overall H_{prod} is manipulated with regards to changing BAT activity.

Skeletal muscle accounts for ~45-55% of body mass in humans, meaning muscle activation during physical activity, as well as during activities of daily living, can significantly alter H_{prod} in humans (Periasamy, Herrera, & Reis, 2017). With regards to H_{prod} during cold exposure, skeletal muscle is mainly considered as the most significant contributor to thermogenesis in humans, while BAT is often considered the sole or main specialized mechanism for NST. Despite these beliefs, researchers continue to pursue other areas of interest with regards to NST. In still developing field of muscle NST, Bal *et al*. (2012) demonstrated that skeletal muscle sarcoplasmic/endoplasmic reticulum Ca^{2+} (SERCA)-based thermogenesis will compensate for BAT-deficient transgenic mice. Despite lacking BAT, the mice were able to adapt and compensate for the loss in H_{prod} via suspected muscle-based thermogenesis. Overall, this rodent study also showed that skeletal muscle based NST is upregulated as BAT activity is minimized. (Periasamy *et al*., 2017).

Skeletal muscle can be a contributor to NST via the activation of futile Ca^{2+} cycling pathways, through SERCA ATPase. SERCA pumps are essential to controlling muscle contraction, maintaining normal cytosolic and intracellular Ca^{2+} levels. Studies have previously examined how specialized heater organs in fish produce heat via continuous SERCA transport due to a constant 'leak' of Ca^{2+} via ryanodine receptor 1. This process continually replenishes cystolic levels of Ca^{2+}, which are actively pumped back into sarcoplasmic reticulum (Bal *et al*., 2012; Periasamy *et al*., 2017). This is the same process previously identified in endothermic mammals SERCA-based NST. SERCA activity in muscles is regulated largely by sarcolipin, a small protein that has been identified as a regulator of the SERCA pump. Increases in sarcolipin levels can block Ca^{2+} transport into sarcoplasmic reticulum, leading to SERCA uncoupling Ca^{2+} transport from ATP-hydrolysis. Ultimately this means that Ca^{2+} is released back into cytosol by

SERCA rather than crossing the membrane, and hydrolyzed ATP energy is released only as heat (Bal *et al*., 2012; Pant, Bal & Periasamy, 2016; Nowack, Giroud, Arnold, & Ruf, 2017).

SERCA-based thermogenesis in humans allows for skeletal muscle to contribute to human endothermy through a pathway independent of ST. In mammals where BAT is only found in insignificant quantities, or contributes minimally to NST, SERCA based NST in skeletal muscle may be responsible for much of the observed glucose uptake during cold exposure (Hanssen *et al*., 2015; Rowland, Bal, & Periasamy, 2015). These studies indicate that NST in humans may not solely dependent on BAT thermogenesis, and that skeletal muscle may be another significant contributor to H_{prod} during cold exposure.

It is important to realize that while primarily highlighting BAT, and SERCA in skeletal muscle, there is nothing to suggest there are not other processes that are contributing in a significant manner to H_{prod} in humans during cold exposure. NST is a universal term that encompasses all aspects of heat production in the human body outside of shivering. The specialized mechanisms observed in BAT and SERCA appear to be the two leading candidates as significant contributors to NST that can be modulated and upregulated during repeated cold exposures.

The actual process of measuring NST during any cold exposure is inherently challenging as both ST and NST are simultaneously contributing to H_{prod} (Gosselin & Haman, 2013; Jansky, 1973; van Marken Lichtenbelt & Daanen, 2003). To confound matters, increases in NST may also lead to decreases in ST, as shown by Cannon & Nedergaard (2011) in small mammals. As well, no direct method is currently available to quantify the independent contributions of NST to H_{prod}, but by combining several methods, we can create an accurate assessment of NST throughout a cold exposure. H_{prod} can be estimated by using indirect calorimetry which

calculates this based on VO_2. As well, measurement of shivering intensity can be quantified via EMG. Using the EMG signal as a percent of maximal voluntary contraction (MVC) provides an unparalleled standard for evaluating changes in ST. Measurement of specific NST mechanisms can be done indirectly as well, as demonstrated by quantification of BAT activity using positron emission tomography/computerized tomography (PET/CT), which can measure glucose uptake through use of radioactive tracers. Multiple studies have repeatedly confirmed a positive correlation between increased BAT oxidative metabolism and increases in H_{prod} (Ouellet *et al*., 2012; van der Lans *et al*., 2013; Yoneshiro *et al*., 2011). Indirect assessment of NST and ST contributions to H_{prod} can be done by comparing levels of EMG activity pre- and post-acclimation when H_{prod} is maintained between the two sessions using a standardized cold exposure. We can infer the relative contributions of NST and ST to H_{prod} as a decrease in muscle activity as measured by EMG with the same total H_{prod} suggests NST is increasing its relative heat contribution to H_{prod}. This methodology is limited in that the exact mechanism of NST cannot be identified, and we can only theorize as to which of the cold induced NST mechanisms is increasing H_{prod}, and to what extent they are doing so. As well, if H_{loss} is not monitored and quantified, changes in vasoconstriction, core temperature, and heat retention may impact how results can be interpreted.

1.4. Cold shock response

During an intense, quick decrease in ambient temperature such as sudden cold water immersion, a 'cold shock' response (CSR) will be triggered. CSR is an immediate and severe threat for all individuals, driving a powerful response of the cardiovascular and respiratory systems. This includes gasping, hyperventilation, hypertension, as well as accelerated heart rate, know as tachycardia (Tipton, 1988; 1989), which represents a serious risk to individuals.

Ventilation can increase up to 5 times baseline levels during an intense cold immersion, which leads to a decrease in CO_2 which can eventually cause blood alkalosis (Wittmers & Savage, 2001). Tachycardia occurs, which may lead to a heart attack, and the reflex to gasp or inspire when falling into cold water has been deemed as a significant contributor to cold water drownings (Mekjavić, Prairie, Burke, & Lindborg, 1987). During prolonged exposure, cold water immersions eventually lead to bradycardia, which is a slowing of heart rate, despite the initial CSR which triggers a short spike in HR. Some of these CSR mechanisms may be related to mammalian evolutionary traits in humans. Leblanc, *et al*. (1975) conducted cold face tests among adults, immersing just the face in cold water. The results showed a marked decrease in HR, or bradycardia, a parasympathetic response, along with simultaneous increase in blood pressure, a sympathetic response. Leblanc's paper suggested it may be due to the mammalian diving response, and cites research by Irving, Scholander & Grinnell (1941) on bradycardia in diving seals. Seals have a decreased HR during water immersion as a defense mechanism to hypothermia. The diving response is typically concomitant with intense vasoconstriction, and bradycardia reduces oxygen requirements while maintaining circulation to the heart and brain. This slowing of the HR and decrease in oxygen requirements from the mammalian diving reflex, or cold water exposure, is what makes inducing a mild hypothermia a standard of care for emergency room physicians when treating a patient after suffering cardiac arrest (Hypothermia after Cardiac Arrest Study Group, 2002). Cold water immersion has also been investigated as a method for sport recovery and is regularly used for this purpose. A review by Wilcock, Cronin and Hing (2006) examined cold-water immersion, or cryotherapy, as a recovery modality, and suggest a large part of this as a recovery is due to the perceived benefits of hydrostatic pressure in stimulating fluid movement, substrate movement from muscles and increased cardiac output.

As well, cold water specifically, as it induces vasoconstriction, decreases the inflammation response of any intense physical activity, helping to improve the rehabilitation process.

1.5. Classifying physiological changes

Acclimation, acclimatization and adaptation are terms used to describe changes in a system to better respond to a prolonged or repeated stress. In the case of human cold exposure, these three terms refer to how the physiological responses to cold exposure of vasoconstriction, ST and NST change following repeated stresses. Acclimation typically refers to controlled and regulated cold exposures in a laboratory setting; acclimatization refers to natural, outdoors, seasonal or otherwise unavoidable cold exposures. Adaptation is generally a term used to describe genetic predispositions from generations of evolution to confer favourable traits for thermoregulation (Kaciuba-Uscilko & Greenleaf, 1989). These terms have been used interchangeably throughout the academic literature, but for the purposes of this research, the above definitions will be used for reference.

Acclimatization to a natural cold northern environment has been demonstrated in Gaspé fishermen, who show an attenuated sympathetic and parasympathetic response to face and hand cold exposure, allowing for improved circulation during cold exposures (LeBlanc, 1962). A similar study by Leblanc *et al*., (1975) examined differences in response to cold hand and cold face tests between northern Indigenous populations, white males from southern Canada, and white males living in northern Canada. Their experimental results showed a spectrum of adaptation and acclimation, as Indigenous individuals showed a much smaller sympathetic-driven CSR, both blood pressure and HR remained close to baseline levels during cold stimulus, while white males from northern Canada had a larger increase in blood pressure, but a significantly smaller increase in blood pressure than those living in southern Canada, who

demonstrated the most significant shifts. Despite the divergence in sympathetic response, these groups showed a similarly strong vagal, parasympathetic drive which presented as bradycardia. Leblanc *et al*. (1975) suggested that the decreased sympathetic response observed in Indigenous populations is related to acclimatization to the cold environment since a significant difference was observed between white males living in the Arctic and those living in the south.

Cold acclimation is the process of repeating exposures to cold in a laboratory setting and has a long history in the scientific literature. Studies have reported acclimation protocols that have produced significant changes in the human physiological response to cold exposure. Davis (1961) showed that an acclimation protocol involving sustained cold exposure for 8 h/day for 31 days, noticeably decreased shivering measured by electromyography (EMG), and these physiological changes were recorded as early as 14 days into the protocol. Davis (1961) also showed that VO_2, a direct correlate to metabolic rate and overall H_{prod}, remained the same pre- to post-cold acclimation, an indicator that NST likely increased to compensate for decrease in H_{prod} from muscle. Research by Blondin, *et al*. (2017) showed that a 4 week cold acclimation protocol was able to elicit a decrease in whole-body shivering intensity of ~20%, measured via EMG. In previous research (van Marken Lichtenbelt *et al*., 2009; Yoneshiro *et al*., 2011), subjective visual measures or experimenter-led questioning were used to quantify shivering activity, but lack the objectivity, standardization, and acute detail provided by EMG. This can lead to incorrect assumptions of decreased shivering activity if not carefully supported with quantitative methodology (Blondin *et al*., 2015).

With the increased interest in BAT in the past decade, recent research has shown that cold acclimation can increase the volume of BAT depots in humans, as well as increase BAT oxidative metabolism during a mild cold exposure. These results indicate an increased

contribution to H_{prod} via NST (Blondin *et al*., 2013; Blondin, *et al*., 2014; van der Lans *et al*., 2013). As mentioned above in detail, repeated cold exposures may also be one of the key approaches for stimulating the SNS and in turn leading to the 'browning' of white adipose tissues, increasing overall capacity of UCP1-mediated NST in humans (Cypess & Kahn, 2010; Nedergaard & Cannon, 2014). Increasing the activity of NST mechanism through cold acclimation can also have potential benefits beyond simply increased daily energy expenditure (EE) and increasing overall NST, but has been reported to increase insulin sensitivity in type 2 diabetes (Hanssen *et al*., 2015; van Marken Lichtenbelt *et al*., 2015).

It is well documented in the scientific literature that the human body will respond to cold exposures differently depending on the type, duration and intensity of exposure. Young *et al*. (1986) discusses three unique patterns of human response to repeated cold stresses. This was recently updated in a review by Castellani & Young (2016), which lays out the three distinct categories of changes in the human physiological responses to repeated cold exposures: habituation, metabolic acclimation, and insulative acclimation.

Habituation, also referred to as hypothermic acclimation, is characterized by blunted responses to cold exposure, including decreased shivering and vasoconstriction (Castellani & Young, 2016). This type of acclimation was also defined by Bittel (1992), who compared responses to a 1°C cold air stress test pre- and post- a 63 day journey to the North Pole. The study found that rectal temperature (T_{rec}) was more susceptible to decreasing in a significant manner following the acclimatization provided by the 63 day trip. Similar observations were made in a pre- and post-acclimation study by Brazaitis *et al*., (2014) who looked at physiological response following 6 and 17 days of intermittent daily cold water immersion at 14°C. Following

6 days of this protocol of intermittent exposure, a hypothermic type of acclimation was evidenced by a lower T_{core}, and a quicker decrease in T_{core} over time, following acclimation.

Generally, metabolic changes have been observed in small non-hibernating mammals, characterized primarily by a specialized ability to increase H_{prod} when exposed to cold (Scholander, Hammel, Andersen & Loyning, 1958). In these small mammals, this is due to BAT stimulated via the norepinephrine sympathetic pathway (Kaciuba-Uscilko & Greenleaf, 1989). As discussed above, originally BAT was solely attributed to these small mammals, but we now know these specialized tissues exist in adult humans. Human research by Scholander, Hammel, Andersen, & Loyning (1958) demonstrated an average ~50-55% increase in 8 individuals' H_{prod} following 6 weeks of outdoor cold exposure. This is a perfect example of how a metabolic acclimation effect would present itself. Similarly, Radomski & Boutelier (2001) identified metabolic acclimatization in individuals following 16 days in the Arctic. A generalized increase in overall H_{prod} among these individuals was identified following their Arctic expedition.

Finally, an insulative type of acclimation is described as improving an individual's ability to effectively use peripheral tissues to decrease H_{loss} to the environment during cold exposure. This acclimation may present itself as a more intense, or efficient vasoconstrictive response, which leads to less blood flow to extremities, and therefore less H_{loss} to the environment. In an insulative acclimatized state, you may also observe a lower T_{skin} than in the non-acclimated state, which protects and maintains T_{core} with little to no increase in metabolic H_{prod} (Young *et al*., 1986). Although not directly quantified in the present study, an insulative response may be responsible for any observed increases in T_{core}.

Repeating cold exposures may be done in at a lower temperature for multiple hours over several weeks, or with shorter, more intense sessions, such as short half-hour cold water

immersion three or four times a week. Radomski & Boutelier (1982) suggest that the continuous acclimation process at a lower temperature will help induce a metabolic type of acclimation, while the intense, shorter duration will lead to insulative changes. Many studies over the years have examined the effect of cold water immersion on the physiological response of the human body. It is well known that H_{loss} in humans is much higher during cold water immersion than a cold air exposure due to the much greater conductive heat transfer of water. This means T_{rec}, or T_{core}, drops quicker and by a larger extent during cold water exposures than cold air exposures (Young *et al*., 1986), providing a more intense stimulus to skin and core thermoreceptors, and making some type of acclimation more likely, although responses vary among individuals.

SUMMARY AND PURPOSE OF INVESTIGATION

The human body is vulnerable to cold and relies on specific mechanisms to avoid critical and dangerous decreases in T_{core}. Depending on the modality of cold exposure, the severity, and the duration, H_{prod} via NST and ST is stimulated to prevent a critical decrease in body temperature to prevent cold injuries such as frostbite, hypothermia, muscle impairment, and potentially death. Repeated cold exposures or sustained environmental exposures have been shown to alter the human physiological response to cold stimuli, including changes to cardiovascular response, thermal comfort, as well as NST and ST mechanisms (Davis, 1961; Young *et al*., 1986; Brazaitis *et al*., 2014). Physiological changes stimulated via cold acclimation may help attenuate the negative effects of the CSR, including the gasp reflex and the tachycardia which both may pose life-threatening risks in cold water.

The purpose of my thesis is to investigate the effect of cold water immersion acclimation on reflex physiological responses and the relative contribution of NST and ST to total H_{prod} during a compensable cold exposure. This will be achieved through two overall objectives:

1) to quantify the effects of a 6 day, high-intensity, non-compensable cold acclimation protocol on thermal comfort as well as thermal and cardiovascular responses;
2) to quantify the effects of a 7 day, high-intensity, cold acclimation protocol on changes in relative contribution of NST and ST to total H_{prod} during an acute, compensable cold exposure. Specifically, we will look at: 1) quantifying changes in ST and H_{prod} during an acute compensable cold exposure at an intensity of ~1.5 times resting metabolic rate, before and after the 7 day cold acclimation protocol, and, 2) assess the changes in ST muscle recruitment pattern during the compensable cold exposure, including any change in onset of shivering.

I hypothesize that:

1) The non-compensable H_{loss} from cold water immersion during the cold acclimation protocol will: 1) induce changes in objective and subjective measures of cold tolerance via TCS and T_{core} response, and both measures will improve, increasing overall tolerance of the intense cold, and 2) core cooling rate and HR response will attenuate from day 1 to day 7.
2) During the acute, compensable cold exposure, ST will decrease while H_{prod} remains steady following the cold acclimation protocol, and recruitment pattern for ST will not change significantly, while intensity decreases during the acute cold exposure.

The aim of Chapter 2 is to quantify the changes of 6 repeated 1h cold water immersions on T_{core}, thermal comfort and HR using esophageal probes, subjective scales and a HR strap, respectively. Chapter 3 will quantify further physiological changes from the full 7 days of cold acclimation by examining ST and NST during a compensable, acute cold exposure, with the use

of EMG and indirect calorimetry. Finally, general conclusions are presented in Chapter 4, summarizing the observed changes in cardiovascular response, ST and NST following several days of a high-intensity, short-term, cold acclimation protocol.

CHAPTER 2:

CHANGES IN SUBJECTIVE AND OBJECTIVE MEASURES OF COLD TOLERANCE DURING 7 DAY COLD-WATER IMMERSION ACCLIMATION PROTOCOL

INTRODUCTION

During a sudden intense cold exposure, the human body experiences the SNS 'cold-shock' response (CSR). In humans, the physiological changes associated with the CSR include: tachycardia, increased ventilatory rate, peripheral vasoconstriction and hypertension (Tipton, 1989). As well, cold exposures that are prolonged are associated with cold injuries including frostbite, loss of dexterity, and hypothermia. Any human involved in an unexpected, accidental or prolonged cold-water immersion is at serious, life-threatening risk, and this intense cold exposure contributes to 372,000 drownings worldwide (Barwood, *et al*., 2017). Consequently, a better understanding of all the physiological responses associated with cold exposures provides important information that can be used to help prevent cold related injuries, and deaths. Tipton (1989) reviewed CSR and cold-water immersion and identified that unlike other physiological 'reflex' responses, the CSR does not appear to serve a beneficial purpose. Tachycardia has been observed when individuals are immersed in cold-water within as little as 2-3 s of exposure. Subjects immersed in 10°C water have a mean increase in HR of ~20 beats·min^{-1} greater than an individual in 27°C water. The initial responses to cold-water immersion were further examined in another study by Tipton, Eglin, and Golden (1998). Two groups underwent repeated 3 min immersions in 10°C water. Despite similar T_{skin} responses between groups, the group that was habituated to the cold exposure had a lower HR and respiratory rate. Based on their study design, Tipton *et al*. (1998) concluded that this was indicative of habituation to the cold-water shock response being centrally mediated.

Following the initial CSR, another physiological cold response mediated by the parasympathetic nervous system (PNS) is observed, known as the mammalian diving response. The diving response is characterized by bradycardia and vasoconstriction of the extremities.

These physiological responses are concurrent with an increase in H_{prod} to offset H_{loss} to the environment, which includes an increase in ST, the primary heat producing mechanism in the human body. Depending on the intensity of the exposure, NST mechanisms are also initiated to compensate for H_{loss} (van Ooijen, *et al*., 2005; Blondin, *et al*., 2014b). A non-compensable cold exposure is one where H_{prod} is less than H_{loss} to the environment, and H_{loss} cannot be offset by defense mechanisms, resulting in a net decrease in body temperature. Understanding these concepts allows for better understanding of the methodology for effective cold acclimation. Cold-water provides a unique modality for cold acclimation, as the convective properties of water results in more rapid H_{loss}, and more substantial decline than during cold air exposure. This can more readily provide a non-compensable H_{loss} resulting in significant decreases in T_{core}, regardless of acclimation status. It is believed that a decrease in T_{core} will provide a strong stimulus for eliciting a physiological acclimation effect in participants undergoing repeated 1h cold-water immersions (Stocks, *et al*., 2004; Imbeault, Mantha & Haman, 2013). Non-compensable cold exposure may be most effective for altering the subjective and objective measures of cold tolerance (Blondin, *et al*., 2014; Sramek, *et al*., 2000) potentially through increased upregulation of NST mechanisms. This could downregulate the sympathetic-regulated CSR, while improving dexterity, maintaining T_{core}, as well as reducing the undesirable perceived cold sensations. This may improve overall outcomes, functionality in the cold and reduce drowning deaths related to sudden, accidental and unexpected cold-water immersions.

Glaser (1950) provided early evidence that physiological changes to cold environments does occur in humans, despite the lack of definitive science prior to 1950. Their research project identified how acclimatization alters blood circulation, and their early trials showed that 3 days in a moderately cold environment led to a lesser drop in T_{skin} and T_{rec}, leading to higher levels of

comfort. Davis (1961) expanded research into cold acclimation by looking at the effect of a 31 day environmental cold exposure among a group of young men who were living outdoors with minimal layers of clothing in an attempt to elicit changes in physiological response to a cold air stress test (CAST). Davis (1961) examined the ST and NST contributions via EMG and H_{prod} calculations, respectively, and identified a significant decrease in shivering activity pre- and post-acclimation. Recently, Blondin *et al.* (2017) conducted a 4 week cold acclimation study which demonstrated that whole-body shivering intensity decreases from pre- to post-acclimation, following up on previous research that demonstrated increases in BAT activity and NST overall, using proven cold acclimation protocols (Blondin *et al*., 2015). Similar studies from van der Lans *et al* (2013) ran cold acclimation without water immersion as well and focused on changes in NST. They found that 10 consecutive days of cold exposure significantly increased NST, through recruitment of brown fat, as evaluated with PET/CT.

Several other studies specifically used intense cold-water as the modality of choice for cold acclimation. Radomski and Boutelier (1982) conducted a study with 9 days of daily cold-water immersion for 20-40 min at 15°C. They found that following cold acclimation, participants had no elevation in overall H_{prod}, and a simultaneous decrease in T_{rec} during cold exposures. They concluded this was evidence of a hypothermic type of acclimation, known as habituation, which blunts the initial physiological responses of the CSR. These results also corroborate research from Young *et al*. (1986), where participants were also acclimatized via cold-water immersions. Their study included 5 immersions a week, for 5 consecutive weeks, at 18°C for 90 min. They showed a decrease in T_{rec} during a standardized cold air stress test from pre- to post-cold acclimation, again concluding a habituation type of acclimation response occurred. A third similar cold acclimation protocol was employed by Brazaitis *et al* (2014),

where participants were immersed in a 14°C bath for 17 total sessions over 20 days. Participants were kept in the water until T_{rec} reached 35.5°C, or for a total immersion period of 2h. This protocol was intermittent in nature, and involved the participant leaving the bath at 20 min intervals for a duration of 10 min. Again, a hypothermic type of acclimation was said to be observed, as cooling rate increased along with the number of cold acclimation sessions.

The overall objective of the current chapter is to quantify changes in cold tolerance, as well as physiological and subjective changes in human cold response following an intense, 6 day cold acclimation protocol. Specifically, cold tolerance will be measured objectively via core temperature and subjectively via a thermal comfort scale. We believe that cold tolerance will be highly variable as quantified by objective changes in T_{eso} and subjective evaluations of thermal comfort. We expect cold tolerance on day 7 will have improved over the course of 6 full days of acclimation, in both measures. The second specific objective of this chapter is to quantify changes in cooling rate and HR response to cold-water immersion. It is expected that on day 7, following the 6 days of cold-water immersion, these responses will become attenuated. We predict that core cooling rate, based on T_{eso} will decrease, and the tachycardia that is normally observed from the CSR of cold-water immersion will also decrease, as has been observed in individuals who become habituated to cold exposures.

MATERIALS AND METHODS

Subjects

Seven healthy, non-acclimatized adult males volunteered for this study. The study protocol was approved by the Health Sciences Ethics Committee of the University of Ottawa, done in accordance with the *Declaration of Helsinki*. All participants filled out a health questionnaire and gave informed consent for their participation. Exclusion criteria included cold or heat acclimated/exposed participants (ex. Outdoor workers), age outside of 18 to 35 years, smokers and anyone using dietary supplements or stimulants. Anthropometric measurements (height, weight), body fat as measured by hydrostatic weighing and VO_{2max} (CSEP treadmill protocol) were taken before the first experimental session (Table 2.1). During the 7 day cold-water immersion, no restriction was placed on participants' diets, although participants were asked to refrain from alcohol throughout the trial and caffeine prior to cold acclimation sessions. Participants were also advised to maintain their physical activity routines, although they were asked to avoid exercising before and up to 2h following each cold-water immersion.

Cold Acclimation Design

Each of the non-cold acclimatized men participated in a 7 day cold acclimation protocol consisting of a cold-water exposure lasting 1h/day. All the acclimation sessions were conducted in a laboratory at Montpetit Hall at the University of Ottawa. On these days, participants were encouraged to arrive as early as possible, typically between 0700 and 1000. Participants changed into swim trunks and were outfitted with a Polar HR monitor (Polar Electro Canada, QC) and neoprene gloves and boots. Baseline measures of HR, blood pressure (BP), tympanic and esophageal (T_{eso}) temperatures, as well as thermal comfort scale ratings were taken. To begin, participants submerged themselves up to their clavicles in a 14°C cold-water bath. Bath

temperature was maintained by adding ice every 10-15 min to return the bath to 14°C. T_{core} was monitored and recorded every 5 min, as described below. Once 1h was complete, or T_{core} reached 35°C, participants exited the water, were dried immediately and helped into dry clothing. Participants were wrapped in blankets and provided warm headwear but were otherwise given time to rewarm through their own physiological processes. After a minimum of 15 min, and a significant rise in T_{core} (as assessed by either tympanic or esophageal probe), participants were stripped of all equipment and could leave the laboratory.

Core temperature

T_{core} can be measured with different techniques, commonly: ear (tympanic), esophageal, or rectal. During the 1h cold-water immersions was monitored using two different methods. As a safety precaution to avoid T_{core} dropping below our lower cut-off of 35°C, a medical grade tympanic thermometer (Tyco Kendall Genius™ 2 Infrared Thermometer, Tyco International, Ireland) was used in both the left and right ear prior to entry into the cold-water bath, and subsequently at 5 min intervals throughout the entire 1h cold-water immersion, or until T_{core} reached 35°C.

During day 1 and day 7, in addition to the tympanic infrared thermometer, a Mon-a-therm™ General Purpose Temperature Probe (Mallinckrodt, Thames, UK) was inserted nasally to measure T_{eso}. While T_{rec} is frequently used in acclimation studies (O'Brien, *et al*., 2000; Young *et al*., 1986; Bittel, 1992), esophageal can provide a sensitive measure of core organs, as it is placed more centrally in the thoracic cavity, ~30 cm down the esophagus.

Core cooling rate was calculated based off T_{core} measurements obtained via tympanic thermometer and esophageal probe. Change in T_{core} (Δ°C) was divided by elapsed time (in min) for every 5 min segment of the cold-water immersion.

Heart Rate

HR was collected using a Polar Electro wrist watch which remotely recorded HR per one second from a HR strap worn directly over the sternum, just below the pectoral muscles, on the participants skin and would submerge with the participant. HR was recorded once 5 min before cold-water immersions, and continuously throughout the 1h immersion, as well as a minimum of 15 min post-immersion. Mean values for HR were calculated using the second-by-second recordings provided for each cold acclimation session to calculate a mean value for each 5 min interval.

Thermal Comfort Scale

Each day, prior to immersion in the cold-water bath, as well as at 5 min intervals throughout the 1h cold-water immersion, participants were asked to rate their thermal comfort and perception of the cold-water stimulus using a categorical +4 (very hot) to -4 (very cold) scale (Fanger, 1970), to assess subjective perception of cold intensity before and after acclimation. Only 5 participants had subjective ratings of perceived cold recorded for all 7 days of the cold-water immersions.

Statistical analysis

Data are expressed as the mean ± SEM. Changes in 1h mean values of T_{eso}, HR, and TCS ratings were analyzed using a two-tailed paired Student's *t* test to determine significant

differences in physiological responses between day 1 and day 7 of cold-water immersion. Cooling rate was calculated based on the change in T_{eso} over each 5 min interval, and a mean value, as well as peak cooling, were compared between day 1 and day 7 of cold-water immersion. Statistical differences were considered significant when $p \leq 0.05$. Analyses were performed using either Microsoft Excel 2016 (Microsoft Corporation, WA, USA) or SPSS, version 22 (IBM, NY, USA).

RESULTS

Cold-water immersions

Seven participants completed 7 days of cold water immersions, 1h/day, in the 14°C water bath. Of the 49 total 1h cold-water immersions conducted during this study, only three individual sessions (2 participants) had to be terminated before completion of the full 1h cold-water immersion due to T_{core} dropping below 35°C. None of these early terminations occurred on day 1 or day 7.

Core temperature

Mean $T_{core,}$ as measured by esophageal probe (T_{eso}), during the 1h cold-water immersion increased from 36.6 ± 0.2°C to 36.9 ± 0.1°C ($p<0.0005$), from day 1 to day 7 of the acclimation protocol. The results are illustrated in figure 1. Based on tympanic measurements collected throughout the 7 days of cold water immersion, a daily decrement in T_{core} was induced during every 1h water immersion.

Cooling Rate

Mean cooling rate was calculated based on change in T_{core} across the 1h cold acclimation (Δ°C/min). Results show that there was no difference between mean cooling rate among participants from a mean of -0.014 ± 0.008 °C·min^{-1} to -0.011 ± 0.012 °C·min^{-1} on day 1 and day 7, respectively. This equated to a mean decrease in T_{core} from start to finish of the 1h cold-water immersion of 0.81°C ± 0.55°C and 0.67°C ± 0.75°C on day 1 and day 7 ($p = 0.15$).

Peak cooling rate was calculated based on the change in T_{core} during each 5 min segment. Peak cooling was -0.043 °C·min^{-1} on day 1 and -0.034 °C·min^{-1} on day 7. No difference was observed in cooling rate from day 1 to day 7.

Heart Rate

Figure 1 shows participant mean HR through the entirety of the 1h cold acclimation decreased significantly from 76.9 ± 5.9 b·min^{-1}to 73.4 ± 6.2 b·min^{-1} (p=0.002) from day 1 to day 7 of cold-water acclimation.

Mean HR following initial immersion, which illustrates changes to the initial CSR, at the 5 min mark in the cold water on day 1 was 87.8 ± 6.0 b·min^{-1} while day 7, was significantly lower at 76.5 ± 8.3 b·min^{-1} ($p < 0.005$).

Thermal Comfort Scale

Five participants completed the thermal comfort scale on all 7 days of cold-water immersion, on a scale from +4 (very hot) to -4 (very cold), with mean TCS for the duration of the 1h immersion increasing from -2.82 ± 1.00 to -2.24 ± 1.02 ($p<0.05$) from day 1 to day 7 of cold. This value is based off mean TCS scores for only 5 participants for each 5 min segment. At no point during the 1h immersion does TCS cold score on day 7 exceed the self-reported cold score on day 1 at the same relative time point.

Table 2.1 Participant characteristics measured pre cold acclimation

N	7
Age (years)	24.1 ± 1.5
Height (cm)	178.2 ± 8.4
Weight (kg)	82.2 ± 4.9
BSA* (m^2)	1.98 ± 0.08
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	57.0 ± 7.7
HR_{max} ($b \cdot min^{-1}$)	194.5 ± 1.4
Siri Body Fat (%)	15.7 ± 2.3

Data are expressed as mean ± SEM

BSA, Body surface area; VO_{2max}, maximal oxygen consumption during CSEP treadmill protocol; HR_{max}, maximal heart rate during treadmill protocol.

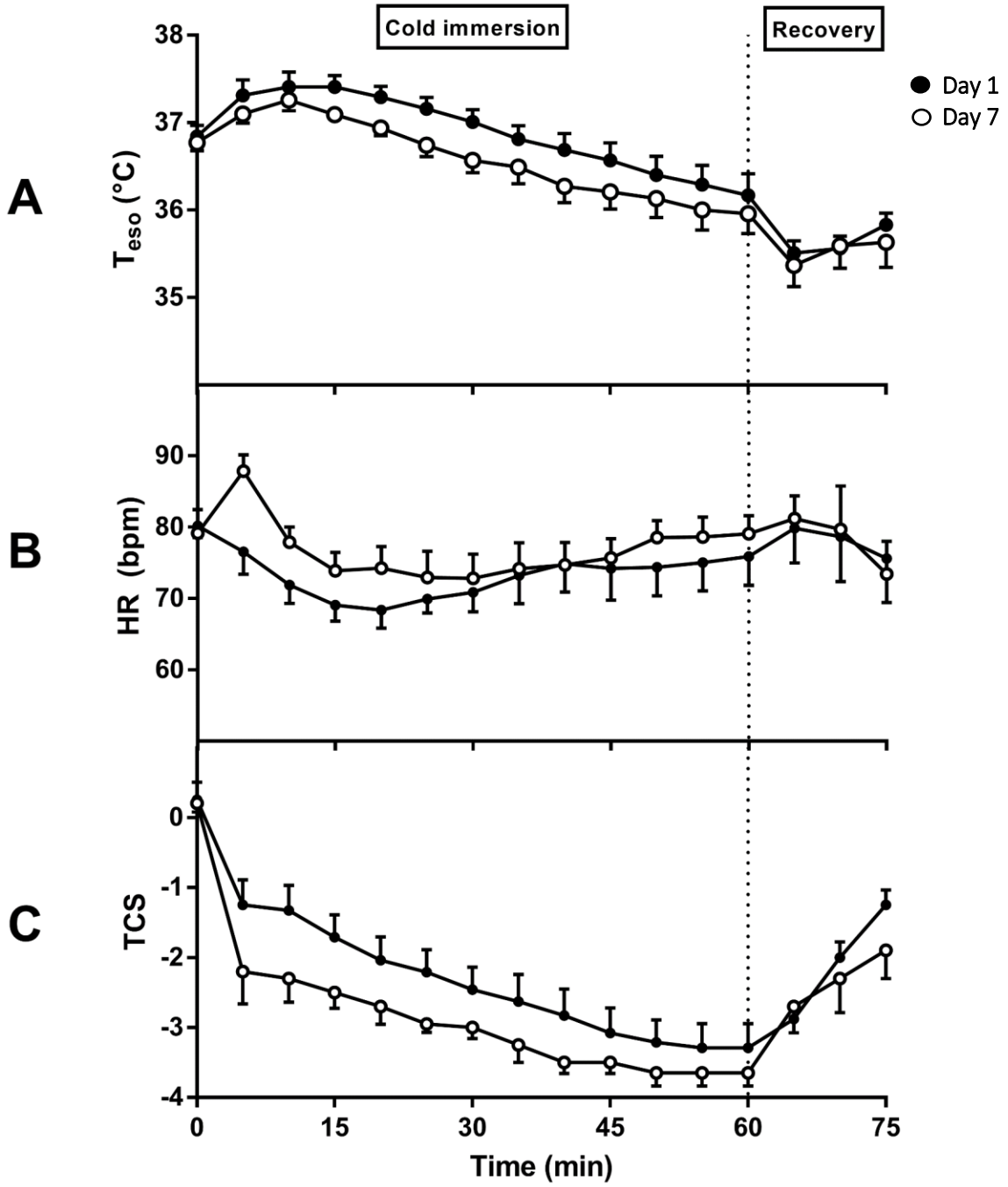

Figure 1. Cold water immersion acclimation. (A) T_{eso}, (B) heart rate and (C) thermal sensation during an acute 14°C cold water immersion and during a rewarming period on Day 1 and Day 7 of the cold acclimation protocol. Values are means ± SEM.

DISCUSSION

The cold acclimation protocol used in this study showed significant changes in physiological responses in just 6 days of 1h cold-water immersions at 14°C. This includes an increase in mean T_{eso} during cold-water immersion from day 1 to day 7, as well as a significant increase in mean thermal comfort rating and a significant decrease in mean HR during the first 5 min of immersion. These results indicate that physiological responses to cold exposure such as core temperature, thermal comfort, and heart rate can be attenuated to different degrees through repeated cold water immersions.

The CSR has been documented during intense cold exposures such as cold-water immersion, and represents a risk to survival, as evidenced by the number of drownings associated with the negative physiological responses observed (Barwood *et al*., 2017; Tipton, 1989). Our study shows that a relatively short and intense (6 day, 1h, at 14°C) exposure protocol induces cold acclimation in young male participants. Objective and subjective measures of cold tolerance among participants during the final 1h 14°C water immersion showed improvement in resilience to the cold. Previous cold acclimation protocols have been based on weeks of repetition at moderate temperatures to achieve physiological changes, and a short-term, high intensity protocol with natural rewarming had not been sufficiently evaluated for its potential to acclimate adults to cold. Attenuation of CSR has been demonstrated following cold acclimation that involves a decrease in both T_{core} and T_{skin} in humans. This includes an increase in sympathetic nervous activity, characterized by increased norepinephrine circulation, but an opposing decrease in tachycardia and ventilatory response during the initial immersion of cold-water (Castellani & Young, 2016; Golden & Tipton, 1988; O'Brien *et al*, 2000; Tipton *et al*., 2013). Many previous cold-water acclimation protocols involved longer duration immersions,

with numerous sessions. Young *et al* (1986) involved 5 weeks of immersions at 18°C for 5 days per week. O'Brien *et al*. (2000) also used a cold-water immersion acclimation protocol of 5 weeks, with daily 1h immersions, but at 20°C. Jansky *et al*. (1995) allowed for more rest during each week, with 3 cold water acclimations at 14°C for 1h each week, for 4 to 6 weeks. Another unique factor for our acclimations was the rewarming protocol for each participant following cold water immersion. While studies by Tipton *et al*. (2013) and Golden & Tipton (1988) involving cold water as the modality for acclimation, sustained for upwards of 90 min, they also finish by rapidly rewarming participants in water at 40°C back to pre-immersion T_{core}. The current acclimation protocol allowed for unassisted rewarming relying solely on thermogenic processes, blankets and clothing to rewarm. Rewarming times varied between individuals, and participants reported anecdotally that shivering lasting up to several hours post-immersion. This was intentional to allow for a prolonged cold stimulus beyond the 1h exposure, which we hypothesized would contribute to a stronger physiological acclimation response. It was the belief in designing this study that an esophageal probe is more likely to accurately reflect the temperature of critical organs in the thoracic cavity such as the heart and lungs. Tympanic and T_{rec}, while representative, are more indirect and can be more susceptible to variation due to environmental factors and vasoconstriction as they are more distal from core organs. Most reviewed cold acclimation protocols used measurements of T_{rec}, which may be a limitation to other studies.

Core temperature changes

Cold-water immersion is known to provide a strong cold stimulus, as the human body is unable to compensate for the heat lost to the environment (water). We observed a significant decrease from initial T_{eso} to the end of cold immersion at 1h everyday throughout our

acclimation protocol. The changes from day 1 to day 7 of the study show that there can be a change in physiological response to this cold exposure. In particular, the T_{eso} recorded shows that mean T_{eso} is sustained at a higher temperature on day 7 relative to day 1. A difference was found between mean T_{eso} on day 1 and day 7. As well, the mean onset of decrease in T_{eso} observed during individual cold-water immersions was delayed, and while T_{eso} dropped below baseline value after only 25 min on day 1, T_{core} did not drop below baseline levels until the measurement at 35 min on the final day, day 7.

This result is opposite from reported values of T_{core} during CAST from Young *et al* (1986), who found a decrease in T_{core} following several weeks of cold water acclimations, although this value was obtained via T_{rec} measurement. T_{rec} was lower during their standardized cold exposure following multiple days of cold-water acclimation, explained as a habituation response, which allowed for a delayed onset of ST. This decrease in T_{rec} during cold exposure following water acclimation was also supported by research from O'Brien *et al*. (2000), and Brazitis *et al.* (2014), who also reported a lower T_{core}, measured rectally following acclimation. It was suggested that acclimation led to a delayed metabolic response, and less intense vasoconstrictor response since the body was habituated to the cold. This delayed response and habituation could lead to a dangerous decrease in body temperature, and hypothermia. This may be due to increased whole-body insulative capacity, as well as hypothermic or habituation responses. A combination of insulative and hypothermic acclimation was observed by Brazaitis *et al*. (2014) after 16 days of acclimation, although the acclimation stimulus used for their study was less intense than the protocol in this study. Collectively, this provides strong evidence for a regional cooling model in humans following acclimation that could improve survival outcomes or comfort by maintaining optimal body temperature around key organs for longer.

Cooling rate

There is no difference in cooling rate ($°C \cdot min^{-1}$) from the first day of cold immersion, to the final day (Day 7), when T_{eso} was again collected. Despite no statistical significance, it also demonstrated how there was a decrease in the overall rate of T_{core} cooling, as measured by the esophageal probe. This is again contrary to what has been observed in similar cold acclimation protocols that measure T_{core} rectally. Brazaitis *et al*. (2014) reported an increase in cooling rate through 17 days of cold acclimation sessions in 14°C water. In the study, they hypothesize that the observed decrease in T_{rec} following 6 days of acclimation is an example of a habituation response, which has been typically characterized as more likely to lead to hypothermia, as H_{loss} is accelerated due to a decline in the vasoconstrictive response. The results of the current study show that T_{eso} does not follow the same pattern in T_{core} changes as previously reported in the scientific literature.

Thermal comfort scale (TCS)

An improved thermal comfort rating during cold exposure was also observed following 6 days of water immersions. Mean TCS score during the full 1h immersion increased from -2.8 to -2.2 ($p < 0.05$). This subjective measure shows that acclimation decreases discomfort and cold sensation. Improvements in cold sensation have been previously reported as well following other cold acclimation protocols (Brazaitis, 2014; van der Lans *et al*., 2013). This is likely an impact of the habituation response that occurs, and a decreased response from the adrenal hormones during a cold exposure.

Heart rate response

Observations of HR throughout all 7 days of cold water immersion showed a strong attenuation of the CSR, driven by the SNS that also elicits tachycardia. A significant decrease

was observed in mean HR across the whole 1h immersion, from 76.9 $b \cdot min^{-1}$ to 73.4 $b \cdot min^{-1}$ as well as at the 5 min mark of immersion, which decreased significantly from a mean 87.8 $b \cdot min^{-1}$ on day 1 of acclimation to a mean 76.5 $b \cdot min^{-1}$ on day 7. Tipton (1989), in describing the CSR, discusses how habituation through repeated exposures to intense cold can decrease the SNS response and increase the parasympathetic response, which leads to a decreased HR. This was also supported by research from Mäkinen *et al*. (2008) that showed a significantly lower increase in norepinephrine during cold exposure following 10 days of cold acclimation, suggesting less activation of the SNS and subsequent decreased HR response. This type of change in HR has been commonly observed following cold acclimations (Brazaitis, *et al*., 2014; Young *et al*., 1986). It is important to also consider other confounding factors that may play a role in HR. Research has shown that anxiety can inhibit habituation to cold exposure and increase heart rate despite cold acclimation. This is likely due to the involvement of the frontal and prefrontal cortices of the brain in both habituation-type acclimations as well as in anxiety (Barwood *et al*., 2017), and this inhibition may influence how acclimated individuals respond physiologically to an unexpected cold water immersion.

CONCLUSION

Together, all these results are consistent with an increased cold-induced habituation, or hypothermic-type response that is also concurrent with an insulative type of acclimation (Young *et al*., 1986; Castellani & Young, 2016). The cold acclimation protocol employed was sufficient to elicit changes in the reflex physiological responses of participants. The increase in T_{core}, the decrease in HR upon immersion and throughout the 1h immersion, as well as the decrease in subjective cold sensation, all indicate the success of cold acclimation. We did not quantify the changes in metabolic H_{prod} or H_{loss} throughout the 7 day cold acclimation protocol, therefore the exact mechanisms or processes responsible for the observed changes are not clear. Despite this, given the results observed, it follows that the 6 days of successive cold immersions at 14°C improved either H_{prod} or insulative capacity. The increase in thermal comfort, in conjunction with the higher T_{core} indicates a habituation to the cold exposure. This suggests that cold tolerance increased following cold acclimation, as hypothesized. Furthermore, while cooling rate did not change in a significant manner, we were able to identify that mean HR immediately following immersion decreased following cold acclimation. This provides evidence of an attenuation of the sympathetic response to intense cold, which is another trademark physiological response of cold habituation, which was expected following 6 days of cold acclimation. More broadly, while it is unclear how these laboratory results could translate into a real, life-threatening situation, this provides evidence that cold acclimation could theoretically help reduce the negative physiological effects of the CSR, and potentially increase survival in dangerous, cold situations.

CHAPTER 3:

CHANGES IN HEAT PRODUCTION DURING A STANDARDIZED ACUTE COLD EXPOSURE PRE- AND POST COLD ACCLIMATION

INTRODUCTION

During a mild cold exposure, the initial response is detected in the skin, as cutaneous thermoreceptors depolarize and initiate a SNS response via the pre-optic area of the brain, even without a decrease in T_{core} (Imbeault *et al*., 2013). This stimulus triggers the release of norepinephrine via the hypothalamus which in turn leads to activation of heat producing pathways, as well as a vasoconstrictive response within the peripheral vasculature. Vasoconstriction is observed in the peripheral tissue which pushes blood into deeper veins and arteries, increasing pressure, cardiac output and stroke volume, leading to a decrease in heart rate (Stocks *et al*., 2004). Simultaneously, mild cold exposure will increase EE through H_{prod}, specifically the two divisions of metabolic H_{prod}; ST and NST (Blondin *et al*., 2014).

ST is accepted as the primary contributor to H_{prod} in humans, and is stimulated quickly upon decrease in ambient temperature as detected by skin thermoreceptors, even during a mild cold exposure (Imbeault *et al*., 2013). A close correlation between shivering intensity and VO_2 as measured by indirect calorimetry, previously led researchers to believe that all metabolic H_{prod} necessary to maintain homeostasis came from ST (Bell, Tikuisis & Jacobs, 1992; Iampietro, *et al*. 1960). This was before the discovery of active BAT in adults, as it was believed humans had only a minimal capacity for NST, although humans do rely on BAT during infancy due to a lack of shivering capacity (Kaciuba-Uscilko & Greenleaf, 1989; Nedergaard; Bengtsson & Cannon, 2007). While not relied on primarily for thermogenesis during a net negative heat exchange with the environment, NST mechanisms have been shown to contribute in a significant manner to H_{prod} during mild cold exposure. The contribution of NST was originally identified in other mammals, particularly in cold exposed rats, and thoroughly reviewed in the past. Research by Hardy (1961) reported on the physiology of temperature regulation, and further work by Himms-

Hagen (1984) focused solely on NST, and BAT in mammals. Both reviews summarized how the process of increasing H_{prod} in response to cold exposure was identified as a sympathetically mediated process with increases in circulating catecholamines immediately following cold exposure.

BAT is an NST tissue and was recently identified as physiological present and relevant in adult humans (Nedergaard, Bengtsson, & Cannon, 2007). The tissue incorportates a unique protein, UCP1, in high quantities which releases potential energy in mitochondria purely as heat (Cannon & Nedergaard, 2004; Nedergaard *et al*., 2001; Ouellet *et al*., 2012; Virtanen *et al*., 2009). BAT is activated by cold exposure, sensing increases in norepinephrine from SNS activation (Bartness, Vaughan, & Song, 2010; Chechi, Carpentier, & Richard, 2013; Cypess & Kahn, 2010; Nedergaard & Cannon, 2014) and BAT has been shown to be impacted by acclimation status. Skeletal muscle has also been a focus of research examining H_{prod}, but primarily as a contractile organ (ST) and not for NST. More recent advances show not only H_{prod} through the contraction of muscle fibers (ST), but meaningful contributions via NST mechanisms, specifically sarcoplasmic endoplasmic reticulum Ca^{2+} ATPase (SERCA). SERCA works to transport cystolic Ca^{2+} into the muscle lumen during the contraction-relaxation process, which costs ATP, with heat as a by-product (Bal *et al*., 2012; Pant, Bal & Periasamy, 2016; Nowack, Giroud, Arnold, & Ruf, 2017). Bal *et al*. (2012) demonstrated that SERCA-based thermogenesis will compensate for the loss of BAT in genetically modified mice.

Research into cold acclimation has shown throughout the year that contributions of ST and NST mechanisms to H_{prod} can be altered. Davis (1961) demonstrated that shivering activity decreases following acclimatization, though H_{prod} remains the same, implying changes in the relative contributions of ST and NST. Similarly and more recently, Blondin *et al*., (2017)

showed a ~21% decrease in total shivering intensity during a sustained cold exposure from pre- to post- 4 week cold acclimation. This acclimation protocol simultaneously showed that metabolically active BAT tissue increased in volume by ~45%, and cold-induced BAT oxidative metabolism increased by ~182%, demonstrating that NST can upregulate when ST decreases. Similar studies by van der Lans *et al* (2013) showed an increase in NST through recruitment of BAT, and through the 'browning' of white adipocytes to present more UCP1 in their makeup. Their research showed an increase by 37% in BAT volume following cold acclimation. These studies show that both ST and NST pathways can be modulated following cold acclimation, although it is unknown if the short-term, intense, 7 day cold water immersion acclimation protocol employed in the present study will have an observable effect on relative contributions of ST and NST, and to what extent.

The overall objective of this chapter is to quantify thermogenesis and the relative contributions of NST and ST to H_{prod} during an acute, compensable cold exposure, following 7 days of cold acclimation. Specifically, we will look at: 1) quantifying changes in ST and H_{prod} during an acute compensable cold exposure at an intensity of ~1.5 times resting metabolic rate, before and after the 7 day cold acclimation protocol, and, 2) assess the changes in ST muscle recruitment pattern during the compensable cold exposure, including any change in onset of ST. It is hypothesized that: 1) ST will decrease while H_{prod} remains steady following the cold acclimation protocol, and 2) recruitment pattern for ST will remain the same, while intensity decreases throughout the acute cold exposure.

MATERIALS AND METHODS

Subjects

The same group of participants as described in Chapter 2 were used in this current chapter as well, to further quantify the effects of the cold acclimation protocol on heat production and shivering during a mild cold exposure. All 7 individuals participated in the mild cold exposure pre- and post-cold acclimation. Rather than a non-compensable cold exposure that elicited intense physiological responses as was used during acclimation (Chapter 2), this acute exposure was longer and milder, allowing for quantification of normal physiological responses to cold exposure and the ST and NST contributions to H_{prod} when core temperature did not drop.

Cold acclimation protocol

The cold acclimation design was described in detail in Chapter 2. It involved 7 days of cold water immersion for 1h at 14°C between the two mild cold exposures.

Mild cold exposure protocol

Acute cold experimental sessions wearing a liquid-conditioned suit (LCS) that allowed for clamping T_{skin} at 26°C were conducted immediately before and after the 7 day cold acclimation to assess muscle-specific ST and H_{prod}. Each acute cold experimental session started between 0700 and 0930, following a 24h period with no strenuous physical activity, caffeine or alcohol consumption. The evening before the test, participants were provided with a standardized meal (2489 kJ or 595 kcal; 55% CHO, 21% fat, 24% protein) and subjects were asked to arrive at the laboratory following a 12-14h fast. Upon arrival subjects changed into either undergarments or shorts. They were then instrumented with T_{skin} sensors and surface electromyography (EMG) electrodes. Participants were then instructed to complete a series of

muscle contractions to measure maximal voluntary contractions (MVC) of each muscle being measured for shivering activity. Participants would then don the LCS and be asked to void their bladders. Subjects would then lay reclined for 1h in ambient conditions (23-25°C) for baseline measures. Following this, the LCS would be perfused with cold water, initially -10°C to accelerate T_{skin} reaching the target of 26°C. Water temperature was regulated using feedback from T_{skin} and a custom designed MATLAB (Mathworks, Natick, MA, USA) program along with a temperature and flow controlled circulation bath, to maintain subject T_{skin} at 26°C throughout the 2.5h exposure. Thermal responses, including tympanic temperature; shivering activity; H_{prod} and fuel selection were measured during baseline and throughout the 2.5h of LCS cold exposure.

Thermal responses

T_{core} was measured at regular intervals throughout the acute cold using a medical grade tympanic thermometer. Mean T_{skin} during the 2.5h acute cold exposure was monitored continuously using heat flux transducers fixed to the skin (area-weighted equation from twelve sites: forehead, chest, biceps, forearm, abdomen, lower and upper back, front and back calf, quadriceps, hamstrings and hand) (Haman, *et al*., 2004; Dubois & Dubois, 1916).

T_{skin} during both the pre- and post-cold acclimation sessions was clamped at 26.0°C. To achieve this, we used a feedback system controlling the circulation water bath. At the beginning of the session, -10°C water was perfused in the LCS, which facilitated a quick drop in T_{skin} to 26.0°C. The 12 sites weighted T_{skin} provided feedback through the temperature sensors to the computer and on to the water bath, allowing for continual warming or cooling of the circulating

water, to best maintain a continuous exposure of 26.0°C T_{skin}, which was achieved quickly and maintained continuously throughout the 2.5h mild cold exposure.

Metabolic measures and Heat production

H_{prod} and fuel selection were quantified by indirect calorimetry using a Field Metabolic System, a flow-through open circuit respirometry system (Sable Systems, Las Vegas, USA) measuring VO_2 and carbon dioxide production (VCO_2). The following equations were used to calculate total carbohydrate (CHO_{ox}) and lipid (FAT_{ox}) oxidation rates:

(1) CHO_{ox} ($g \cdot min^{-1}$) = $4.59VCO_2$ ($l \cdot min^{-1}$) – $3.23VO_2$ ($l \cdot min^{-1}$)

(2) FAT_{ox} ($g \cdot min^{-1}$) = $-1.70VCO_2$ ($l \cdot min^{-1}$) + $1.70VO_2$ ($l \cdot min^{-1}$)

The values of VCO_2 ($l \cdot min^{-1}$) and VO_2 ($l \cdot min^{-1}$) were corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1.010 and 0.843 $l \cdot g^{-1}$, respectively). Protein oxidation was estimated at 66 mg/min based on previously published urinary urea extraction measurements (Haman, Legault, and Weber, 2004; Haman *et al*., 2002).

Composition of inspired (ambient) and expired (canopy) air, barometric pressure, and water vapor were measured using a Field Metabolic System, an open circuit respirometry system (Sable Systems, Las Vegas, USA). Flow rate through the canopy was regulated using a mass flow pump (Flow generator, Sable Systems, Las Vegas, USA).

Substrate oxidation calculations for CHO and lipids from non-protein. Protein oxidation was assumed to contribute 12.5% of H_{prod} during both baseline and cold as previously observed by Haman *et al* (Haman, Legault, Weber, 2004). Vallerand & Jacobs (1989) found that there is no increase in protein oxidation during a mild cold exposure.

Shivering intensity

Shivering activity was measuring using a wireless EMG system (Delsys Myomonitor III, USA). EMG surface electrodes were placed on 4 muscles: *m. trapezius superior* (TRA), *m. pectoralis major* (PEC), *m. recturs femoris* (RF), and *m. vastus lateralis* (VL). Each skin site was prepared using 3M Red Dot Trace Prep (3M Canada, London, ON, Canada) and ethanol swabs (Alcohol Prep Pad, Dukal Corporation). The EMG electrodes were placed on the right side of the body, directly over each muscle belly, parallel to muscle fibers. This was marked with an indelible skin marker to allow consistent placement between experimental sessions. Raw EMG signals were collected at 1000Hz, filtered to remove spectral components below 20Hz and above 500Hz, as well as 60 Hz contamination from related harmonics, using custom-designed MATLAB algorithms (Mathworks, Natick, MA, USA). Shivering activity of the 4 individual muscles was measured for 10 min during baseline and continuously throughout the acute cold exposure. Participants were encouraged and reminded to minimize voluntary muscle activity during recording periods.

Shivering intensity of individual muscles was determined from root-mean-square (RMS) values calculated from raw EMG signals using a 50 ms overlapping window (50%). Baseline RMS values (5 min average measured prior to cold exposure) are subtracted from the shivering RMS values and the RMS values obtained from MVCs. Shivering intensity was normalized to RMS_{mvc}.

Statistical analysis

Data are expressed as the mean ± SEM. A paired Student's *t* test was used to compare between acute cold experimental sessions (pre v post cold acclimation), as well as from start to finish of

cold acclimation (day 1 v day 7). Analyses were performed using either Microsoft Excel 2016 (Microsoft Corporation, WA, USA) or Prism, version 6.00 (GraphPad, San Diego, CA, USA).

RESULTS

Thermal Responses

There was no significant difference in mean tympanic temperature during the mild cold exposure from pre- to post- cold acclimation sessions ($p>0.05$, NS). T_{skin} during mild cold was clamped at 26.06°C ± 0.02°C and 26.09°C ± 0.01°C for the pre-and post-acclimation sessions, respectively. Mean bath temperature during the 2.5h exposure did not change between day 1 and day 7.

Heat Production

Pre-to post-cold acclimation H_{prod} values are shown in Fig. 3.2 and 3.3. Area under the curve (AUC) was calculated using Riemann's midpoint sum to estimate H_{prod} throughout the entire 2.5h acute cold exposure. There was no significant difference ($p=0.28$) in changes in mean H_{prod} AUC from pre-cold (1304.2 ± 77.81 kJ), to post-cold acclimation (1226.9 ± 102.7 kJ). No significant difference in metabolic fuel selection means or AUC were observed during the 2.5h acute cold exposure ($p=0.88$).

Shivering Response

Changes in shivering intensity during the mild cold are shown in Figure 2.2 and 3.3. Shivering intensity increased throughout the mild cold exposure in both pre- and post-acclimation conditions. AUC was calculated using Riemann's midpoint sum to estimate shivering intensity (%MVC) throughout the entire 2.5h acute cold exposure. There was a significant difference in mean AUC shivering intensity ($p<0.005$), which decreased by 38% from

pre- (214 ± 9 %MVC x 150 min) to post-cold acclimation (134 ± 12 %MVC x 150 min). Significant differences in AUC from pre- to post-cold acclimation were found in individual muscles ($p<0.005$), including the *trapezius* (234 ± 85 to 167 ± 73 %MVC x 150 min), *pectoralis* (338 ± 83 to 209 ± 57 %MVC x 150 min), the *rectus femoris* (122.5 ± 30 to 58 ± 17 %MVC x 150 min). No significant difference was seen in AUC pre- to post-acclimation in the *vastus lateralis* ($p=0.09$).

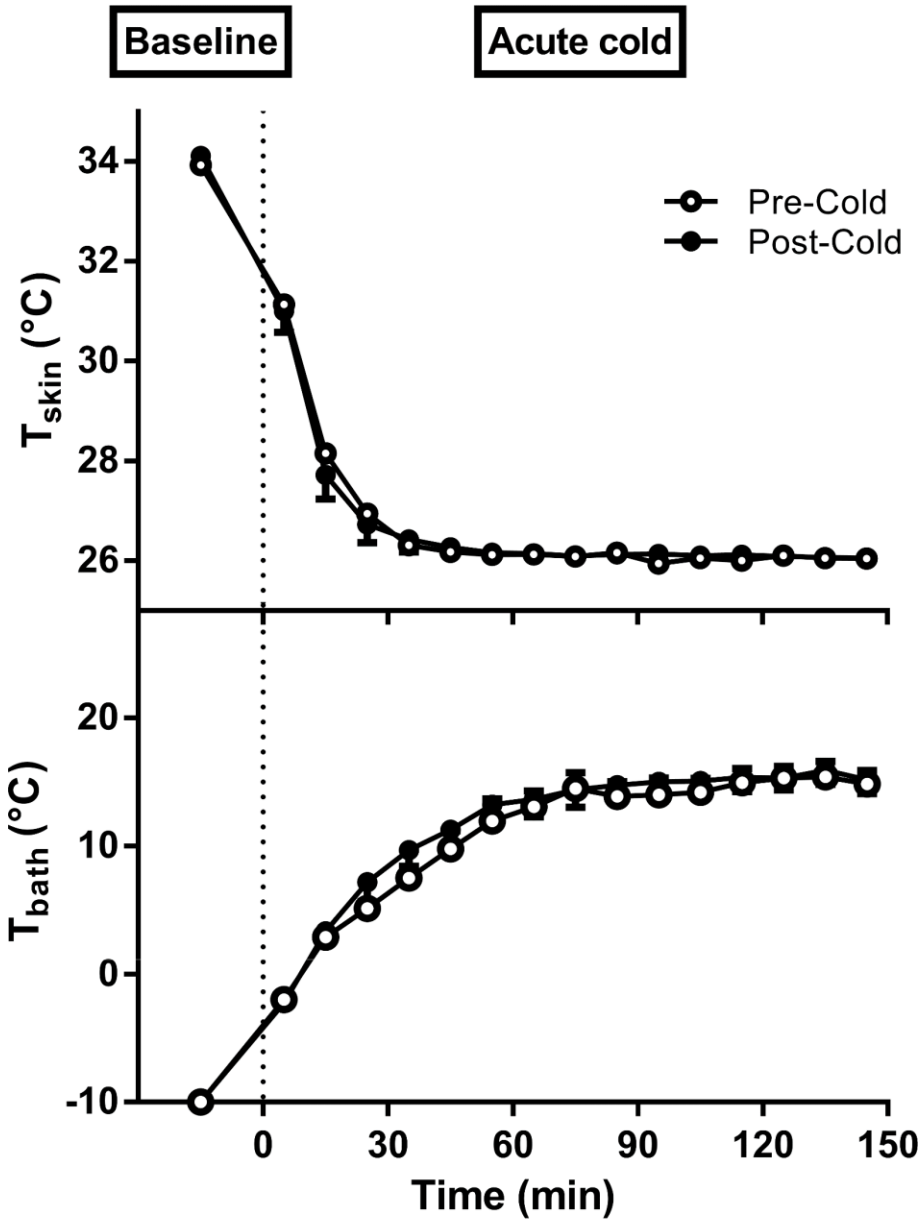

Figure 2.1. Mean skin and water bath temperatures. Changes in mean skin temperature (Tskin, °C) and circulating water bath temperature (Tbath, °C) measured during an acute cold exposure pre- (○) and post- (●) cold acclimation. Values are means ± SEM.

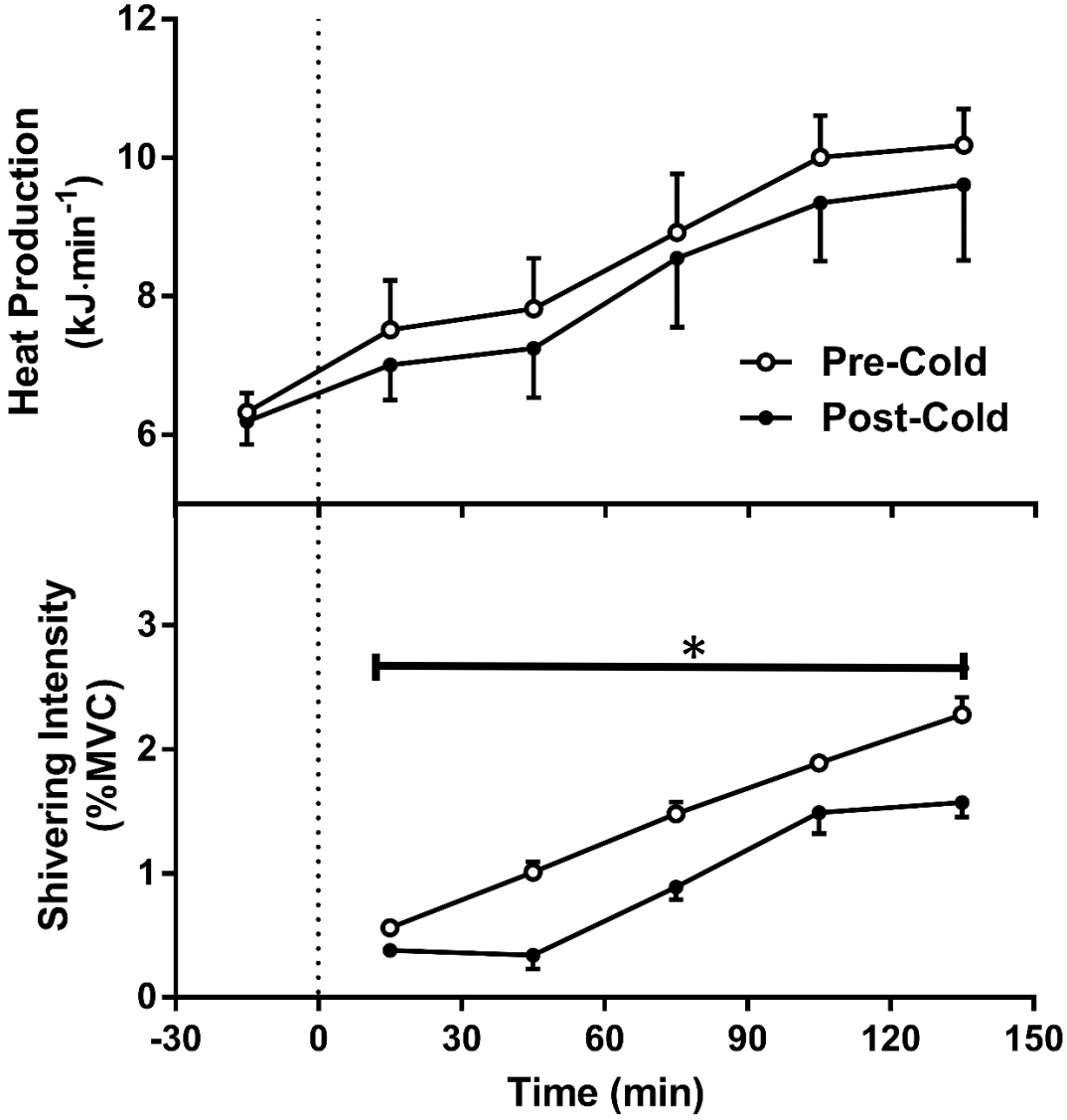

Figure 2.2. Thermogenic responses to acute cold exposure. Changes in rate of metabolic heat production ($kJ \cdot min^{-1}$) and shivering intensity (%MVC) during an acute cold exposure at a mean skin temperature of 26°C before and after cold acclimation. Values are means ± SEM.

*Significant difference in AUC for shivering intensity, $p < 0.05$.

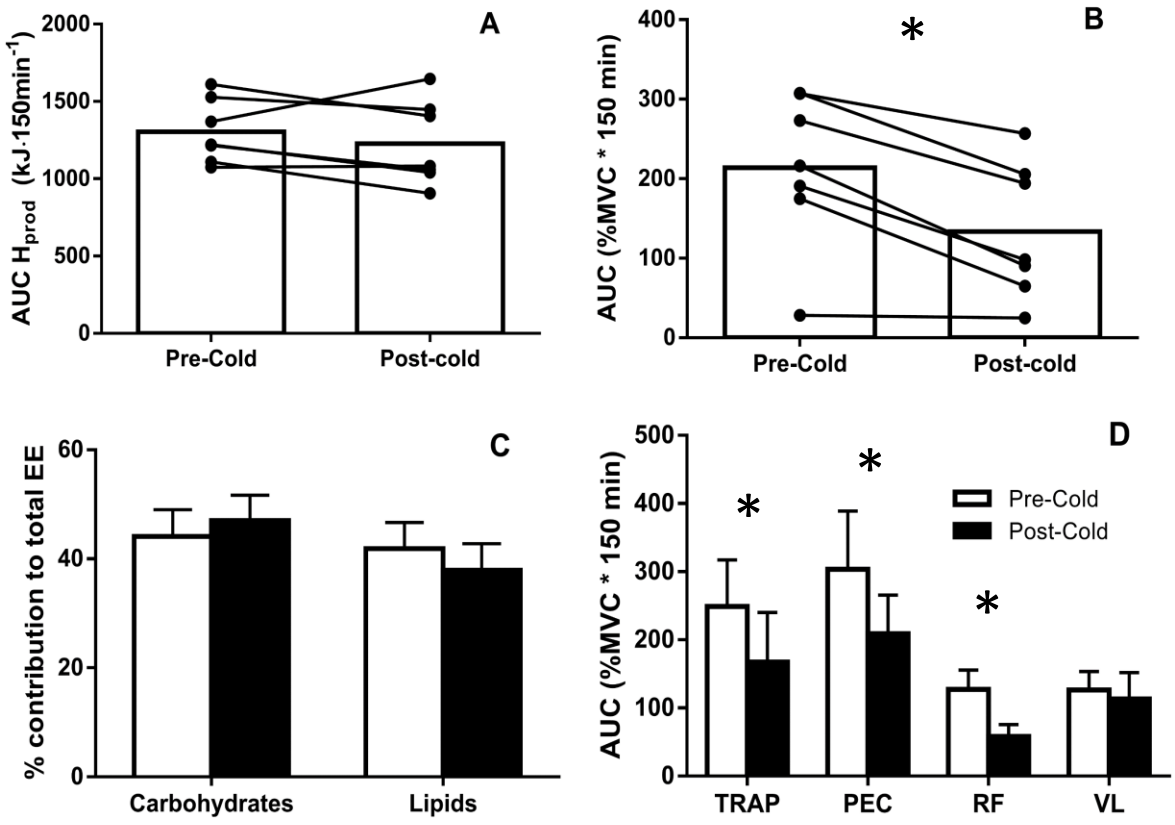

Figure 2.3. Overview of heat production and shivering (A) Area under the curve (AUC) heat production (kJ·150 min), (B) total shivering AUC (%MVC·150 min), (C) fuel selection by % contribution to total energy expenditure (EE), and (D) mean individual muscle total shivering by AUC (%MVC·150 min), during an acute cold exposure at a mean skin temperature of 26°C before and after cold acclimation. Values are means ± SEM. * Pre- vs. post-cold significant difference, $p < 0.05$.

DISCUSSION

This study provides evidence that NST can increase, leading to reduced ST following just 7 days of cold acclimation in 14°C water. We observed a decrease in AUC shivering, as well as mean shivering intensity during the 2.5h mild cold pre-to post-cold acclimation, while H_{prod} was maintained. While not directly quantifying NST activity, this provides the indirect evidence that NST mechanisms upregulated to maintain H_{prod} when ST decreased. Reducing ST during cold exposure, while maintain H_{prod}, may provide a survival advantage by reducing the discomfort of involuntary muscle contractions and its effects on locomotion and dexterity (Meigal, 2002).

Thermal Responses

Clamping the cold stimulus during the 2.5h LCS exposure based on a T_{skin} of 26°C was meant to provide a standardized and reproducible cold exposure for pre- and post-acclimation. Our results showed that we were able to reproduce the stimulus between both acute cold sessions. The decrease in T_{skin} to 26°C was not so intense to decrease T_{core}, which did not change significantly during either pre- or post-cold 2.5h acute cold exposures. We did observe a significant increase in H_{prod} from start to finish of both acute cold sessions, which can be attributed to cold induced ST and NST activation.

Heat production

T_{core} was monitored throughout the duration of the acute cold exposure, with simultaneous quantification of H_{prod} via indirect calorimetry and ST indirectly via EMG. H_{prod} during cold exposure includes the increase observed via ST and NST, naturally initiated to prevent decreases in body temperature. Previous studies have observed a significant increase in metabolic heat production via ST, reaching up to 5 times metabolic rate (Eyolfson, *et al*. 2001).

By quantifying H_{prod} from pre- to post-acclimation, we observed that no significant change occurred overall, and H_{prod} increased on a similar trajectory during the 2.5h pre- and post-cold acclimation acute cold LCS exposures. With no observed change in heat production following acclimation, and a significant decrease in ST of ~40%, it is necessary to evaluate NST contributions to human H_{prod} following acclimation.

Nonshivering Thermogenesis (NST)

As discussed previously, in mammals, thermogenic BAT is known as the greatest contributor to total NST. Following a cold acclimation protocol consisting of 10°C exposure for 2h for 4 weeks (5 days/week) using a LCS, Blondin *et al*., (2017) showed that a ~20% decrease of ST was associated with a ~45% increase in BAT volume and a ~2.2-fold increase in its metabolic activity. Unfortunately, in the present study, changes in BAT volume and metabolic activity were not assessed and therefore, the potential role of BAT in reducing ST by ~40% remains undetermined. Previously, studies have shown that NST that can increase through repeated mild-cold exposure in humans by increasing BAT thermogenesis (Blondin *et al*., 2014; Blondin *et al*., 2017a; Blondin *et al*., 2017b). Despite increasing BAT volume by 45% and its thermogenic capacity by 150-182% in these previous studies, there was no observed effect on fuel selection in the current study. It is possible that the thermogenic contribution of BAT increased in this study but did not impact fuel selection when exposed to a mild acute cold. Due to the relatively small size of BAT (<1% of body weight in humans) (van der Lans *et al*., 2014; Blondin *et al*., 2014) and generally low thermogenic contribution (<1% of H_{prod}) (U Din *et al*., 2016), it is more probable that another source of NST, or a combination of sources is responsible for the improved thermal comfort and reduced body cooling during the acclimation in cold-water and the increased NST during the acute mild cold sessions wearing the LCS.

One of these other sources is skeletal muscle, and it constitutes another important target for cold-induced NST as muscle accounts for 42% of total body weight (Kim *et al*., 2002). Studies have shown that acute cold exposure increases proton leak in muscle, which functions as a form of NST (Wijers *et al*., 2008; Blondin *et al*., 2017a). The increased association between shivering intensity and cold-induced thermogenesis previously described following a 4 week mild cold acclimation, was previously attributed to a potential whole-body improvement in the coupling of oxidative phosphorylation across skeletal muscles (Blondin *et al*., 2017a). As there was no change to the strength of the relationship post-acclimation during our standardized acute cold exposure, this may indicate that perhaps proton leak was not abolished in the present study. Finally, in contracting muscles, cytosolic Ca^{2+} levels rapidly increase and sarcoplasmic reticulum SERCA must pump Ca^{2+} back into the SR to maintain its store, accounting for 24-58% of tissue metabolic rate in contracting muscles in rodents (Rolfe & Brown, 1997). This Ca^{2+} release is critical for muscle contractions during shivering, but in cold-acclimated rodents Ca^{2+} leaks into the cytosol via ryanodine receptors leading to excessive activation of SERCA to pump Ca^{2+} back into the SR, generating heat in the process (Bal *et al*., 2012). With humans possessing significantly greater levels of the SERCA regulatory proteins (Babu *et al*., 2007) compared to rodents and relying to a greater extent on skeletal muscle to produce heat, this thermogenic mechanism could be significant in humans and serve as a form of NST following cold acclimation. Further investigations are required to determine the contribution of these skeletal muscle-derived thermogenic processes following such a cold acclimation.

In summary, we have demonstrated that 7 days of cold water immersion can decrease shivering intensity by ~40%, nearly double what we previously described in response to a 4 week compensable cold acclimation. The decreased shivering intensity for the same H_{prod} in

response to a mild acute cold exposure, suggests a significant increase in the contribution of NST following 7 days of cold water immersion. This had no effect on whole-body fuel selection, which suggests that the source of NST must rely on a similar fuel mixture to produce this heat. Although several studies have examined the effects of repeated cold exposure on H_{prod} in humans, few have ever simultaneously quantified the contribution of shivering and by extension the contribution of NST to this heat production.

CONCLUSION

Our study confirms that NST can be substantially modulated and decrease ST contribution to H_{prod} following a 7 day cold acclimation protocol in 14°C water. As in other mammals, such a reduction in ST during cold exposure provides a survival advantage by reducing the discomfort of involuntary muscle contractions and its effects on dexterity and voluntary movements. However, it remains that identifying the exact mechanisms involved in increasing NST during acute cold exposure or following cold acclimation is particularly difficult at the tissue or at the whole-body level and was not within the scope of this study. This study not only fills a gap to improve our understanding of the physiological and metabolic responses to chronic cold, but it also provides critical information to develop strategies to improve human survival and tolerance in cold climates. Further work studying this subject is necessary to understand the thermogenic processes that may be modulated under such a short but intense cold acclimation and determine the minimal acclimation duration and cooling temperature required to elicit similar metabolic changes.

CHAPTER 4:

GENERAL CONCLUSION

Results from Chapter 2 and 3 show that reflex physiological responses to cold water can be attenuated following 7 days of cold acclimation. It was also observed that this cold acclimation was able to alter the contribution of ST and NST to overall H_{prod} during acute cold exposure. Future research in this area should continue to explore the effect of cold water acclimation on physiological heat production mechanisms. Specifically, it would be beneficial to understand which NST mechanisms increase heat production and to what extent they are able to upregulate activity. Accurate measurement of heat production via BAT and skeletal muscle mechanisms would help to better understand the pathways through which cold water exposure alters cold response physiology. It will be important to continue further research in cold water acclimation with various age groups, body types, and between genders to fully understand potential physiological and confounding factors that may influence thermoregulation. From a practical standpoint, it is important to understand how this research may be applied in a real-world setting. Measuring changes in dexterity and fine motor skills pre- and post-acclimation could answer some fundamental questions on how useful acclimation may be for translating to jobs with regular cold exposure. In terms of survival, understanding how acclimatized individuals differ in terms of surviving extended cold exposures may be valuable. Does a decreased shivering rate decrease glycogen depletion and maintain muscle glycogen reserves for a longer period of time? Or does a true survival scenario offset and potential benefits from a laboratory-induced acclimation? There are many possible avenues to better understanding acclimation and cold physiology in human populations, with many important questions. Future work in the field of cold and cold survival will help us understand what limits human functionality and survival in the cold.

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APPENDIX: JOURNAL ARTICLE PUBLICATION

Attached is the published article, "Seven days of cold acclimation substantially reduces shivering intensity and increases non-shivering thermogenesis in adult humans."

Citation:

Gordon, K., Blondin, D. P., Friesen, B. J., Tingelstad, H. C., Kenny, G. P., & Haman, F. (2019). Seven days of cold acclimation substantially reduces shivering intensity and increases non-shivering thermogenesis in adult humans. Journal of Applied Physiology. Retrieved from https://www.physiology.org/doi/abs/10.1152/japplphysiol.01133.2018.

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RESEARCH ARTICLE | *Passive Properties of Muscle*

Seven days of cold acclimation substantially reduces shivering intensity and increases nonshivering thermogenesis in adult humans

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Gordon K, Blondin DP, Friesen BJ, Tingelstad HC, Kenny GP, Haman F. Seven days of cold acclimation substantially reduces shivering intensity and increases nonshivering thermogenesis in adult humans. *J Appl Physiol* 126: 1598–1606, 2019. First published March 21, 2019; doi:10.1152/japplphysiol.01133.2018.—Daily compensable cold exposure in humans reduces shivering by ~20% without changing total heat production, partly by increasing brown adipose tissue thermogenic capacity and activity. Although acclimation and acclimatization studies have long suggested that daily reductions in core temperature are essential to elicit significant metabolic changes in response to repeated cold exposure, this has never directly been demonstrated. The aim of the present study is to determine whether daily cold-water immersion, resulting in a significant fall in core temperature, can further reduce shivering intensity during mild acute cold exposure. Seven men underwent 1 h of daily cold-water immersion (14°C) for seven consecutive days. Immediately before and following the acclimation protocol, participants underwent a mild cold exposure using a novel skin temperature clamping cold exposure protocol to elicit the same thermogenic rate between trials. Metabolic heat production, shivering intensity, muscle recruitment pattern, and thermal sensation were measured throughout these experimental sessions. Uncompensable cold acclimation reduced total shivering intensity by 36% ($P = 0.003$), without affecting whole body heat production, double what was previously shown from a 4-wk mild acclimation. This implies that nonshivering thermogenesis increased to supplement the reduction in the thermogenic contribution of shivering. As fuel selection did not change following the 7-day cold acclimation, we suggest that the nonshivering mechanism recruited must rely on a similar fuel mixture to produce this heat. The more significant reductions in shivering intensity compared with a longer mild cold acclimation suggest important differential metabolic responses, resulting from an uncompensable compared with compensable cold acclimation.

NEW & NOTEWORTHY Several decades of research have been dedicated to reducing the presence of shivering during cold exposure. The present study aims to determine whether as little as seven consecutive days of cold-water immersion is sufficient to reduce shivering and increase nonshivering thermogenesis. We provide evidence that whole body nonshivering thermogenesis can be increased to offset a reduction in shivering activity to maintain endogenous heat production. This demonstrates that short, but intense cold stimulation can elicit rapid metabolic changes in humans, thereby improving our comfort and ability to perform various motor tasks in the cold. Further research is required to determine the nonshivering processes that are upregulated within this short time period.

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cold acclimation; cold-water immersion; energy metabolism; nonshivering thermogenesis; shivering thermogenesis

INTRODUCTION

Humans exposed to a cold environment rely on the recruitment of heat-conserving and heat-producing cold-defense responses to limit and counteract the heat lost to the surrounding environment. In nonexercising humans, metabolic heat production ($\dot{H}_{prod}$) is sustained by the combined activation of nonshivering thermogenesis (NST) and shivering thermogenesis (ST). Both brown adipose tissue (BAT) (22, 29) and skeletal muscle proton leak (3, 31) have been identified as significant contributors to NST during acute cold exposure in lean, young, unacclimated individuals. However, ST remains the predominant form of heat production in cold-exposed adult humans. The recruitment of ST, in particular, can affect thermal comfort and work performance in the cold by impairing gross and fine neuromuscular performance and coordination (9, 21), ultimately impacting the odds of survival. Consequently, over several decades, investigators have attempted to identify a cold acclimation protocol that could help elicit significant changes in thermoregulatory, metabolic, and neuromuscular responses as a means of improving thermal comfort and work performance in the cold (9, 32).

Despite the breadth of research examining the effects of repeated cold exposure on the thermoregulatory responses to an acute cold, only two studies have quantified the effects of cold acclimation on changes in ST in young healthy humans. In 1961, Davis showed that 31 days of cold air exposure (~12°C, 8 h/day) resulted in an ~80% reduction in ST and ~15% reduction in whole body $\dot{H}_{prod}$ in healthy men previously acclimatized to summer conditions (seasonal average of ~20–30°C (10). More than five decades later, Blondin et al. (3) showed that 4 wk of daily compensable cold exposure in unacclimatized men using a liquid conditioned suit (LCS) (for 2 h/day at 10°C, 5 days/wk) was sufficient to elicit a ~20% decrease in ST response for the same given $\dot{H}_{prod}$. In addition, by combining isotopic and nuclear imaging methods in these same individuals, the authors also showed that BAT volume and thermogenic capacity increased following cold acclimation by 45% and 182%, respectively, and concomitantly reduced skeletal muscle proton leak (3). Although these results are promising as a means of improving cold tolerance, identifying a cold acclimation protocol that can shorten the time required

to increase the relative contribution of NST to total $\dot{H}_{prod}$ but reduce that of ST would be particularly valuable in this field of physiology.

Therefore, the purpose of this study was to determine whether a short-duration, high-intensity cold acclimation would reduce the contribution of ST to total $\dot{H}_{prod}$ for the same absolute cold stress. More specifically, we quantified whether seven consecutive days of 14°C water immersion for 1 h, resulting in a maximum of 2°C decrease in core temperature, would result in a significant decrease in ST and improve thermal sensation between a preacclimation compared with a postacclimation acute cold exposure at the same given $\dot{H}_{prod}$. In 1996, Young (32) suggested that the greatest metabolic effects resulting from repeated cold exposure would require a sufficiently uncompensable cold exposure stimulus that would result in a decrease in core temperature. By using such a cold acclimation approach, we predict will result in a greater cold-induced stimulation of nonshivering thermogenic pathways and reduce the contribution of shivering to total heat production, compared with what was previously shown from a compensable cold acclimation protocol (3).

METHODS

Participants

Seven healthy, nonacclimatized adult males between the ages of 20 and 29 yr old volunteered for this study, which was performed in accordance with the Declaration of Helsinki and approved by the Health Sciences Ethics Committee of the University of Ottawa. All participants filled out the Physical Activity Readiness Questionnaire (PAR-Q+) and gave written informed consent before their participation. Individuals that were cold or heat acclimatized, taking any medications or dietary supplements, or who had known metabolic, respiratory, or cardiovascular disease were excluded.

Body height, mass, surface area, and density, as well as maximal oxygen consumption ($\dot{V}O_{2max}$) were measured during the screening session. Body height was measured using an eye-level physician stadiometer (model 2391; Detecto Scale Company, Webb City, MO), while body mass was measured using a digital weight scale (model CBY150X; Mettler Toledo) with a high-performance weighing terminal (model IND560; Mettler Toledo). Measurements of body height and mass were subsequently used to calculate body surface area (11). Body density was measured using the hydrostatic weighing technique and used to estimate body fat percentage (27). Maximal oxygen consumption ($\dot{V}O_{2max}$) was determined by incremental treadmill exercise to volitional fatigue. Breath-by-breath oxygen consumption was measured by an automated indirect calorimetry system (Medgraphics Ultima, Medical Graphics, St. Paul, MN), and $\dot{V}O_{2max}$ was taken as the highest average oxygen consumption recorded over 30 s (Table 1).

Table 1. *Participant characteristics*

Parameter	Value
n	7
Age, yr	24 (4)
Height, cm	178.2 (8.4)
Weight, kg	82.2 (12.9)
BSA, m^2	1.98 (0.19)
$\dot{V}O_{2max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	57.0 (7.7)
HR_{max}, $beats \cdot min^{-1}$	195 (3)
Body fat_{Siri}, %	15.7 (5.6)

Data are expressed as means (SD). BSA, body surface area; HR_{max}, maximal heart rate during treadmill protocol; $\dot{V}O_{2max}$, maximal oxygen consumption.

Cold Acclimation

Volunteers participated in a 7-day cold acclimation protocol consisting of daily cold water immersions lasting no more than 1 h a day. On these days, participants arrived between 0700 and 1000 to begin the water immersions. They changed into swim trunks and were fitted with a heart rate monitor (V800 watch with H7 wearlink, Polar Electro Oy, Kempele, Finland), neoprene gloves, and boots. After baseline measures, participants were submerged up to their clavicles in a 14°C circulated cold water bath. Bath temperature was maintained by adding ice every 10–15 min. On *days 1 and 7*, esophageal temperature (T_{es}) was measured using a pediatric thermocouple probe of ~2-mm diameter (Mon-a-therm general purpose, Mallinckrodt Medical, St. Louis, MO) inserted ~40 cm past the entrance of the nostril and confirmed every 5 min with aural canal temperature measurement (T_{aural}; Welch Allyn Braun ThermoScan Pro 6000; Braun, Kronberg, Germany). On *days 2–6* of the cold water immersion acclimation, only T_{aural} was used to monitor changes in core temperature, to reduce the discomfort for participants. Once the 1-h immersion was completed or the core temperature reached 35.0°C (T_{es} for *days 1* and *7*, T_{aural}, for *days 2–6*), participants exited the bath, were dried, and passively warmed before changing into warm clothing.

Preacclimation and Postacclimation Acute Cold Exposure

Acute cold experimental sessions were conducted the day before and the morning immediately following the 7-day cold acclimation (*day 8*) using a LCS combined with a mean skin temperature ($\overline{T}_{skin}$) clamping system set at 26°C for the duration of the cold exposure, as described previously (8). These sessions were started between 0700 and 0930, following a 24-h period, with no strenuous physical activity, caffeine, or alcohol consumption. The evening before the test, participants were provided with a standardized meal (2489 kJ or 595 kcal; 55% CHO, 21% fat, 24% protein), and subjects were asked to arrive at the laboratory following a 12–14-h fast. Upon arrival, subjects changed into undergarments and were then instrumented with T-type (copper/constantan) thermocouples integrated into heat flow sensors (Concept Engineering, Old Saybrook, CT) and surface electromyography electrodes (Myomonitor, Delsys, Natick, MA). Participants were then instructed to complete a series of muscle contractions to measure maximal voluntary contractions (MVC) of the muscles being measured for shivering activity. Participants were then fitted with the LCS and asked to void their bladders. Subjects then lay reclined for 60 min in ambient conditions (23–25°C), following which, baseline measures were taken. Thereafter, the LCS was perfused with cold water, initially at −10°C to accelerate $\overline{T}_{skin}$ reaching the target of 26°C. Water temperature was regulated as described previously using feedback from skin temperature sensors and a custom-designed program along with a temperature and flow-controlled circulation bath, to maintain subject skin temperature at 26°C throughout the 150-min exposure (8) (Fig. 2*B*). Thermal responses, including tympanic temperature, shivering activity, and metabolic rate were continually measured during baseline and throughout the 150 min of cold exposure.

Thermal responses. Core temperature during the mild acute cold exposures was measured from T_{aural}. Mean $\overline{T}_{skin}$ during the 150-min acute cold exposure was monitored continuously using heat flux sensors fixed to the skin [using area-weighted equation from 12 sites (weighting) (18): forehead (7%), chest (9.5%), biceps (9%), forearm (7%), abdomen (9.5%), lower back (9.5%), upper back (9.5), front calf (8.5%), back calf (7.5%), quadriceps (9.5%), hamstrings (9.5%), and hand (4%)], and maintained around 26.0°C.

Metabolic measures and heat production. Whole body metabolic rate and fuel selection were quantified continuously by indirect calorimetry using a Field Metabolic System, a flow-through open

circuit respirometry system (Sable Systems, Las Vegas, NV) measuring oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). Carbohydrate and lipid utilization rates were calculated using the following *Eqs. 1* and *2*):

$$CHO_{ox}(g/min) = 4.59\dot{V}CO_2(l/min) - 3.23\dot{V}O_2(l/min) \quad (1)$$

$$FAT_{ox}(g/min) = -1.70\dot{V}CO_2(l/min) + 1.70\dot{V}O_2(l/min) \quad (2)$$

where $\dot{V}CO_2$ (l/min) and $\dot{V}O_2$ (l/min) were corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1.010 and 0.843 l/g, respectively). Protein oxidation was estimated at 66 mg/min (14–16). Energy potentials of 16.3, 40.8, and 19.7 kJ/g were used to calculate the amount of heat produced from glucose, lipid, and protein oxidation, respectively (20, 24).

Thermal sensation. Subjective thermal sensation was determined every 30 min by asking the subjects to identify their perception of the exposure temperature on the basis of a 9-point Likert scale, with −4 and +4 being the coldest and warmest ever experienced, respectively, and 0 feeling neither cold nor warm. This is a modification of the scale described previously (23).

Shivering intensity. Shivering activity was measured using a wireless EMG system (Myomonitor IV, Delsys, Natick, MA). Surface EMG electrodes were placed on four muscles: musculus trapezius superior, musculus pectoralis major, musculus rectus femoris, and musculus vastus lateralis. Each skin site was prepared and cleaned using 3M Red Dot Trace Prep (3M Canada, London, ON, Canada) and ethanol swabs (Alcohol Prep Pad, Dukal Corporation). The EMG electrodes were placed on the right side of the body, directly over each muscle belly. This was marked with an indelible skin marker to allow consistent placement between experimental sessions. Raw EMG signals were collected at 1,000 Hz, filtered to remove spectral components below 20 Hz and above 500 Hz, as well as 60-Hz contamination from related harmonics, using custom-designed MATLAB algorithms (MathWorks, Natick, MA). Shivering activity of the four individual muscles was measured for 10 min during baseline and continuously throughout the acute cold exposure. Participants were encouraged and reminded to minimize voluntary muscle activity during recording periods.

Shivering intensity of individual muscles was determined from root-mean-square (RMS) values calculated from raw EMG signals using a 50-ms overlapping window (50%). Baseline RMS values (5-min average measured before cold exposure) are subtracted from the shivering RMS values and the RMS values obtained from maximum voluntary contractions (MVCs) (RMS_{mvc}). Shivering intensity was normalized to RMS_{mvc}.

$$\text{Shivering intensity}(\%MVC) = \frac{RMS_{shiv} - RMS_{baseline}}{RMS_{mvc} - RMS_{baseline}} \times 100$$

Statistical Analysis

Data are expressed as means ± SD ($n = 7$), unless indicated otherwise. Paired Student's *t*-test was used to compare between acute cold exposure experimental sessions. A repeated-measures ANOVA with acclimation status, temperature, and their interaction as independent variables was used to analyze acclimation- and temperature-dependent differences in thermal responses (T_{es}, $\overline{T}_{skin}$, and water bath temperature), metabolic responses ($\dot{H}_{prod}$, CHO_{ox}, and FAT_{ox}), EMG activity (shivering intensity) over time (Time = 0, 30, 60, 90, 120, and 150 min used for analysis). Bonferroni's multiple-comparisons post hoc test was used, where applicable. Statistical differences were considered significant when the P value was <0.05. Pearson correlation coefficient was used to determine correlations between variables. All analyses were performed using SPSS for Windows (version 21; SPSS, Chicago, IL) or GraphPad Prism (version 7; GraphPad, San Diego, CA).

RESULTS

Cold Acclimation

Changes in T_{es}, HR, and thermal sensation over the 60 min of cold water immersion for *day 1* and *day 7* are presented in Fig. 1. Water temperature was not different between *day 1* and *day 7*, averaging 14.5°C (SD 0.1) and 14.4°C (SD 0.1), respectively ($P = 0.36$). Esophageal temperature decreased more significantly during the water immersion on *day 1* [36.8°C (SD 0.2) to 35.6°C (SD 0.7)] than on *day 7* [36.8°C (SD 0.3) to 36.2°C (SD 0.6)] ($P = 0.03$) (Fig. 1, *A* and *B*). Average HR throughout cold water immersion was higher on *day 1* [76 beats/min (SD 6)] compared with *day 7* [73 beats·min^{-1} (SD 7)] ($P = 0.004$) (Fig. 1*C*). Thermal sensation was found to decrease continuously during cold water immersion but was significantly lower throughout *day 1* compared with *day 7* [*day 1* at −3.1 (SD 0.3) to *day 7* at −2.5 (SD 0.7), $P = 0.02$] (Fig. 1*E*).

Preacclimation and Postacclimation Acute Cold Exposure

Thermal responses. A computer-controlled system set at a $\overline{T}_{skin}$ of 26°C (SD 0.1) was used to standardize the stimulus for $\dot{H}_{prod}$ pre- and post-cold acclimation. The resulting changes in $\overline{T}_{skin}$ and water bath temperature over the 150 min of acute cold exposure are presented in Fig. 2. By design, $\overline{T}_{skin}$ fell significantly during cold exposure, stabilizing at 26.1°C (SD 0.04) before and 26.0°C (SD 0.08) after the cold acclimation ($P = 0.91$) (Fig. 2*A*). Water temperature circulating through the suit increased progressively, from −6.2°C (SD 0.8) °C at the start of cooling and stabilizing at 14.9°C (SD 2.5) by the end of cold exposure preacclimation and from −6.8°C (SD 1.2) to 15.0°C (SD 2.6) postacclimation (Fig. 2*B*). There was no difference in starting ($P = 0.32$) or steady-state water temperature ($P = 0.89$).

Heat production and fuel selection. Changes in $\dot{H}_{prod}$ pre- to post-cold acclimation are shown in Fig. 3. Time course and total heat production (as area under the curve, AUC) were the same before and after cold acclimation (Fig. 3, *A* and *C*, effect of time $P < 0.0001$). The $\dot{H}_{prod}$ increased on average 1.54-fold from 6.3 kJ/min (SD 0.3) [304 ml O_2/min (SD 35)] at baseline to 10.2 kJ/min (SD 1.4) [482 ml O_2/min (SD 67)] in the last 30 min in the cold before acclimation and from 6.2 kJ/min (SD 0.3) [292 ml O_2/min (SD 39)] at baseline to 9.6 kJ/min (SD 2.6) [458 ml O_2/min (SD 125)] in the last 30 min in the cold after acclimation (Fig. 3*A*, $P < 0.0001$). The AUC for $\dot{H}_{prod}$, or the total amount of heat produced, averaged 1304 kJ·150 min (SD 205) before cold acclimation and 1227 kJ·150 min (SD 272) after cold acclimation (Fig. 3*C*, $P = 0.28$). Similarly, as shown in Fig. 4, there was no significant difference in CHO and lipid use (rate or AUC) during the 150 min in the cold. Total CHO utilization over the 150 min averaged 35.6 g (SD 11.7) [580 kJ (SD 191) and 51%$\dot{H}_{prod}$ (SD 11)] before cold acclimation and 34.8 g (SD 12.4) [568 kJ (202) and 44%$\dot{H}_{prod}$ (SD 8)] after cold acclimation ($P = 0.88$). Total lipid utilization over 150 min averaged 13.6 g (SD 2.4) [553 kJ (SD 98) and 38%$\dot{H}_{prod}$ (SD 12)] before cold acclimation and 12.0 g (SD 4.3) [488 kJ (SD 176) and 43%$\dot{H}_{prod}$ (SD 9)] after cold acclimation ($P = 0.37$).

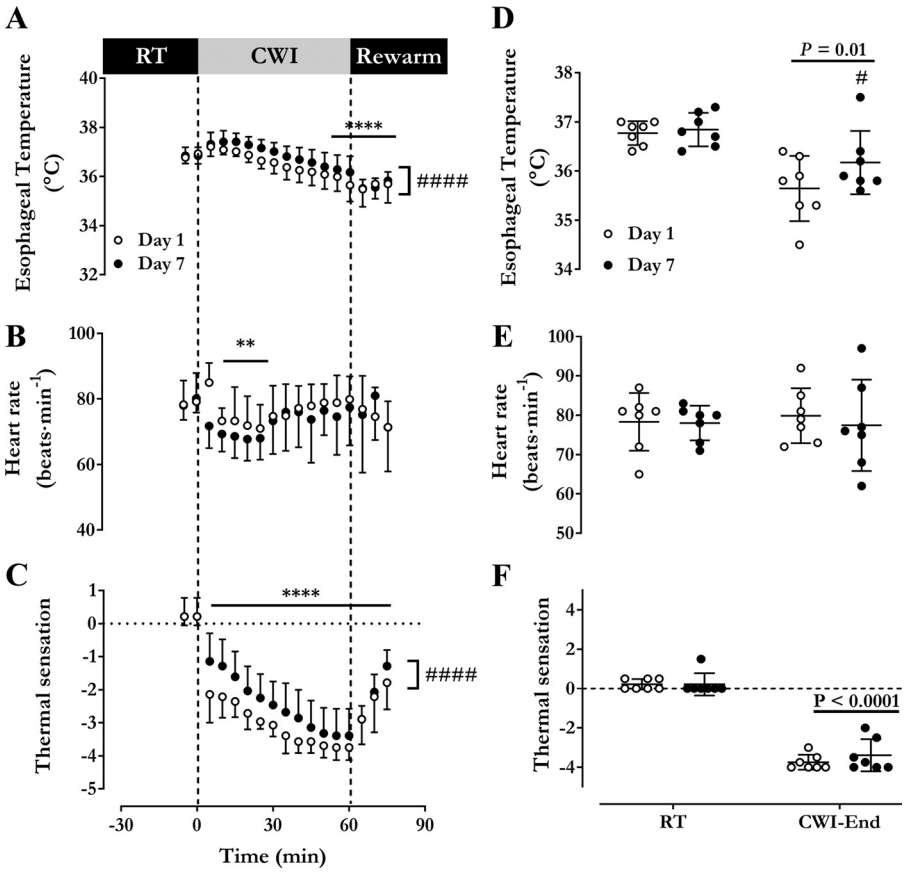

Fig. 1. Cold water immersion acclimation. Time course of esophageal temperature (*A*), heart rate (*B*), and thermal sensation at room temperature (RT) (*C*), during an acute 14°C cold water immersion (CWI) and during a rewarming period on *day 1* and *day 7* of the cold acclimation protocol. Average esophageal temperature (*D*), heart rate (*E*), and thermal sensation (*F*) of final 5 min at room temperature and cold water immersion. Values are expressed as means ± SD (n = 7 men). Repeated-measures ANOVA with Bonferroni post hoc test. $^{**}P < 0.01$, $^{****}P < 0.0001$ vs. Time = 0 min. $\#P < 0.01$, $\#\#\#\#P < 0.0001$ vs. *day 1*.

Shivering response. Changes in shivering intensity during the mild cold are shown in Fig. 3. Shivering intensity increased throughout the mild cold exposure in both preacclimation and postacclimation conditions (Fig. 3*B*; $P \leq 0.0001$). Total shivering intensity (AUC) decreased by 36% (SD 20) from 205% MVC·150 min (SD 92) preacclimation to 132% MVC·150 min (SD 85) postacclimation ($P = 0.003$). This postacclimation decrease in shivering activity occurred primarily in the musculus pectoralis [2.3% MVC (SD 1.5) to 1.5% MVC (SD 1.2), $P = 0.001$] (Fig. 5*A*). There was no association between the acclimation-induced change in total heat production and shivering intensity ($r = 0.07$; $P = 0.88$) (Fig. 5*B*). There was a strong association observed between cold-induced thermogenesis and shivering intensity both preacclimation and postacclimation ($r = 0.84$ preacclimation versus $r = 0.84$ postacclimation, $P < 0.0001$ (Figs. 5, *B* and *C*). The slopes of the regression lines were not different preacclimation compared with postacclimation (1.6 kJ·min^{-1}·%MVC^{-1} preacclimation versus 1.9 kJ·min^{-1}·%MVC^{-1} postacclimation; $P = 0.31$).

DISCUSSION

Previous investigations have shown that daily mild cold exposure can increase the thermogenic contribution of NST and decrease ST within 10–20 days (3, 10). In one instance, ST was reduced by 50% within 10 days of daily cold air exposure (10). Others, however, have posited that the greatest metabolic effects resulting from a cold-acclimation protocol would likely require a daily stimulus sufficiently cold or long enough in duration to elicit a decrease in core temperature (32). We show that during an individualized acute cold exposure designed to elicit a ~1.5-fold increase in metabolic rate both before and after the cold acclimation, ST is 36% lower following a 7-day cold water immersion acclimation protocol (Fig. 5). This observation confirms that NST can be increased substantially by as little as 7 days of cold exposure and can compensate for any decreases in ST to maintain thermogenesis. Together, these observations exemplify the important metabolic flexibility of humans to sustain $\dot{H}_{prod}$ under compensable cold conditions using various thermogenic mechanisms (3) and confirm that changes in the contribution of NST can occur even with only a week of daily cold water immersion.

Seven-Day Cold Water Immersion Acclimation Protocol

When compared with heat acclimation, far less is known on the capacity of humans to modify thermoregulatory processes in response to repeated cold exposure. Following up on our previous cold acclimation work (3, 5, 8), we opted to use in this study an uncompensable cold acclimation protocol consisting of 7 days of 14°C water immersion in an attempt to induce faster and larger increases in the contribution of NST to total $\dot{H}_{prod}$ than previously obtained from a milder cold acclimation. As such, the present 7-day cold acclimation protocol was designed to induce daily decreases in core temperature of ~1°C [1.2 (SD 0.8) on *day 1* and 0.7°C (SD 0.7) on *day 7*], resulting in intense shivering during the water immersion and the subsequent passive rewarming after the immersion (17, 25). Although the cooling stimulus was colder than our previous compensable cold acclimations, which used 10°C water

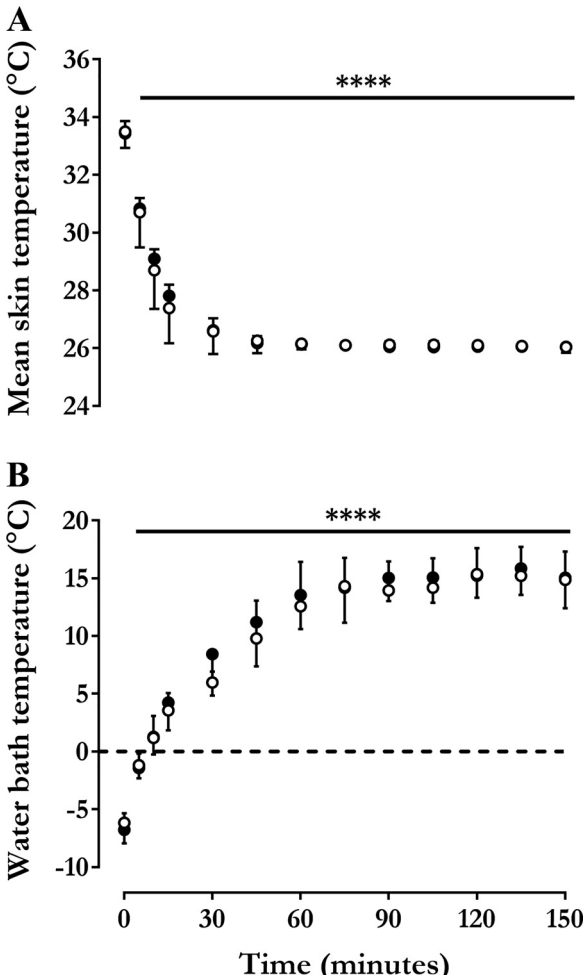

Fig. 2. Mean skin and water bath temperatures. Changes in mean skin temperature ($\overline{T}_{skin}$, °C) (*A*) and circulating water bath temperature (T_{water}, °C) (*B*) measured during an acute cold exposure before (○) and after (●) cold acclimation. Values are expressed as means ± SD (n = 7 men). Repeated-measures ANOVA with Bonferroni post hoc test. ****$P < 0.0001$ vs. Time = 0 min.

through the LCS (3, 5) or $\overline{T}_{skin}$ maintained at 28°C (8), all participants in the present study were able to tolerate the complete immersion duration of 60 min, and core temperature never reached the critical limit for water extraction of 35°C.

Given the water temperature used in this acclimation protocol, the heart rate upon initial water immersion (first 5 min) rose rapidly preacclimation to 85 beats/min (SD 6) [from 78 beats/min (SD 7) at room temperature], a marker of the sympathetic stimulation resulting from "cold shock" (28), but fell immediately to 72 beats/min (SD 7) postacclimation [from 78 beats/min (SD 7) at room temperature] (Fig. 1*C*). Our results also indicated that T_{es} cooling rate was attenuated, on average by ~0.01°C/min, from *day 1* to *day 7* (Figs. 1, *A* and *B*), while thermal sensation was significantly warmer following cold acclimation (average rating of −3.1 vs. −2.5) (Figs. 1, *E* and *F*. These results suggest that the heat-loss defense mechanisms (vasoconstriction) and/or the thermogenic responses were increased postacclimation, resulting in slower cooling and improved thermal sensation. Unfortunately, in the present study, we did not quantify changes in either $\dot{H}_{prod}$ or $\dot{H}_{loss}$ throughout the 7-day cold-water immersion protocol. Consequently, the exact processes responsible for the reduced rate of body core cooling remain unknown. However, these results appear to suggest a potential blunted sympathetic response upon entering the cold water and that NST, likely from skeletal muscles, was significantly upregulated to support a greater thermogenic rate. This increased thermogenic response would be consistent with a "metabolic" acclimation phenotype that would be anticipated from an uncompensable cold acclimation protocol (32).

Contribution of ST and NST Following Intense 7-Day Cold Acclimation

Quantifying the relative contribution of ST and NST to total $\dot{H}_{prod}$ during cold exposure in humans presents a number of important challenges (see topical review in Ref. 4). The greatest challenge relates to the fact that whole body NST cannot be quantified directly because it involves all cold-induced stimulation of thermogenic processes in various cells of the body that are not linked to muscle contractions. For this reason, we can typically only qualitatively assess modifications in NST based on changes in whole body ST activity at the same given $\dot{H}_{prod}$ (3, 5, 12). To achieve this objective, we elected to use a liquid-cooled suit coupled with a computer-controlled chiller to clamp $\overline{T}_{skin}$ and associated changes in $\dot{H}_{prod}$ during an acute cold exposure before and after cold acclimation. Using this method, we clamped average $\overline{T}_{skin}$ at 26°C to achieve the same time course and overall ~1.6-fold increase in $\dot{H}_{prod}$ before and after cold acclimation (Fig. 3*A*). Under these standardized conditions, we showed that whole body ST was reduced by ~40% after the cold acclimation protocol when compared with responses measured before the cold acclimation (Fig. 3*D*). This change can be explained by a combined slower progressive rise in ST and a more than ~30% reduction in average ST intensity measured in the last 30 min of cold exposure (Fig. 3). This large reduction of ST at the same $\dot{H}_{prod}$ clearly indicates that cold acclimation resulted in a major stimulation in NST processes. This has significant implications for cold endurance, tolerance and ultimately, survival. Whether such significant changes in the recruitment of NST processes can be accomplished with a shorter similar acclimation protocol or slightly longer but milder cold-water immersion is also of importance. This 7-day cold acclimation protocol was selected to account for what we believed would be the minimum amount required to observe physiologically relevant differences in $\dot{H}_{prod}$ and ST, based on the only other cold acclimation protocol to examine these outcomes simultaneously using moderate cold exposure (10). It is important to note that changes in shivering intensity observed in the present study may be specific to conditions where increases in heat loss induced by cold exposure may be matched by increases in $\dot{H}_{prod}$, termed compensable cold exposure. It is unclear whether these changes in thermogenic responses would also be observed during uncompensable cold exposure such as cold-water immersion.

Of great interest is also the effects of a cold acclimation and associated reduction in ST on the reliance on CHO to fuel heat production (13). Previous work has shown that muscle glycogen accounts for ~80–85% of all the glucose required to sustain total CHO oxidation, at cold exposures eliciting a

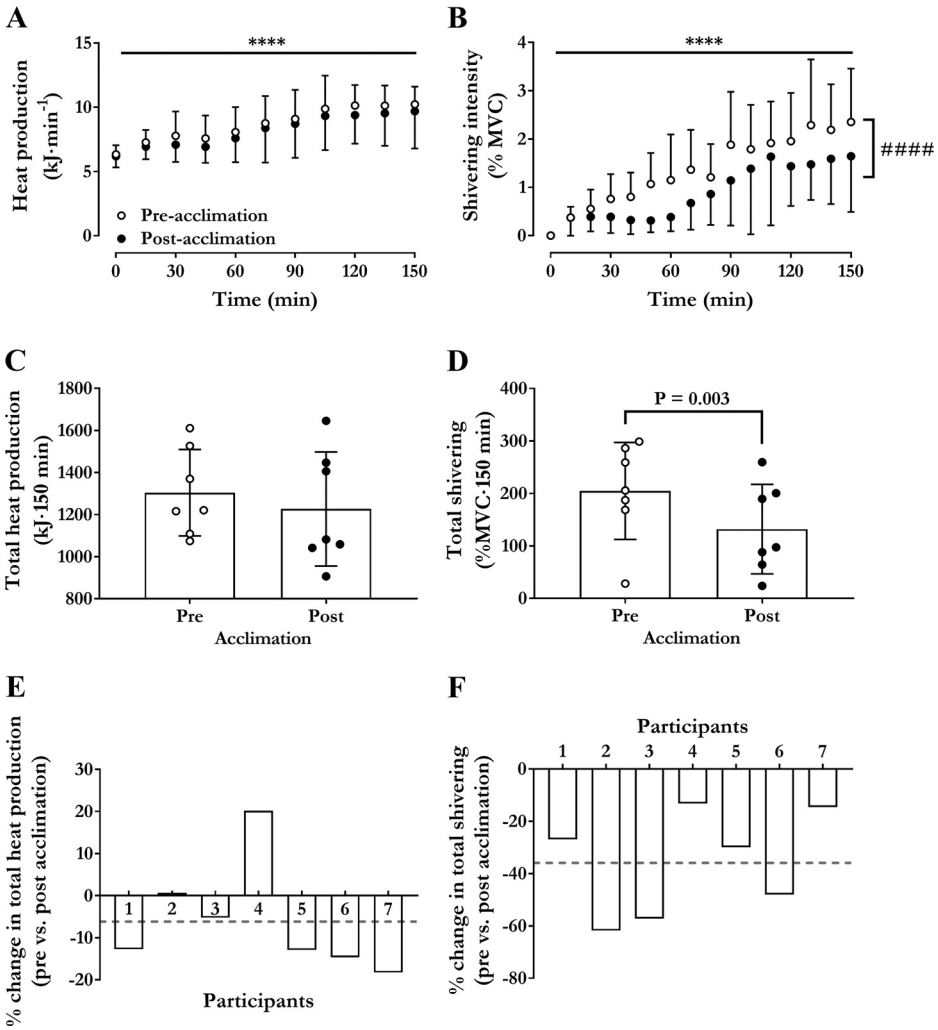

Fig. 3. Thermogenic responses to acute cold exposure. Changes in rate of metabolic heat production (kJ/min) (*A*), shivering intensity [% maximum voluntary contraction (MVC)] (*B*), total heat production (kJ·150 min) (*C*), and total shivering (%MVC·150 min) (*D*) during an acute cold exposure at a mean skin temperature of 26°C before and after cold acclimation. Percent change in heat production (*B*) and shivering intensity (*F*) from before to after cold acclimation for each participant. Values are expressed as means ± SD (n = 7 men). Repeated-measures ANOVA with Bonferroni post hoc test. ****$P <$ 0.0001 vs. Time = 0 min. ####$P < 0.0001$ vs. preacclimation. Student's t-test used in *D*.

metabolic heat production that is 2.0 to 3.5 times above basal levels (13). Therefore, we were interested in determining whether the ~40% decrease in shivering intensity following cold acclimation found here could preserve the limited CHO reserves, which despite its significant reliance in the cold only represent ~1% of total energy reserves (7). Fuel selection can be modified by recruiting different muscle groups, different subpopulations of muscle fibers within the same muscle, and different metabolic pathways within the same muscle fibers. Here, we show that shivering intensity was reduced in all muscles examined following cold acclimation, but decreased most significantly in the pectoralis, falling by 35% (Fig. 5). Interestingly, this overall reduction in ST and increase in NST did not modify absolute rates and relative contribution of CHO and lipid to total $\dot{H}_{prod}$ during cold exposure (Fig. 4). This finding implies that the NST processes that were involved in modulating ST must necessarily use a similar fuel mixture to sustain $\dot{H}_{prod}$.

Contribution of Various Tissues and Mechanisms to NST

Our study confirms that NST can increase substantially to reduce ST following a 7-day cold acclimation in 14°C water. As in other mammals, such a reduction in ST during cold exposure confers a survival advantage by reducing the discomfort of involuntary muscle contractions and its effects on locomotion and motor control (9, 21). However, identification of the exact tissues and mechanisms involved in increasing NST during acute cold exposure or following cold acclimation is particularly difficult at the tissue and/or whole body level. Previously, we have shown that one source of NST that can increase through repeated mild-cold exposure in humans is BAT thermogenesis (3, 5, 8). Despite increasing BAT volume by 45% and its thermogenic capacity by 150–182% in these previous studies, this had no effect on fuel selection. Therefore, it is possible that the thermogenic contribution of BAT increased in the present study but did not impact fuel selection when exposed to a mild acute cold. Given its small relative size (<1% of body weight in humans) (6, 30) and thermogenic contribution (<1% of whole body heat production) (29), it is more probable that another source of NST is responsible for the improved thermal sensation and reduced body core cooling in the cold water immersion and the increased NST in the acute mild cold.

Skeletal muscles constitute another important target for cold-induced NST, as it accounts for 42% of total body weight

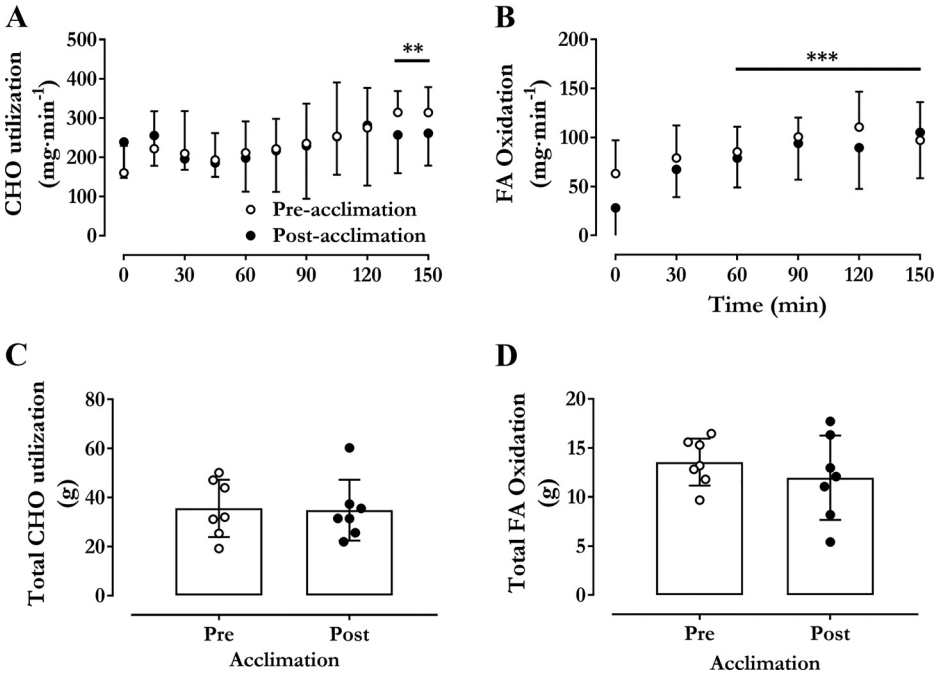

Fig. 4. Substrate utilization. Changes in rate of carbohydrate (CHO) (*A*), lipid oxidation (*B*), total carbohydrate (*C*), and lipid oxidation (*D*) during an acute cold exposure at a mean skin temperature of 26°C before and after cold acclimation. Values are expressed as means ± SD (n = 7 men). Repeated-measures ANOVA with Bonferroni post hoc test. ***$P < 0.001$, **$P < 0.01$ vs. Time = 0 min.

(19). We and others have shown that acute cold exposure increases proton leak in the vastus lateralis (3, 31), another source of NST, but 4 wk of mild daily cold exposure abolishes this response (3). In addition, in contracting muscles cytosolic Ca^{2+} levels rapidly increase, and sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) must pump Ca^{2+} back into the SR to maintain its store, accounting for ~24–58% of tissue metabolic rate in contracting muscles in rodents (26). This Ca^{2+} release is critical for muscle contractions during shivering, but in cold-acclimated rodents, calcium leaks into the cytosol via ryanodine receptors, leading to excessive activation of SERCA to pump Ca^{2+} back into the SR, generating heat in the process (2). With humans possessing significantly greater levels of the SERCA regulatory proteins (1) compared with rodents and relying to a greater extent on skeletal muscle to produce heat, this thermogenic mechanism could likely be quite significant in humans and serve as a form of NST following cold acclimation. Further investigations are required to determine the contribution of these skeletal muscle-derived thermogenic processes in such a cold acclimation.

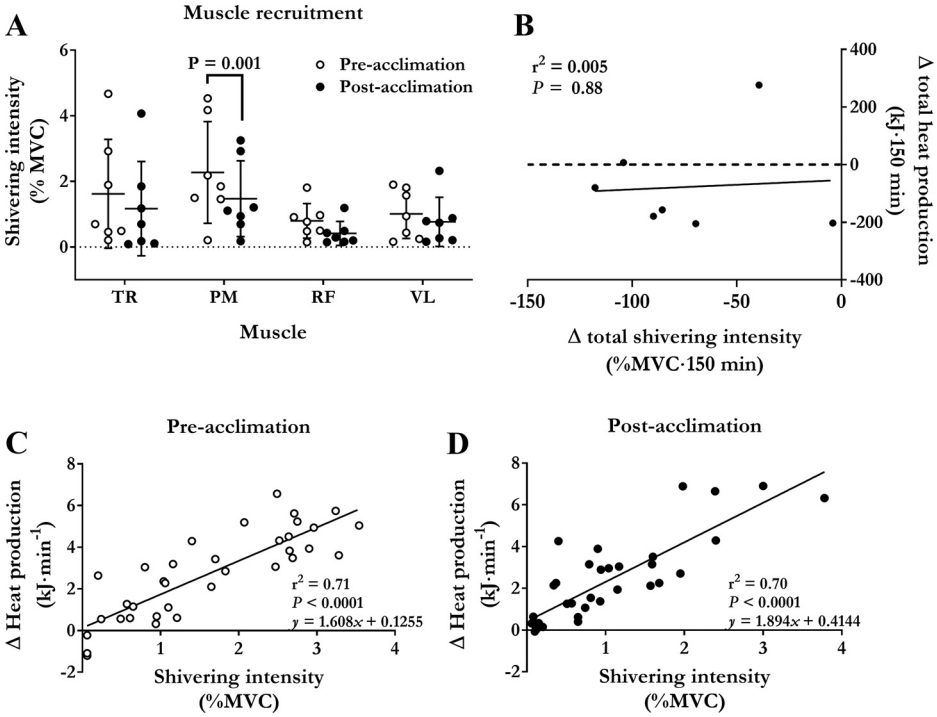

Fig. 5. Muscle recruitment pattern contribution of ST to heat production. *A*: differences in shivering intensity for trapezius (TR), pectoralis (PM), rectus femoris (RF), and vastus lateralis (VL) during an acute cold exposure at a mean skin temperature of 26°C before and after cold acclimation. Values are expressed as means ± SD (n = 7 men). Repeated-measures ANOVA with Bonferroni post hoc test. *B*: relationship between acclimation-induced change in total heat production and total shivering intensity (difference between preacclimation and postacclimation total heat production and shivering intensity) in men exposed to an acute cold. Relationship between cold-induced changes in heat production and shivering intensity in men exposed to a cold eliciting a mean skin temperature of 26°C for 150 min before (*C*) and following a 7-day (*D*) 14°C cold-water immersion acclimation protocol. Values presented are from five sampling intervals during cold exposure (time = 30, 60, 90, 120, and 150 min) from all subjects.

In summary, we have demonstrated that seven consecutive days of cold-water immersion can decrease shivering intensity by 36%, nearly double what we previously described in response to a 4-wk compensable cold acclimation. The decreased shivering intensity for the same whole body heat production in response to a mild acute cold exposure, suggests a significant increase in the contribution of NST following 7 days of cold water immersion. This had no effect on whole body fuel selection, which suggests that the source of NST must rely on a similar fuel mixture to produce this heat. Although several studies have examined the effects of repeated cold exposure on whole body heat production in humans, few have ever simultaneously quantified the contribution of shivering and by extension the contribution of NST to this heat production. This not only fills a critical gap to improve our understanding of the physiological and metabolic responses to chronic cold, but it also provides critical information to develop strategies to improve human survival and tolerance in cold climates. With the growing naval traffic expected in arctic regions as a result of the exploration of natural resources, research, military operations, tourism, and expansion of shipping lanes for cargo ships, there is an increase in occupational exposure to cold and increased risk for ship groundings. The grounding of naval vessels poses several risks, as survivors may be required to wait 5–7 days before being rescued. In addition, there remain critical knowledge gaps in our understanding of the cold acclimation responses in vulnerable populations, such as the elderly or individuals with impaired motor function, as well as in individuals originating from warm climates, which clearly need to be addressed in future studies. Consequently, further work is necessary to understand the thermogenic processes that may be modulated under such a short, but intense, cold acclimation and determine the minimal acclimation duration and cooling temperature required to elicit metabolic changes that may be critical for survival and performance in cold environments.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

K.G., D.P.B., G.P.K., and F.H. conceived and designed research; K.G., B.J.F., and H.C.T. performed experiments; K.G., D.P.B., B.J.F., and H.C.T. analyzed data; K.G., D.P.B., B.J.F., H.C.T., G.P.K., and F.H. interpreted results of experiments; K.G. and D.P.B. prepared figures; K.G. and D.P.B. drafted manuscript; K.G., D.P.B., B.J.F., H.C.T., G.P.K., and F.H. edited and revised manuscript; K.G., D.P.B., B.J.F., H.C.T., G.P.K., and F.H. approved final version of manuscript.

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