DOCTOR OF PHILOSOPHY

Thermoregulation and mucosal immunity : the effects of environmental extremes

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Award date: 2013

Awarding institution: Bangor University

Link to publication
CHAPTER ONE

General Introduction

Increasing numbers of people travel to cold and mountainous regions for work, recreation and physical activities (e.g. soldiers, mountaineers and skiers) (Young et al. 1998; Oliver et al. 2012). In such cold and hypoxic climates, accidental hypothermia is a perpetual hazard for casualties of mountain incidents (Kornberger and Mair, 1996) and is a significant contributor to fatalities (Sallis and Chassay, 1999; Sharp, 2007). The likelihood of developing a peripheral cold injury (e.g. frost nip and frost bite) or upper respiratory tract infection (URTI) has been reported to be increased in mountaineers and individuals exposed to cold and high altitude for prolonged periods (Giesbrecht, 1995; Felicijan et al. 2008; Oliver et al. 2012), however whether acclimatisation to altitude reduces these risks is unknown. Anecdotal evidence suggests a relationship exists between exertional fatigue, often experienced during outdoor pursuit activities, and susceptibility to hypothermia (Thompson and Hayward, 1996; Pugh, 1966; Young et al. 1998). Ultimately, ill health and cold injury are likely to reduce cognitive function and motor performance (Patil et al. 1995), causing confusion and lethargy so that the casualty is forced to remain sedentary. Furthermore, loss of motor control makes it difficult to execute survival procedures (Vanggaard, 1975).

Factors found to alter human thermoregulation and immunity in a cold environment include, but are not limited to: prolonged exposure to cold-wet conditions (Thompson and Hayward, 1996), hypoxia (Golja et al. 2004), prolonged fatiguing exercise, sleep deprivation and negative energy balance (Castellani et al. 1998; Young et al. 1998). Nevertheless, the effect of prolonged (> 2 h) exposure to cold and hypoxia on thermoregulatory and immune responses remains equivocal (Cipriano and Goldman, 1975; Blatteis and Lutherer, 1976; Giesbrecht, 1995; Savourey et al. 1997; Gleseson, 2000). Since URTIs are associated with a reduction in the concentration of salivary
immunoglobulin A (s-IgA) (Gleeson, 2000) and research surrounding the typical mucosal immune response to cold, with and without hypoxia, is sparse, further investigation in this area is required.

Although mortality rates are relatively low with severe non-trauma related hypothermia (<28°C), they rise alarmingly with traumatic injury where mortality is 100% at 32°C (Moss, 1986; Jurkovich et al. 1987). Rescue times for casualties depend on global location and weather conditions. Yet even where distances are relatively short between emergency services and a casualty (e.g. Scottish Highlands) the reported times for evacuation are 2.25 h by helicopter and approximately 3.5 h when individuals are evacuated by others means (e.g. on foot) (Crocket, 1995; Hindsholm et al. 1992).

While superior re-warming methods are available (i.e. forced air warmer, inhalation re-warming) these require medically trained personnel and a power supply. Therefore, simple methods that prevent heat loss and promote re-warming that casualties or first-responders can administer instantly whilst they wait for evacuation will likely reduce hypothermia related fatalities. Current water and wind-proof survival bags aim to reduce core cooling by decreasing heat loss through convection and radiation (Light and Norman, 1990), yet accidental hypothermia still affects approximately 10% of casualties reported in mountainous environments. It is pertinent therefore to establish an optimal lightweight device to increase the rate of core re-warming compared to shivering alone (Giesbrecht et al. 1987; Williams et al. 2005).

Given prolonged exposure to cold and/or hypoxia poses detrimental effects to thermoregulation and immune function, it is of benefit for those individuals travelling to high altitudes and low temperatures to know which self-administering survival product will offer optimal protection against peripheral and central cold injury if a situation occurred where they were forced to remain sedentary.
Thesis objectives

With this information in mind, the objectives of this thesis were to:

I. Determine the effects of acute and prolonged (18 days) exposure to a simulated high altitude of 4000 m on physiological and behavioural thermoregulation during mild cold exposure.

II. Examine the mucosal immunity responses to acute and prolonged (18 days) exposure to a simulated high altitude of 4000 m

III. Compare the effectiveness of four field re-warming methods for the treatment of cold casualties upon thermoregulation and metabolism during a three hour ‘awaiting rescue’ scenario in 0°C following cold water immersion to reduce core temperature to 36°C.

IV. Examine the mucosal immunity response to prolonged cold exposure (0°C) in pre-cooled casualties.

V. Investigate the efficacy of three field protection methods for the treatment of non-shivering cold casualties using an in vitro torso model exposed to -18.5°C, 0°C and 18.5°C for four hours.
CHAPTER TWO

Literature review

2.1 Ill health in cold and hypoxic environments

2.1.1 Hypothermia

Hypothermia occurs through a combination of increased heat loss and decreased heat production. Heat loss is often increased in cold air when wet and windy conditions are also present (Thompson and Hayward, 1996). The majority of accidental hypothermia cases are reported to result from cold-water immersion or cold-water submersion (Golden et al. 1997), cold-air exposure (Pugh, 1966) or traumatic injury with haemorrhage (Moss, 1986; Jurkovich et al. 1987). Hypothermia is strongly related to coagulopathy and shock (Beilman et al. 2009) and it is this ‘triad’ that significantly contributes to the mortality of trauma patients (Arthurs et al. 2006). Since the term ‘hypothermic’ is often misleading regarding severity, systems exist to classify the different stages of hypothermia (Table 2.2).
Table 2.1. Signs, symptoms and physiological changes associated with progressive hypothermia. Adapted from Danzl and Pozos, (1994).

<table>
<thead>
<tr>
<th>Stage of Hypothermia</th>
<th>Rectal Temperature (°C)</th>
<th>Signs and symptoms</th>
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<tbody>
<tr>
<td>Mild</td>
<td>37</td>
<td>Normal rectal temperature</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>Increased metabolic rate due to shivering</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>Maximum shivering thermogenesis</td>
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<tr>
<td></td>
<td>34</td>
<td>Amnesia and judgement problems</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>Ataxia and apathy</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>Shivering stops</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Possible cardiac arrhythmias</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>Decreasing consciousness</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>Increased sensitivity to ventricular fibrillation</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>Loss of voluntary motion</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>No response to pain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cerebral blood flow decreased to 30% of normal</td>
</tr>
<tr>
<td>Moderate</td>
<td>25</td>
<td>Maximum risk of ventricular fibrillation</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>Asystole</td>
</tr>
<tr>
<td>Severe</td>
<td>22</td>
<td>Maximum risk of ventricular fibrillation</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Asystole</td>
</tr>
</tbody>
</table>

2.1.2 Peripheral cold injury

In the instance of peripheral cold injury, manual dexterity is impaired and work capacity deteriorates (Holmer, 1994). For example, military operations in cold, snowy environments increase the chances for minor cold injuries that limit military unit effectiveness due to lost man-hours. Cold weather also increases the difficulty of performing tasks related to eating, drinking, and normal hygiene. This subsequent inability to protect oneself or practise basic survival skills while awaiting rescue in a cold environment may increase the likelihood of hypothermia related fatalities.

Peripheral ‘freezing’ cold injuries resulting from acute cold exposure include frostbite and frost-nip. The milder of the two, frost-nip, is a precursor to frostbite and occurs via skin contact with cold surfaces. With frost-nip, only superficial skin is frozen and tissues are not permanently damaged.
Frostbite, on the other hand, develops as a function of the body’s protective mechanisms to maintain core temperature. Warm blood is shunted from cold peripheral tissues to the core by vasoconstriction of arterioles that supply capillary beds and venules to the extremities and face, especially the nose and ears. Frostbite progresses from distal to proximal and from superficial to deep. As the temperature of these areas continues to decrease, cells begin to freeze. Damage to the frostbitten tissue is due to electrolyte concentration changes within the cells, resulting in water crystallization within the tissue (Conway et al. 1998).

Frequent or prolonged exposure to moderate cold has been demonstrated to precipitate or exacerbate shoulder and extremity pain, respiratory infections (Griefahn, 1995) and the ‘non-freezing’ cold injuries of chilblains (perniosis) and trench (immersion) foot (Herrington, 1996; Conway et al. 1998). Muscle and tendon tears may also be more likely in cold environments. Raynaud’s syndrome and the related white finger syndrome cause severe arterial vasoconstriction with digital blanching, and severe cases may lead to ulceration and tissue loss (Lloyd, 1994). Any form of peripheral cold injury may have the potential to inhibit individuals from carrying out simple behavioural thermoregulatory activities.

### 2.1.3 Upper respiratory tract infections (URTI)

Upper respiratory tract infection (URTI) is a nonspecific term used to describe acute infections involving the nose, paranasal sinuses, pharynx, larynx, trachea, and bronchi. Therefore URTI include, but are not limited to: nasopharyngitis, pharyngitis, laryngitis, tonsillitis, sinusitis and bronchitis. Upper respiratory tract infections represent the most common acute illnesses evaluated in the outpatient setting. Viruses cause the vast majority of acute upper respiratory tract infections (Neemisha et al. 2001; Monto, 2002; Fendrick et al. 2003). The common cold is a viral illness with
the most associated cause being rhinovirus; others are parainfluenza, respiratory syncytial, coronavirus, adenovirus, echovirus, coxsackievirus and parainfluenza virus (Turner, 1995). Rhinovirus accounts for up to 60% of infections but generally resolves spontaneously without antimicrobial therapy (Ashes, 1999). Symptoms include nasal discharge, nasal obstruction, and throat irritation. The National Institute of Allergy and Infectious Diseases reports that people in the USA suffer 1 billion colds each year, with an incidence of two to four for the average adult, and six to ten for children (Nieman et al. 2011). URTIs impose an estimated $40 billion cost on the US economy (Fendrick et al. 2003). Lifestyle habits and demographic factors such as mental stress (Cohen, 2005), lack of sleep (Cohen et al. 2009), poor nutrition (Wolvers et al. 2006), and old age (Aw et al. 2007) have all been associated with impaired immune function and elevated risk of infection.

Infections and acute mountain sickness (AMS) are common at high altitude, yet their precise etiologies remain elusive and the potential for misdiagnosis is considerable (Bailey et al. 2003). The misdiagnosis of altitude illness is likely due to the similarity of nonspecific constitutional symptoms (e.g. fatigue, fever, shortness of breath on exertion, decreased appetite and headache) associated with infection and AMS (Bailey et al. 2003). Both conditions are characterised by changes in peripheral biomarkers related to free radical, skeletal muscle damage and amino acid metabolism (Bailey et al. 2003). While not establishing cause and effect, free radical-mediated changes in peripheral amino acid metabolism, known to influence immune and cerebral serotonergic function, may enhance susceptibility to and delay recovery from altitude illness (Bailey et al. 2003). While AMS is not dangerous, at altitudes over 4000 m it can progress unpredictably to one of two potentially fatal forms (high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE)) that may co-exist with each other and be misdiagnosed (Wright and Fletcher, 1987). Symptoms of HAPE include a cough, frothy sputum and lung crackles but without definite abnormal cardiac signs. These features may be misdiagnosed as pneumonia, which sometimes
present as an aggravating factor (Wright and Fletcher, 1987). Further, upper respiratory symptoms (URS) may present in the absence of illness and infection (Giesbrecht, 1995).

2.2 Thermoregulation in cold

During thermoregulatory homeostasis human core temperature ($T_c$) is maintained close to 37°C (Widmaier et al. 2004). The central control mechanism in the hypothalamus determines mean body temperature ($T_b$) and compares this to a pre-determined set-point temperature by integrating thermal signals from peripheral and core receptors (Sessler, 1994; Guyton, 1996). Changes in ambient temperature generate thermo-receptor impulses that are propagated to the anterior hypothalamus via afferent pathways in the spinal cord (Pocock and Richards, 2006). Efferent responses are mediated via the posterior hypothalamus to initiate heat loss and heat production mechanisms.

2.2.1 Behavioural responses

Behavioural responses to an increased perception of cold include wearing extra clothing, closing an open window and turning up the heating. During outdoor pursuits in cold, mountain regions individuals will often seek cold and wind-proof shelters to make a hot drink or meal. If shelter is unavailable or stopping is not an option, increasing the intensity of any physical activity or exercise will help to increase heat production.

2.2.2 Physiological responses – Heat balance

The mechanisms of heat transfer include evaporation, radiation, convection and conduction. Each mechanism contributes proportionally to heat transfer and fluctuates depending on the air/water
temperature and humidity. For a healthy human of normal body core temperature in a temperate environment considered comfortable however, evaporation, the conversion of water to its gaseous phase, accounts for 20-30% of total body heat loss; radiation, a loss of heat to the environment via infra-red radiation, accounts for 55-65% of total body heat loss; convection, the transfer of heat to the external environment by air or water in contact with the body, is accountable for ~12% of total body heat loss and conduction, the transfer of heat to another object via direct contact accounts for only 3% of total body heat loss (Kanzenbach and Dexter, 1999).

An involuntary heat transfer response to maintain core temperature in the cold involves the control of cutaneous vascular smooth muscle tone (e.g. vasoconstriction) combined with a rapid fall in $T_{sk}$ (Wagner et al. 1974). This reduced blood flow to peripheral vessels decreases convective heat loss and conserves $T_c$. Peripheral vasoconstriction is a powerful mechanism to reduce the heat loss, but results in strong cooling of the extremities (Daanen, 2003). However, the extremities (i.e. fingers, hands, ear lobes and toes) possess the ability to prevent the occurrence of local cold extremes with a specialised pattern of circulation known as arteriovenous anastomoses. These have smooth muscle in their walls and are deep to the tips of the capillary loops, close to the surface of the skin. The arteriovenous anastomoses open up when $T_b$ is above normal during reflexively induced vasodilation and during cold induced vasodilation (CIVD) when $T_b$ is reduced. Upon opening, a large flow of blood passes through the shunts increasing heat transfer (Abramson, 1967). Since blood flow increases substantially in the fingers during CIVD, and this increases muscle temperature and blood circulation in the large vessels of the forearm (Lewis, 1930; Ducharme et al. 1991), it is likely that CIVD reduces the risk of local cold injuries (Iida, 1949; Wilson and Goldman, 1970), improves manual dexterity and enhances tactile sensitivity during work in the cold by improving muscle function (Daanen, 2003). Individuals with a higher CIVD response are considered to be less susceptible to local cold injuries due to the maintenance of a higher rate of extremity blood flow during cold exposure (Daanen and van Ruiten, 2000). Beyond the specialised
circulation at the extremities further heat transfer systems exist in the human forearm. Veins running alongside the ulnar and radial arteries, called venae comitantes (Bazett et al. 1948), create a counter-current heat exchange system between arteries going to the periphery of a limb and venous blood returning from the periphery.

2.2.3 Physiological responses – Heat production

In addition to a complex vascular system helping to maintain heat balance in the cold, an increase in metabolic rate (MR) via shivering thermogenesis (ST) or non-shivering thermogenesis (NST) evokes heat production and attenuates further decrements in $T_c$ (Lindahl, 1997; Potkanowicz et al. 2003). Consequently, the two main physiological adjustments that occur during cold exposure are insulative and metabolic (van Ooijen et al. 2004). Nonetheless, such physiological adjustments are particularly reliant upon individual characteristics given that they may be modified by cold acclimatisation (Scholander et al. 1958), amount of body fat (Wade et al. 1979), relative humidity (RH) (Burton et al. 1955), wind speed, physical fitness (Adams and Heberling, 1958; Bittel et al. 1988) and age (Horvath et al. 1955; Wagner et al. 1974).

The level of thermogenesis that humans can achieve and maintain is important for survival during severely cold weather (Vallerand and Jacobs, 1989). During mild hypothermia ($T_c = 35\,^\circ C$) it has been suggested that ST remains the only physiological process available for maintaining $T_c$ in a non-exercising casualty void of a re-warming device (Folk, 1974; Neufer et al. 1988; Haman et al. 2002). Metabolic rate, as measured by oxygen consumption ($VO_2$), increases proportionally to shivering intensity of the whole body or specific muscle groups (Horvath et al. 1956; Tikuisis et al. 1991). Peak shivering intensity can increase heat production up to five times the basal metabolic rate (BMR) (Eyolfson et al. 2001). The intensity of shivering is determined by the severity of cold
exposure, the amount of fat-free mass (FFM), the subject’s fitness (Eyolfson et al. 2001) and the rate of change of the skin temperature (Fiala et al. 2001).

Non-shivering thermogenesis is a heat-production mechanism that involves no muscular contractions. Heat production under the conditions of basal metabolism is mostly obligatory NST. Obligatory NST serves to maintain the basic energy demands of the homoeothermic organism (Jansky, 1973). During cold exposure NST is defined as an increase in heat production in the absence of shivering (van Ooijen et al. 2005). This additional heat production that occurs is called regulatory NST (Jansky, 1973).

Non-shivering thermogenesis has been found to occur in the newborn of some species (e.g. humans and sheep), hibernating animals, and in other animals such as the rat (Horovitz, 1971). Brown fat is the primary energy source for NST, separating itself from ordinary fat by having much smaller cells that contain greater amounts of mitochondria (Saito et al. 2009). Studies in animals indicate that brown adipose tissue (BAT) is important in the regulation of body weight, and it is possible that individual variation in adaptive thermogenesis can be attributed to variations in the amount or activity of BAT (van Marken Lichtenbelt et al. 2009). Until recently, the presence of BAT was thought to be relevant only in small mammals and infants, with negligible physiologic relevance in adult humans (Astrup et al. 1985; Himms-Hagen, 2001). On the contrary, new evidence has observed a high rate of occurrence of BAT in healthy young males that is mainly activated during cold exposure (van Marken Lichtenbelt et al. 2009). The activity of BAT is reported to be greater in lean males in comparison to those who are overweight or obese (van Marken Lichtenbelt et al. 2009).
2.3 Thermoregulation in hypoxia

2.3.1 Behavioural responses

Hypoxia has been shown to alter thermal sensation during cold exposure (Golja et al. 2004) which has been related to a lowered neural activity and delayed signal conduction (Astrup, 1982). This blunted thermal sensation may cause inappropriate behavioural thermoregulation (e.g. neglecting to add extra layers of clothing) and therefore be a major implication in cold injury. Anecdotal reports have also suggested hypoxia alters thermal comfort. However these early reports describe the pain to single limb, cold water immersion (Mathew, 1979) and thermal discomfort to whole body mild cold air exposure (Blatteis and Lutherer, 1976) to be greater at altitude than at sea level which did not abate with altitude acclimatisation (Mathew, 1979; Blatteis and Lutherer, 1976). Further investigation into the effect of whole body cooling on perception of thermal comfort during acute hypoxia and following acclimatisation may help provide clarity.

2.3.2 Physiological responses – Heat balance

The effect of hypoxia on typical thermoregulatory responses to the cold remains equivocal. Whilst some research suggests hypoxia causes greater heat loss and lower core temperatures during cold exposure compared to normoxia (Cipriano and Goldman, 1975; Kottke et al. 1948, Table 2.2), other research has reported that hypoxia does not affect thermoregulation (Blatteis and Lutherer, 1976; Robinson and Haymes, 1990, Table 2.2). Those studies that show greater reductions in core temperature have reported greater increases in skin temperature (Cipriano and Goldman, 1975; Kottke et al. 1948). Nonetheless, at altitude a significant reduction in CIVD is found where cold co-exists with systemic hypoxia (Mathew et al. 1977; Daanen and van Ruiten, 2000). Consequently, the risk for local cold injuries may be enhanced at altitude (Daanen and van Ruiten, 2000).
2.3.3 Physiological responses – Heat production

It is well established that heat production via the cold-induced increase in VO$_2$ is suppressed during hypoxia (Blatteis and Lutherer, 1976; Kottke et al. 1948; Savourey et al. 1997) and is coupled with a reduced time to shivering onset as well as an overall increased shivering intensity (Blatteis and Lutherer, 1976; Bullard, 1961; Robinson and Haymes, 1990). The occurrence at altitude of a reduction in the cold-induced increase in VO$_2$ without a diminution of shivering activity suggests hypoxia depresses NST (Blatteis and Lutherer, 1976) which is related to an inhibition of the aerobic catabolism of FFA (Robinson and Haymes, 1990). Nonetheless, given the cold-induced increase in VO$_2$ of high altitude residents has been reported as significantly greater than un-acclimatised lowlanders, indicates that recovery from the hypoxic depression of the metabolic response to cold occurs following prolonged exposure to altitude (Blatteis and Lutherer, 1976). However, acclimatised lowlanders, following a 6 week residence at altitude, showed no reversal in the hypoxia-induced reduction of the metabolic response to cold. On the contrary, shivering intensity decreased as altitude exposure continued, yet total VO$_2$ in the cold was not further decreased. This suggests therefore that some NST recovery may have occurred during the 6-week altitude stay and helped to replace the reduced ST (Blatteis and Lutherer, 1976).
2.4 The immune system

2.4.1 Innate immunity - External barriers against infection

The main line of defence from infection is the skin which, when intact, is impermeable to most infectious agents; when there is skin loss (e.g. burns) infection becomes a major problem (Davies et al. 2006). Most bacteria fail to survive on the skin because of the direct inhibitory effects of lactic and fatty acids in sweat and sebaceous secretions and the low pH which they generate.

Mucus secreted by membranes lining the inner surfaces of the body, acts as a protective barrier to block the adherence of bacteria to epithelial cells. Microbial and other foreign particles trapped within the adhesive mucus are removed by mechanical stratagems such as ciliary movement and coughing and sneezing. If microorganisms do penetrate the body, two main avenues of defence exist to destruct the problem. These are the destructive effect of soluble chemical factors such as bactericidal enzymes and the mechanism of phagocytosis.

2.4.2 Innate immunity - Phagocytosis

After inoculation, viruses and bacteria encounter several barriers, including physical, mechanical, humoral and cellular immune defenses. Adenoids and tonsils contain immune cells that respond to pathogens. Humoral (Immunoglobulin-A) and cellular immunity reduce infections throughout the entire respiratory tract. Resident and recruited macrophages, monocytes, neutrophils, and eosinophils coordinate to engulf and destroy the virus or bacteria in the process known as phagocytosis. A host of inflammatory cytokines mediates the immune response to invading pathogens. Phagocytic cells have a system of receptors that recognise molecular patterns expressed by pathogens. Normal nasopharyngeal flora, including various staphylococcal and streptococcal
species, help defend against potential pathogens. After adherence of the microbe to the surface of the neutrophil or macrophage the resulting signal initiates the ingestion phase.

### 2.4.3 Innate immunity - Soluble chemical factors

The spread of infection may be limited by enzymes released through tissue injury which activate the clotting system. Of the soluble bactericidal substances elaborated by the body, the most abundant and widespread is the enzyme lysozyme. Like the α-defensins of the neutrophil granules, the human β-defensins are peptides derived by proteolytic cleavage from larger precursors. The main human β-defensin (hDB-1) is produced mostly in the kidney, female reproductive tract, oral gingiva and particularly the lung airways. Another airway antimicrobial active against Gram-negative and positive bacteria is LL-37.

A number of plasma proteins collectively termed acute phase proteins show a dramatic increase in concentration in response to early ‘alarm’ mediators such as macrophage derived interleukin-1 (IL-1) released as a result of infection or injury. It is likely that the acute phase response enhances host resistance, minimising tissue injury and promoting repair.

### 2.4.4 Specific acquired immunity – Antibody

Antibodies are an adaptor molecule capable of stimulating the phagocytic cells and sticking to an invading microbe. Antibodies have three main regions, two concerned with communicating with phagocytes and one devoted to binding to a microorganism. Each antibody has a recognition portion complementary in shape to some microorganism to which it can then bind firmly to. The molecules in the microorganisms which evoke and react with antibodies are called antigens. Each lymphocyte
of a subset from B-lymphocytes, is programmed to make one antibody on its outer surface to act as a receptor. When an antigen enters the body it binds to the receptor that it fits best. Lymphocytes, whose receptors have bound to an antigen receive a triggering signal and develop antibody-forming plasma cells.

When the body makes an antibody response to a given infectious agent, that microorganism must exist in the environment and is likely to come into contact again. On first contact with an antigen the information received imparts some memory so that the body is prepared to repel any later invasion by that same organism. Upon secondary contact with the antigen the response is characterised by a more rapid and more abundant production of antibody resulting from the priming of the antibody-forming system.

2.5 Mucosal immunity

The mucous membranes covering the aerodigestive and urogenital tracts as well as the eye conjunctiva, the inner ear and the ducts of all exocrine glands are endowed with powerful mechanisms that degrade and repel most foreign matter (Holmgren and Czerkinsky, 2005). In addition, a large and specialised innate and adaptive mucosal immune system protects these surfaces, and thereby also the body interior, against potential insults from the environment. In a healthy human adult this local immune system contributes to almost 80% of all immunocytes (Holmgren and Czerkinsky, 2005). The adaptive immune defense at mucosal surfaces is to a large extent mediated by secretory IgA antibodies, the predominant immunoglobulin in human external secretions. The resistance of secretory IgA to proteases makes these antibodies uniquely suited for functioning in mucosal secretions (Holmgren and Czerkinsky, 2005). Although secretory IgA is the
predominant mucosal defense mechanism, locally produced IgM and IgG can also contribute significantly to immune defense (Holmgren and Czerkinsky, 2005).

The production of natural antimicrobial peptides (AMP) has emerged as an important mechanism of innate immunity in plants and animals. Antimicrobial peptides are polypeptides of fewer than 100 amino acids that are found in host defense settings (Ganz, 2003). Cationic antimicrobial peptides, such as defensins, cathelicidins and thrombocidins, are an important human defense mechanism, protecting skin and epithelia against invading microorganisms and assisting neutrophils and platelets (Peschel, 2002). Individual members of these AMP families have been implicated in AMP activity of phagocytes, inflammatory body fluids and epithelial body secretions. Defensins are diverse members of a large family of AMPs, contributing to the antimicrobial action of granulocytes, mucosal host defense in the small intestine and epithelial host defense in the skin and elsewhere (Ganz et al. 1985; Selsted et al. 1985; Ganz, 2003). Cathelicidins are structurally and evolutionarily distinct AMPs, similar to defensins in abundance and distribution (Zanetti et al. 1995; Lehrer and Ganz, 2002). Defensins however, are particularly prominent in humans, as evidenced by the large number of expressed human genes, the various forms that are present in human tissues, and the ubiquitous occurrence of defensins in inflamed or infected human tissues (Ganz, 2003).

Saliva is essential for the maintenance of oral health (Mandel, 1989). Saliva flow and composition function in lubrication, remineralisation, buffering and digestion, while also possessing anti-viral and anti-bacterial properties (Tenovuo, 1998; West et al. 2006). Saliva is the first line of defense against pathogens invading the oral cavity (Gleeson, 2000, Table 2.3). The importance of mucosal secretions cannot be underestimated given that 95% of all infections are initiated at mucosal surfaces (Bosch et al. 2002). Saliva provides a mechanical washing effect to protect the oral mucosa whilst mucosal secretions (e.g. immunoglobulins, mucins, amylase, lactoferrin) prevent viral
replication and bacterial attachment to the mucosal surfaces (McNabb et al. 1981; Mackinnon and Hooper, 1994; Dowd, 1999).

2.6 Immune system in cold and hypoxia

2.6.1 Cold and immune system

Folklore suggests that exposure to cold ambient temperatures causes humans to contract colds (rhinoviruses) or other URTI (Dowling et al. 1958; Biggar et al. 1984; Castellani et al. 2002). However, data to support a link between cold ambient temperatures, reduced immune function and increased susceptibility to infections in humans is not well delineated (Castellani et al. 2002; Lavoy et al. 2011). On the contrary, URTI are the most common infections worldwide with peaks often observed in cold winter months (Mourtzoukou and Falagas, 2007). Furthermore, URTI substantially increase wintertime morbidity (Hajat et al. 2004) and account for at least 20% of the excess winter mortality (The Eurowinter Group, 1997; Diaz et al. 2005; Nayha, 2005). Inhalation of cold air and cooling of the body surface have both been shown to cause pathophysiological responses that may contribute to increased susceptibility to URTI (Mourtzoukou and Falagas, 2007; Giesbrecht, 1995; Eccles, 2002; Cruz et al. 2006). Albeit, the interaction of exercise and cold exposure on immune function has not been widely studied (Castellani et al. 2002), data have shown cross country skiers commonly contract URTIs during outdoor training periods (Bergland and Hemmingsson, 1990). Secondly, soldiers completing sustained military operations in the Canadian Arctic have displayed an increased incidence and severity of URTI (Sabiston and Livingstone, 1973). It remains unclear however, if increased reports of URTI during prolonged cold exposure are due to high levels of energy expenditure, negative energy balance, cold exposure per se or to a combination (Walsh and Whitham, 2006).
Cold exposure has been found to increase plasma nor-adrenaline concentration, a marker of sympathetic nervous system (SNS) activity (Castellani et al. 2001; Castellani et al. 1999; Castellani et al. 1998). Since the SNS mediates immune function, exposure to cold may indirectly evoke an immune response (Castellani et al. 2001). A change in plasma cortisol has demonstrated modulations in immune function (Shephard, 1997) but at present the effects of cold exposure on plasma cortisol concentrations remain unclear (Frank et al. 1997; Wilkerson et al. 1974; Wittert et al. 1992). Nor-adrenaline and natural killer (NK) cell activity have been shown to increase in men following 30 minutes exposure to 4°C (Lackovic et al. 1988, Table 2.3). However, cell proliferation decreased after mitogen stimulation in almost identical conditions (Jurankova et al. 1995, Table 2.3). Acute cold water immersion (14°C) demonstrated increased white blood cell counts (Jansky et al. 1996) and a cold acclimation period with six weeks of cold water immersion, three times per week increased the percentage of CD14+ and CD25+ cells. Given cold exposure activates various cellular immune markers, a direct causal relationship to illness or infection remains unclear. Furthermore, since URS (i.e. persistent dry cough and nasal drying), with the absence of infection, have been reported following increased ventilation in cold dry air (Giesbrecht, 1995), further studies investigating mucosal immunity during cold exposure may provide some clarity.

In clinical environments, patients undergoing general anesthetic often experience mild hypothermia occurring from a reduced ability to produce heat via shivering. This perioperative model of hypothermia can also be related to a hypothermic casualty in the field who, through additional trauma, may also be unable to shiver. Perioperative hypothermia is associated with disturbances in the cardiovascular and respiratory systems, abnormalities in blood coagulation and platelet function, increased protein catabolism and urinary nitrogen loss following surgery (Forstot, 1995). Consequently, investigators have reported that hypothermia may delay wound healing and predispose patients to wound infections (Kurz et al. 1996).
In laboratory animals, exposure to hypothermia has been found to enhance the sensitivity of animals to bacterial infections as compared with normothermic animals (Sheffield et al. 1994). Similarly, in patients undergoing surgery, mild hypothermia impaired neutrophil oxidative killing during the intraoperative period (Wenisch et al. 1996). Suppression of immune defense mechanisms occurs in the postoperative period as a result of surgery stress and anesthesia (Salo, 1992). Such immune compromise could affect the postoperative infection rate, healing reaction, and the rate and size of tumor metastases disseminated during surgery (Salo, 1992).

### 2.6.2 Hypoxia and immune system

Ascent to high altitude is associated with alterations in physiological and metabolic functions (Mazzeo et al. 1994; Mazzeo et al. 1995; Mazzeo et al. 2001). These adjustments to high altitude are a necessary attempt to maintain homeostasis in the presence of hypoxemia (Mazzeo, 2005). However, the sympathoadrenal responses associated with acute and chronic high altitude exposure can influence immune function which may increase the risk of illness and infection while individuals are a far distance from appropriate medical resources (Mazzeo et al. 1994; Mazzeo et al. 1995; Mazzeo et al. 2001; Pedersen et al. 1994). Short term hypoxia has been reported to induce similar immunosuppression upon the mobilisation of T-cells and natural killer cells (Klokker et al. 1993; Klokker et al. 1995) as that demonstrated during surgery (Lennard et al. 1985) and exercise (Pedersen et al. 1990). Neutrophil concentration and function changes and the regulation of cytokine production and release have also been shown to be reduced in hypoxia (Pyne et al. 2000; Pedersen and Steensburg, 2002).

On the contrary, research remains equivocal regarding the effect of hypoxia on the immune system. For example, a study examining plasma concentrations of IgG, IgA and IgM were found either
unchanged or increased following 40 days between 3200m and 3800m and after two years at an altitude of 3700m (Meehan, 1987). An earlier investigation comparing the effects of chronic hypoxia on antibody production of natives from three different altitudes (3051, 3700 and 4680m) and natives from lower land (150m) (Lopez et al. 1975) found no significant differences between serum concentrations of IgG, IgA and IgM, albeit there was a tendency for greater IgA values in the natives from the highest altitude.

2.6.3 Hypoxia and cold and immune system

Given the limited research regarding the immune response to hypoxia, even less information is available highlighting the immune response to hypoxia in the cold. Nonetheless, it has been reported that AMS and HAPE are notably more common in mountain climbers during seasons of lowest ambient temperatures (Giesbrecht, 1995). Furthermore, it is believed that travelling to regions of high altitude and cold temperatures increases susceptibility to URTI (Walsh and Whitham, 2006). However, the evidence to support this is lacking and the problem is confounded by possible misdiagnosis due to some overlap in the symptoms of AMS and URTI (Bailey et al. 2003). On the contrary, it was reported that soldiers stationed at an altitude of 3692m had a higher prevalence of pneumonia compared with troops stationed at low altitude (Singh et al. 1977). Anecdotal reports and specific case studies also document an increase in URTI symptoms when ascending to altitudes >4000m (Basnyat et al. 2001; Oliver et al. 2012).
2.7 Mucosal immunity in cold and hypoxia

2.7.1 Cold and mucosal immunity

Stress induced immune perturbations have been attributed to the stimulation of the sympatho-adrenal-medullary and hypothalamic-pituitary adrenal axis which cause an increase in circulating stress hormones (catecholamines and cortisol). An influx of these hormones initiates a subsequent increase in secretory IgA production (Glesson, 2007). Cold stress is associated with an increased incidence of bronchorrhea, sinusitis, and other URTI (Clothier, 1974; Giesbrecht, 1995). Increased ventilation in very cold dry air, due to exercise or increased shivering activity, can cause a persistent dry cough and nasal drying (Giesbrecht, 1995). Human mucociliary clearance is slowed on exposure to cold air (Proctor, 1982). This is because the decrease in temperature is likely to reduce both ciliary beat frequency and the rate of mucus secretion (Proctor, 1982). A large decrease in temperature is also likely to cause an increase in the viscosity of respiratory mucus. Following a cross country ski race, a decrease in saliva immunoglobulin-A (s-IgA) concentration was associated with a large inflow of cold air that lowered the temperature of the mucous membranes (Tomasi et al. 1982). On the contrary, exercise in the cold has also been found to have no effect on s-IgA concentration (Housh et al. 1991). However, s-IgA was only reported as a concentration and not secretion rate, thus ignoring the drying effect of heavy breathing during cold exposure and exercise. Nonetheless, performing prolonged exercise in freezing cold conditions did not influence saliva flow rate or s-IgA secretion rate responses (Walsh et al. 2002, Table 2.3). Given research has investigated mucosal immunity during exercise in the cold, surprisingly little is known regarding the effects of sedentary activity in the cold upon the mucosal immunity of shivering, cold casualties. This seems pertinent since heat production arising from shivering activity and subsequent hyperventilation is similar to that during exercise. Furthermore, since heat production during
sedentary activity is eventually outweighed by heat loss, corresponding with a reduction in $T_c$ could evoke an even larger mucosal immune response.

### 2.7.2 Hypoxia and mucosal immunity

Mucosal immunity is suggested to be unchanged by hypoxia, and any reduced IgA and lysozyme levels may be caused by the combined stresses of cold, isolation and reduced humidity (Meehan et al. 1988; Muchmore et al. 1981). Secondly, given physical activity is often undertaken at altitude may also inadvertently affect mucosal immunity. For example, individuals enrolled in a ‘live high, train low’ training camp demonstrated a decline in $s$-IgA levels, which was suggestive of a cumulative negative effect of physical exercise and hypoxia on $s$-IgA (Tiollier et al. 2005, Table 2.3). On the contrary, epidemiologic data has provided indirect evidence that hypoxia may indeed impair immune competence in humans (Meehan et al. 1988). For example, a higher prevalence of pneumonia has been found among military troops stationed at high altitude (Singh et al. 1977) and an increase in infant mortality due to respiratory infections among high altitude natives has also been reported (Chohan et al. 1975, Table 2.3). Exposure to hypobaric hypoxia therefore, may serve as a stimulus to activate the neuroendocrine system and identify the mechanisms responsible for immune modulation during environmental stress in humans (Meehan et al. 1988).

### 2.7.3 Hypoxia and cold and mucosal immunity

Since URTIs are the main cause of illness and missed practise in elite cross country skiers (Berglund and Hemmingson, 1990) and cases of acute mountain sickness and high-altitude pulmonary edema (HAPE) are increased in mountaineers during cold seasons (Giesbrecht, 1995),
cold-induced decrements in immune-surveillance may be a potential problem for individuals at altitude. The additional stressor of cold in hypoxia-exposed humans may evoke a similar suppression on the mucosal immune system as that demonstrated with physical activity in the cold. At present, little attention has been directed toward the influence of hypoxia upon mucosal immunity in the cold. Whether the risk of illness or infection is exacerbated in un-acclimatised expeditioners upon arrival to hypoxic and cold environments remains unknown. Furthermore, it has not been clarified whether such detriments to mucosal immunity are reduced following a period of altitude acclimatisation. This information may have significant implications for spaceflight personnel during long term missions where conditions of low oxygen supply are likely (Cogoli, 1981; Taylor and Dardano, 1983).
2.8 Cold casualty re-warming and protection

The treatment of hypothermia should aim to minimise the post-exposure after-drop and promote a steady continuous rate of re-warming (Giesbrecht et al. 1997). ‘After-drop’ is the term given when removal from the cold induces a further reduction in $T_c$ (Giesbrecht and Bristow, 1992) as such re-warming should be continued until thermal, cardiorespiratory, and metabolic homeostasis can be maintained (Giesbrecht et al. 1997). One explanation for the after-drop phenomenon is proposed by the circulatory theory, which has two variations. The first states that upon re-warming, $T_c$ decreases because of the cold blood returning from vasoconstricted peripheral vessels (Hervey, 1973). However, it is unlikely that vasoconstriction during cooling would preferentially trap blood peripherally but rather force it back to the core, leaving minimal blood in these tissues (Giesbrecht and Bristow, 1992). The second variation is that vasodilation, upon re-warming, results in the flow of cold blood from the core to previously hypoperfused colder superficial and peripheral tissue, resulting in further cooling of the blood and subsequently the core (Burton and Edholm, 1955; Golden, 1973).

Since the movement of cold casualties has been demonstrated to increase after-drop following cold water immersion (Giesbrecht et al. 1987), after-drop rate may be related to the ambulation of subjects from a cold to warm environment. For example, core cooling has been measured at 1.2 – 1.3°C/h in subjects buried in snow and wearing only lightweight clothing insulation systems (Grissom et al. 2004). However, subjects extricated from snow burial after 60 minutes and taken to a warm environment, displayed a core temperature after-drop rate of 3.3°C/h (Grissom et al. 2008). The after-drop rate was more than double the core cooling rate, suggesting that movement is a significant factor of hypothermia severity when individuals are moved from a cold environment to a warm environment (Grissom et al. 2010).
Currently, most technical re-warming methods for the treatment of hypothermia require hospitalisation. However, immediate field methods for treating hypothermia occurring during military operations or outdoor recreation activities are essential when evacuation to hospitals may be difficult (Giesbrecht et al. 1987). Portable central airway re-warming devices have been developed for field use (Lloyd, 1973); however these can only be administered by fully qualified rescue teams. A paucity of information is available for simple, economical and practical re-warming methods that can be administered by a first responder in the field while awaiting evacuation to superior means (Giesbrecht et al. 1987).

2.8.1 Hospital based methods

The safety of external whole body re-warming by warm water immersion has created controversy within the literature. While warm water immersion donates large amounts of heat to promote a rapid rate of re-warming (Romet and Hoskin, 1988), it causes a large initial $T_c$ after-drop (Hayward et al. 1975) and increases the risk of fibrillation (Webb, 1986). This is because direct warming of the periphery promotes an increased inflow of cold blood to the heart which creates large core cooling. Thus, whole body re-warming should not be used with severely hypothermic casualties. By heating core organs first, (e.g. heating blood in an extracorporeal circuit or peritoneal lavage) after-drop can be avoided (Webb, 1986). In comparison to warm water re-warming, core temperature after-drop has been demonstrated to decrease by 30% when forced air warming is used to donate heat by the direct transfer of warm air onto the periphery of the body (Giesbrecht et al. 1994). On the contrary, studies investigating the re-warming capacity of inhaling warm, humidified air in mildly hypothermic subjects removed from the cold have found little (Hayward and Steinman, 1975; Romet and Hoskin, 1988) or no (Collis et al. 1977; Goheen et al. 1997) benefit compared to shivering thermogenesis alone. Given perioperative hypothermia may contribute to
immunosuppression during the postoperative period, the use of non-invasive re-warming devices with patients during surgery may help to reduce this.

2.8.2 Field methods

It is acknowledged that mildly hypothermic victims can be suitably re-warmed in the field if adequate insulation is provided (Pugh, 1964; Grissom et al. 2010). In the field, sources of heat are limited to the ingestion of warm liquid, body-to-body contact (Harnett et al. 1980; Giesbrecht et al. 1994), heating pads (Collis et al. 1977; Giesbrecht et al. 1987) and possibly inhalation of humidified air or oxygen (Giesbrecht, 2001). The charcoal burning ‘Heat Pac’ (Heat Pac, Oslo, Norway) provides a thermal advantage when metabolic heat production is minimal (Hultzer et al. 1999) and is probably the most efficient portable warming device available (Giesbrecht, 2001). It is small, light and produces approximately 250 W for up to 12 – 14 h. Although inhalation warming is often proposed as an effective strategy for body warming, or at least prevention of further body cooling (Weinberg, 1998), the advantage regarding thermal balance is minimal (Geisbrecht, 2001). In shivering subjects, no re-warming advantages were found when re-warming trials were conducted in 20°C (Romet and Hoskin, 1988), 2°C (Sterba, 1991) or –20°C air (Mekjavic and Eiken, 1995). When using a human model for severe hypothermia (shivering inhibited by pethidine in hypothermic subjects) (Giesbrecht et al. 1997) inhalation rewarming still did not provide any core rewarming advantage over spontaneous warming over 150 min of recovery (Goheen et al. 1997).

Early reports suggest body-to-body contact with a minimally clothed euthermic heat donor whilst in an insulated bag may be beneficial for the re-warming of hypothermic victims (Collis, 1976; Mills et al. 1987; Robinson, 1992). However, it has been demonstrated that heat donors blunt the recipients’ shivering thermogenesis and re-warming rates remain the same as that of shivering alone
(Giesbrecht et al. 1994, Table 2.4). Therefore, mildly hypothermic victims who are otherwise healthy and shivering normally should simply be removed from the cold stress to a dry insulated environment (i.e. a sleeping bag). Victims who are not shivering because of severe hypothermia, impaired thermoregulatory control, or depleted metabolic substrates should be evacuated to a medical facility immediately (Grissom et al. 2010). The absence of shivering has been reported to induce a three-fold increase of $T_c$ after-drop and a four-fold increase in the length of the after-drop period compared to when shivering is not inhibited (Giesbrecht et al. 1997). However, research is limited as to the effects of hypothermia on non-shivering casualties due to the ethical boundaries of injecting subjects with shivering depressants.

An alternative way to increase the body’s own heat production is through exercise. However, after-drop amount and length were found to be greater in cold individuals during an exercise re-warming protocol compared to shivering only or externally applied heat (Giesbrecht et al. 1987, Table 2.4). It is likely that before exercise, blood in the muscles is cooler than blood at the core; nonetheless, given that exercise increases circulation, blood at the core is readily cooled (Hudlicka, 1982; Giesbrecht et al. 1987). For this reason exercise should be avoided in an individual whose $T_c$ is unknown or less than 32°C and shows signs of physical and / or metabolic exhaustion, (i.e. incapable of shivering). Additionally, through injury, individuals may be forced to remain sedentary while awaiting rescue.

2.8.3 Single and multi-layer survival products

Metalized plastic sheeting (MPS) is a single layered, water and windproof material thought to benefit the human body by reflecting radiated heat lost from the body surface (Marcus et al. 1977, Table 2.4). Single-layer MPS products were originally favoured for their small (e.g. 9.5cm x 2.5cm
light-weight (e.g. 50g) appeal compared to heavier, bulkier casualty bags made of materials such as goose down (Light et al. 1980, Table 2.4). However, research to date is equivocal as to the thermal protection provided by MPS. For example, MPS and metal foil have been associated with a reduced heat loss in cold conditions (Spencer-Smith, 1977). On the other hand, MPS has been found to be inefficient at preventing heat loss during operative surgery (Radford and Thurlow, 1979), and was reported to provide no additional thermal protection to the periphery of cold exposed humans in comparison to polythene and nylon (Marcus et al. 1977).

Anecdotal reports suggest the light-weight structure and weak adhesive of MPS bags makes them insecure and easily torn. Yet, bulk and weight of a casualty bag is of direct importance to the individual who must survive in an isolated environment using only what they may have carried with them in a rucksack (Light et al. 1980). Single layer MPS may be light-weight but appears to offer little thermal protection in cold environments. However, placing a fibre pile liner inside a casualty bag incorporating MPS seems to evoke significantly smaller reductions in $T_{rec}$ and $T_{sk}$ compared with single layer MPS (Light et al. 1980). In this study, shivering was also noted to be eliminated in the MPS and fibre pile bag, indicating multi-layer products may offer thermal and metabolic advantages. Additionally, multi-layer survival products tested during a one hour cold exposure in $-10^\circ$C and average wind speed $3.0\text{m}\cdot\text{s}^{-1}$, were able to maintain $T_{sk}$ close to normothermia, while single layer MPS was not (Grant et al. 2002, Table 2.4). Despite this, participants reported similar increased perceptions of cold and displayed a rapid fall in $T_{rec}$. Each multi-layer system had a wind and water-proof nylon outer; one contained a fibre pile lining and the other incorporated an inner Duffel bag. Evidently multi-layer systems may better conserve heat via improved insulation in comparison to single-layer systems. However, while $T_c$ continues to decline rapidly in currently available survival products, alternative materials should be researched.
Albeit, multi-layer devices may provide initial re-warming treatment to a cold casualty, subsequent peripheral warming has been reported to evoke a secondary decline in $T_c$ (Grant et al. 2002). Peripheral vasoconstriction therefore, is likely to be limited in casualties wearing thick winter garments inside a survival bag because of a high heat transfer generated from the body’s core to the skin (Grant et al. 2002; Vokac and Hjeltnes, 1981). A temperature gradient between the skin and the air inside the bag may impair peripheral thermoreceptor activity (Grant et al. 2002). To protect ecological validity most research with survival products includes participants wearing typical outdoor clothes (Marcus et al. 1977; Light et al. 1980; Light and Norman, 1990; Grant et al. 2002). However, this could be causing a temperature gradient within the survival bag and thus contributing to the observed decline in $T_c$. At present, the maximal capacity to protect $T_c$ in survival bags remains unknown. Research should therefore examine multi-layer survival products while subjects are minimally clad.

A recent in vitro study comparing cooling times of pre-heated fluid bags protected with different field methods reported that a multi-layered survival bag (Blizzard Survival™) was superior at preventing core heat loss compared with a polythene survival bag, single layer MPS and wool blanket (Allen et al. 2010). Further, an active heating combination of the multi-layered metalized sheeting survival bag (Blizzard Reflexcell™) with chemical heat pads was shown to be superior to all other methods tested. Nonetheless, given that the exposure conditions did not reflect typical cold temperatures experienced during outdoor winter pursuits, it remains unknown how well these devices will protect non-shivering, sedentary humans in the cold.

Reflexcell™ has an elasticised and cellular construction to trap warm air and draw the material to the body. This reduces the number of cold spaces and heat loss by convection. The silvered surfaces of Reflexcell™ reduce heat loss by radiation and provide a wet and wind proof layer. Minimising heat loss by convection and radiation during cold exposure reduces the likelihood of hypothermia.
(Light and Norman, 1990). The Blizzard Survival™ bag, light-weight and easily compressed in to a video cassette package, is a full sized sleeping bag constructed entirely from Reflexcell™ material. The Blizzard Survival Heat™ comprises Reflexcell™ technology with self-activating heat pads which can also be vacuumed to the size of a video cassette. Self-activating heat pads eliminate the need for a power supply, and Velcro sides allow medical attention to be administered efficiently.

Thermal efficiency of sleeping bag materials is generally expressed as a warmth-to-weight ratio by dividing the insulating value of the material (Togs) by the weight of a typical sleeping bag made from that material. The basic unit of insulation coefficient is the RSI (1m²k/watt). One tog is equal to 0.1 RSI. This means the thermal resistance in togs is equal to ten times the temperature difference (in °C) between the two surfaces of a material, when the flow of heat is equal to one watt per square metre. University laboratory tests using a Tog rating of 8 Togs and a weight of 330 g for the Reflexcell™ bag found a value of 24 Togs/kg. This is over twice the value for goose down, which was previously regarded as having the highest possible warmth-to-weight ratio among insulating materials. Further investigation of Reflexcell™ with human participants is required to test the thermal capacity provided by this new technology.
CHAPTER THREE

General methods

3.1 Ethical approval

Approval for all studies was obtained from the local Ethics Committee (School of Sport, Health and Exercise Sciences, Bangor University). The nature and purpose of each study was explained both verbally and in writing to each volunteer (Appendix A). Each participant was made aware that they were free to withdraw from the study at any time and completed an informed consent form (Appendix B). All volunteers were non-smokers and had no significant oral, dental, or systemic disease and were not taking any medication at the time of participating in the studies. To determine eligibility for inclusion into a research study, participants completed a medical and health questionnaire (Appendix C) and reported no incidences of illness in the six weeks prior to beginning the study.

3.2 Anthropometry and body composition

Height was recorded using a wall stadiometer (Bodycare Ltd, Warwickshire, UK) and NBM by digital platform scales accurate to the nearest 50 g (Model 705, Seca, Hamburg, Germany). Whole body skeletal muscle mass, appendicular lean mass, and fat free mass were assessed using segmental multiple frequency bioelectrical impedance (InBody 230, Biospace, Seoul, 3 Korea). For this procedure participants removed shoes and socks and dressed in t-shirt and shorts only. Validity and reliability of this technique to assess regional and whole body composition has been shown previously (Malavolti et al. 2003; Jensky-Squires et al. 2008).
3.3 Experimental procedures

The day prior to each experimental trial participants ingested water equal to at least 35 ml·kg\(^{-1}\) body mass and a prescribed diet. Participants arrived to the laboratory euhydrated. This was verified by urine specific gravity equal to or less than 1.020 g·ml\(^{-1}\) (Casa et al. 2005) and urine osmolality no greater than 800 mOsm/kg (Urine Osmolality Meter PAL-mOsm). Hydration was also inspected against a urine colour chart with hydration accepted as a level 3 or less (Armstrong, 2000).

3.4 Thermoregulatory measures

Core temperature (T\(_{rec}\)) was recorded using a flexible thermistor inserted 12 cm beyond the anal sphincter (YSI 4000A, Daytona, FL, Chapter 4 and 5) (2020 series, Squirrel data logger, Grant) (Chapter 6 and 7). Measurements of core temperature by flexible thermistor was considered the least invasive yet most accurate method. For example tympanic temperature is not as accurate as measurements taken from the rectum, yet eosophageal measurements would have caused a degree of discomfort for participants and they would have been unable to consume fluids (Chapter 6 and 7). Gastrointestinal telemetry pills would have been affected by the ingestion of hot fluids (Chapter 6). Skin temperature was recorded at 8 sites using either iButton\textsuperscript{®} technology (Maxim Integrated Products, Inc., Sunnyvale, CA, USA) (Chapter 4 and 5) or skin thermistors (2020 series, Squirrel data logger, Grant) (Chapter 6 and 7). Weighted mean skin temperature was calculated using an area-weighted formula (ISO-standard 9886, 2004) (eq. 1) (Chapters 4, 5, 6 and 7). Metabolic heat production (M) was assessed and calculated via indirect calorimetry methods (Servomex 1420B, Crowborough, UK and Harvard Apparatus, Edenbridge, UK) (eq. 2) (Chapters 4, 5, 6 and 7). Thermal comfort was measured using the McGinnis Scale of Thermal Comfort (Hollies and Goldman, 1977) (Chapters 4, 5, 6 and 7).
3.5 Sample collection and analysis

Saliva and urine samples were collected at least 15 min after fluid consumption and at least 1 h after the participants had eaten. In addition, saliva samples were obtained after participants had remained seated for 10 min.

3.5.1 Saliva

Each participant was asked to thoroughly rinse their mouth with water and swallow residual before saliva collection of un-stimulated whole saliva samples into a pre-weighed universal container (HR 120-EC, A & D instruments, Tokyo, Japan) (Chapters 5 and 7). All saliva samples were collected for 5 minutes while the participant sat quietly, leant forward and passively drooled with minimal orofacial movements. Saliva volume was estimated by weighing the universal container immediately after collection to the nearest mg and saliva density was assumed to be 1.00 g·ml⁻¹ (Cole and Eastoe, 1988). From this, saliva flow rate was determined by dividing the volume of saliva by the collection time. Saliva was then aspirated into eppendorfs and stored at -40°C prior to analysis.

Saliva IgA concentration was determined by ELISA (Salimetrics Europe Ltd, Suffolk, UK) using an IgA monomer derived from human serum as standard. During the ELISA a constant amount of goat anti-human s-IgA conjugated to horseradish peroxidase was added to tubes containing specific dilutions of standards or saliva. The antibody-conjugate was bound to the s-IgA in the standard or saliva samples. The amount of free antibody remaining was inversely proportional to the amount of s-IgA present. After incubation and mixing, an equal solution from each tube was added, in duplicate, to the microtitre plate coated with human s-IgA. The free or unbound antibody conjugate bound to the s-IgA on the plate. After further incubation, unbound components were washed away.
Bound conjugate was measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine. This reaction produced a blue colour, followed by yellow once the reaction had been chemically stopped. Optical density was read on a standard plate reader at 450 nm. The amount of peroxidase was inversely proportional to the amount of s-IgA present in the sample (Chard, 1990). The intra-assay CV was 1.4% and 2.6% for chapters 5 and 7 respectively. The s-IgA secretion rate was calculated by multiplying the saliva flow rate by s-IgA concentration.

The alpha amylase ELISA method (Salimetrics Europe Ltd, Suffolk, UK) utilized a chromagenic substrate, 2-chloro-p-nitrophenol linked with maltotriose (Wallenfels et al. 1978). The enzymatic action of alpha amylase on this substrate yielded 2-chloro-p-nitrophenol were spectrophotometrically measured at 405 nm. The amount of alpha amylase activity present in the sample was directly proportional to the increase in absorbance at 405 nm. For ease of use, the reaction was read in a 96-well microtiter plate with controls provided. The intra-assay CV was 2.5% and 2.1% for chapters 5 and 7 respectively.

3.5.2 Urine

First morning urine samples were collected in full and determined for volume, urine specific gravity (Atago Uricon-NE; NSG Precision Cells, Farmingdale, NY, USA) and osmalality. Urine colour was determined by a urine colour scale (Armstrong et al. 1994). Provided participants fulfilled the hydrated criteria they were able to take part in the studies. Urine was aspirated into eppendorfs, frozen at -40°C and later thawed for analysis of urinary urea nitrogen content (Chapter 6: Randox Laboratories, Co. Antrim, UK).
3.6 Calculations

Mean skin temperature (T_{sk}) area-weighted 8-site equation, adjusted for regional proportions (ISO-standard 9886, 2004):

\[
\text{Mean } T_{sk} = (0.07 \cdot T_{\text{forehead}}) + (0.175 \cdot T_{\text{right scapula}}) + (0.175 \cdot T_{\text{left upper chest}}) + (0.07 \cdot T_{\text{right arm in upper location}}) + (0.07 \cdot T_{\text{left arm in lower location}}) + (0.05 \cdot T_{\text{left hand}}) + (0.19 \cdot T_{\text{right anterior thigh}}) + (0.2 \cdot T_{\text{left calf}}). \tag{eq. 1}
\]

From expired gas samples, values for the respiratory exchange ratio (RER) and VO\textsubscript{2} were obtained and calculated with body surface area (A\textsubscript{D}) to estimate metabolic heat production (M) in W·m\textsuperscript{-2} using the following equation (Gagge and Gonzalez, 1996):

\[
M = [0.23(\text{RER}) + 0.77] \cdot (5.873) \cdot (\text{VO}_2) \cdot (60/ A_{D}) \tag{eq. 2}
\]

3.7 Statistical analysis

Statistical analyses were completed using Statistical Package for Social Sciences (SPSS Version 14). Data were examined using repeated measures ANOVA. Where assumptions of sphericity and normality were violated appropriate adjustments to the degrees of freedom were made. Effect sizes (\eta\textsuperscript{2}) were determined for main outcome measures and were interpreted as small (0.010), medium (0.060) and large (0.140) (Cohen, 1988). Significance was accepted at \( P < 0.05 \). Where significance occurred Post Hoc Tukey’s HSD was used. All data are presented as Mean ± standard deviation (SD). Sample sizes were estimated using similar data from previous investigations (http://www.dssresearch.com/toolkit/sscalc). Alpha and power levels were set at 0.05 and 80% respectively.
CHAPTER FOUR

Thermal Comfort and Thermoregulatory Responses to Cold Exposure in Hypoxia before and after an Altitude Stay

4.1 Abstract

The purpose of this study was to determine thermal comfort and thermoregulation in relation to potential cold injury during hypoxic and mild cold exposure before and after an 18-day mountaineering expedition. In short, ten males completed 2-hour cold air tests (CAT 15°C, RH 44%) in an environmental chamber in a supine position. Three CATs were performed: 1. Sea level (SL); 2. Un-acclimatized in normobaric hypoxia (UN: ~4000m); 3. Post expedition in normobaric hypoxia (PEX: ~4000m). During the CAT core and mean skin temperature were not different between SL, UN and PEX. However, compared with SL, metabolic heat production was lower during hypoxic exposures (SL 93 ± 36, UN 68 ± 25, PEX 65 ± 25 W·m⁻²; P < 0.001: mean ± SD). Observed shivering activity was also lower after the expedition, which suggests a different contribution of non-shivering and shivering thermogenesis (SL 1.1 ± 0.1, UN 1.0 ± 0.2 and PEX 0.6 ± 0.1; P = 0.002). Thermal comfort was increased PEX compared with SL and UN (SL 5.0 ± 1.4, UN 5.6 ± 1.1, PEX 6.1 ± 0.8; P = 0.004). Following an 18-day mountaineering expedition a lack of change in core and skin temperature despite a decrease in shivering is consistent with a greater reliance on non-shivering thermogenesis. The alterations observed post expedition in thermal comfort suggest a greater thermal tolerance but an increase in susceptibility to cold injury in hypoxia.
4.2 Introduction

High altitude environments may alter perceptual and thermoregulatory responses to the cold that predispose individuals to greater cold injury (Blatteis and Lutherer, 1976; Cipriano and Goldman, 1975; Golja et al. 2004; Johnston et al. 1996; Savourey et al. 1997). Indeed epidemiological studies suggest cold injury rates increase at high altitude (Hashmi et al. 1998; Harirchi et al. 2005). Research studying the effect of hypoxia on thermoregulatory responses to cold is however equivocal.

Compared with cold alone exposure to cold and hypoxia is associated with a decreased thermal sensitivity (Golja et al. 2004), lower core temperature and higher skin temperature (Cipriano and Goldman, 1975; Kottke et al. 1948) which may increase predisposition to cold injury. Nonetheless, some studies have reported no change in thermal comfort (Golja et al. 2005), core temperature (Blatteis and Lutherer, 1976; Robinson and Haymes, 1990) or skin temperature (Brown et al. 1952). An earlier onset of shivering with increased intensity is a common observation in men exposed to hypoxia and cold (Blatteis and Lutherer, 1976; Bullard, 1961; Robinson and Haymes, 1990). This may result from reduced non-shivering thermogenesis (NST), demonstrated by a reduced cold-induced increase in VO₂ (Blatteis and Lutherer, 1976). Hypoxic-induced suppression of NST has been explained by an inhibition of the aerobic catabolism of body fat stores (Robinson and Haymes, 1990). However, patterns of fuel selection during hypoxia in the cold remain to be clarified.

An altered perception of cold sensation has been reported at altitude (Golja et al. 2004) and has been suggested to be related to a lowered neural activity and delayed signal conduction (Astrup, 1982). A diminished cold sensation is likely to predispose individuals to cold injury because they neglect basic behavioural thermoregulatory mechanisms (e.g. putting on extra layers and seeking shelter). However, following acclimation by intermittent exposure to hypoxia, cold and warm sensitivities of the hand were unchanged compared with baseline measures (Malanda et al. 2008).
At present, local and whole body sensations to cold have not been simultaneously measured. In addition to hypoxic-induced alterations in cold sensation, anecdotal reports suggest hypoxia also alters thermal comfort. Early reports describe the pain to single limb cold water immersion (Mathew *et al.* 1979) and thermal discomfort to whole body mild cold air exposure (Blatteis and Lutherer, 1976) to be greater at altitude than at sea level. These same anecdotal reports suggest that the increased pain and discomfort did not abate with altitude acclimatisation (Mathew *et al.* 1979; Blatteis and Lutherer, 1976). Greater pain to single limb cold water immersion has recently been confirmed by a more systematic investigation (Daanen and van Ruiten, 2000). However, the effect of whole body cold exposure during acute hypoxia and following a high altitude stay on perception of thermal comfort has yet to be explored.

Demonstrated by the maintenance of a higher mean body temperature and mean skin temperature during acute cold stress, hypoxic acclimatisation has been shown to induce greater cold tolerance (Blatties and Lutherer, 1976; Mathew *et al.* 1979). Studies also suggest altitude acclimatisation may result in NST recovery (Blatteis and Lutherer, 1976; Mathew *et al.* 1979). The amount of recovery however remains unclear and is complicated by the methods of data collection used (Blatteis and Lutherer, 1976; Cipriano and Goldman, 1975; Mathew *et al.*, 1979). For example, sample sizes in these studies were small and methods to determine shivering activity were not valid. It is plausible to suggest that while un-acclimatised exposure to hypoxia may inhibit the catabolism of fat stores and thus reduce NST, the same might not be true following acclimatisation. Research has examined thermoregulatory responses after acclimatisation during cold exposure at sea level (Blatteis and Lutherer, 1976; Savourey *et al.* 1997), yet no studies have assessed such responses during hypoxia in the cold following a mountaineering expedition.

The effect of hypoxia in the cold on thermoregulatory mechanisms of un-acclimatised men is equivocal. It is unknown whether acclimatisation, resulting from mountaineering activity at altitude, affects thermoregulatory, metabolic and perceptual responses to hypoxia in the cold. The aim of the
present study therefore, was to examine these responses in un-acclimatised and acclimatised individuals after an 18-day Alpine expedition. It was hypothesised that un-acclimatised exposure to hypoxia and cold would impair thermoregulatory responses compared to those reported in normoxia. Secondly, it was hypothesised that acclimatisation due to the expedition would restore the responses toward those observed in normoxic cold exposure.
4.3 Method

4.3.1 Participants
Ten healthy males (mean ± SD: age, 21.7 ± 2.4 years; height, 181.7 ± 5.1 cm; body mass, 76.4 ± 9.9 kg and body fat, 11.6 ± 6.6 %), accustomed to multi-day expedition-type activities were recruited from an 18-day Alpine expedition (Figure 4.1). Participants gave written consent and medical history before the study that had received local Ethics Committee approval.

4.3.2 Study design
Participants completed three experimental trials in a normobaric, temperature and humidity controlled chamber (Dry bulb temperature of the chamber remained at 15°C with a relative humidity (RH) 40% and 0.2 m.s\(^{-1}\) wind velocity; Delta Environmental Systems, Chester, UK) over a 12 week period. Trials were 1. sea-level (SL: F\(_{\text{IO}} \text{O}_2 = 0.209\)); 2. Un-acclimatised in normobaric hypoxia (UN: F\(_{\text{IO}} \text{O}_2 = 0.125\), ~4000m); and 3. post expedition acclimatised in normobaric hypoxia (PEX: F\(_{\text{IO}} \text{O}_2 = 0.125\), ~4000m). To avoid an order effect SL and UN were performed before the expedition in half of the participants and 2 months after the expedition in the remainder. During PEX the mean altitude gained on a daily basis was 2989 ± 867 m.

4.3.3 Experimental procedures
Having consumed no food within 4 hours, participants arrived to the laboratory euhydrated (Chapter 3). After voiding bladder and bowels, anthropometric measures of height and body mass were obtained. Body composition was analysed by segmental multiple-frequency bioelectrical impedance (InBody 230, Biospace, Seoul, S Korea: Malavoliti et al. 2003; Jensky-Squires et al. 2008). Daily energy requirements were determined by body composition and a mean of daily
metabolic rate, hourly basal metabolic rate, eight hours sleep, eight hours of occupational activities (e.g. moving, sitting and standing) and eight hours of residual time (WHO, 2004).

4.3.4 Experimental Trials

Participants completed each trial at the same time of day. Following pre-trial measures, participants began a 10 minute supine rest in a thermo-neutral environment (ambient temperature: 21.3 ± 1.1°C; RH: 52 ± 5.4%). Resting values of core (T_{rec}) and skin (T_{sk}) temperature were recorded. Metabolic heat production (M), thermal comfort, peripheral and central coldness (Borg CR100) (Borg and Borg, 2001), heart rate (HR) (Polar, Electro Kempele, Finland), and arterial oxygen saturation (S_{a}O_{2}% by finger pulse oximetry (Supermon 7210, Kontron, Watford, UK), were also recorded.

Following baseline measures and wearing shorts only, participants entered the chamber and rested supine upon roll mats situated on the floor for 150 minutes. Core temperature, M, S_{a}O_{2}%, HR, whole body thermal comfort and peripheral and central coldness were recorded every 5 minutes. Skin temperature was recorded every minute. To detect potential CIVD, toenail and fingernail bed temperatures were measured with i-buttons taped to the large toe and index finger of the right hand and foot. During the initial 30 minutes, participants were covered with a standard duvet (4.5 togs). This thermo-neutral condition (van Ooijen et al. 2005) ended and the 120 minute CAT began when the duvet was removed. Thermo-neutral conditions were necessary in order to establish any change between non-shivering thermogenesis and shivering thermogenesis.

Electromyograms (AD instruments Powerlab) of the pectoralis major determined shivering activity. Pilot work demonstrated this muscle to provide accurate traces with little background noise compared to abdominal or quadriceps muscles. In addition, acetate traces were taken of each participant’s torso to ensure exact placement of EMG leads during each trial. EMG data was logged every minute and measured in micro-volts. Shivering was also rated using a 4 point scale by an
observer *(Appendix F)* (Blatteis and Lutherer, 1976). Training was given to a maximum of two researchers in order to maintain consistent observational practice. Participants remained in the environmental chamber for 150 minutes unless: 1. $T_{recl}$ decreased to 36°C or fell 1.5°C below resting, 2. Lake Louise scores went above 1 to highlight the presence of headache (hypoxic trials only) or 3. participants chose to withdraw. Upon completion participants were re-warmed and monitored until core temperature was within 0.5°C of resting.

![Figure 4.1](image.png)

**Figure 4.1.** Mean daily altitude of participants during an Alpine expedition.

### 4.3.5 Statistical analyses

A sample size of nine was estimated using rectal temperature data from a previous investigation (Blatteis and Lutherer, 1976). To allow for incompletion, ten subjects were recruited. A one-way fully repeated-measures ANOVA was performed on body mass and composition data and urine osmolality. Two-way fully repeated-measures ANOVA (time x trial) were performed on thermoregulatory, metabolic and EMG data. Data was normally distributed.
4.4 Results

4.4.1 Environmental chamber conditions, hydration status, body mass and composition

Chamber temperature (SL: 15.0 ± 0.1; UN: 15.2 ± 0.2; PEX: 15.2 ± 0.2°C, F_{2, 10} = 1.168, P = 0.350) and relative humidity (SL: 46 ± 4; UN: 43 ± 8; PEX: 39 ± 4%, F_{2, 10} = 0.292, P = 0.753) were not different between trials. Urine osmolality indicated hydration status was similar before each trial (SL: 364 ± 129, UN: 366 ± 129, PEX: 382 ± 202 mOsm·Kg\(^{-1}\), P = 0.670). Body fat and mass were unchanged before and after the expedition (SL: 11.6 ± 6.6; PEX: 11.5 ± 6.3 %, P = 0.745; SL: 75 ± 10; PEX: 76 ± 10 Kg, P = 0.786 respectively).

4.4.2 Core and skin temperature responses

Cold exposure caused T\(_{rec}\) to decline similarly during all CATs (F_{12, 108} = 4.456, P = 0.000, η\(^2\) = 0.326) (Figure 4.2). Cold exposure reduced mean T\(_{sk}\) in all trials (F_{12, 108} = 170.084, P = 0.000, η\(^2\) = 0.942) but was also not influenced by hypoxia (F_{2, 18} = 1.450, P = 0.261, η\(^2\) = 0.136) (Figure 4.3). Skin temperature at the forehead was lower throughout SL compared to UN and PEX (F_{2, 18} = 9.875, P = 0.001, η\(^2\) = 0.521) (Table 4.1). Skin temperature at the left chest was also lower during SL compared to PEX following 10 minutes of cold exposure (F_{1.291, 11.618} = 4.743, P = 0.043, η\(^2\) = 0.324). No differences occurred for any other individual skin sites (Table 4.1).

Temperatures at the fingernail bed were the same in each trial (F_{2, 18} = 0.033, P = 0.967, η\(^2\) = 0.004) (Table 4.2). During the initial exposure, temperatures at the toenail bed were also the same (F_{2, 18} = 1.324, P = 0.291, η\(^2\) = 0.101). However, following 60 minutes, toenail bed temperature was lower during UN compared to SL (F_{2, 18} = 3.793, P = 0.042, η\(^2\) = 0.288) (Table 4.2). An interaction established cold exposure evoked a cooler toenail bed temperature in unacclimatised individuals (F_{24, 216} = 6.882, P = 0.000, η\(^2\) = 0.356).
**Figure 4.2.** The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level (♦) and at 4000 m before (■) and after (△) acclimatisation on $T_{\text{rec}}$ (°C). Values are mean ± SD. *significant difference vs. 0 mins ($P < 0.05$).

**Figure 4.3.** The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level (♦) and at 4000 m before (■) and after (△) acclimatisation on mean skin temperature (°C). Values are mean ± SD. *significant difference vs. 0 mins ($P < 0.05$).
Table 4.1. The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level (SL) and at 4000 m before (UN) and after (PEX) acclimatisation on skin temperature (°C).

<table>
<thead>
<tr>
<th>Skin temperature at individual sites (°C)</th>
<th>SL</th>
<th>UN</th>
<th>PEX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Forehead</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>30.3</td>
<td>32.0†</td>
<td>32.4†</td>
</tr>
<tr>
<td>60</td>
<td>28.7*</td>
<td>30.4*†</td>
<td>31.0*†</td>
</tr>
<tr>
<td>120</td>
<td>28.6*</td>
<td>30.3*†</td>
<td>30.8*†</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>28.9 ± 2.3</td>
<td>30.7 ± 2.0</td>
<td>31.1 ± 1.1</td>
</tr>
<tr>
<td><strong>Right Scapula</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>34.0</td>
<td>34.7</td>
<td>34.0</td>
</tr>
<tr>
<td>60</td>
<td>34.5</td>
<td>35.1</td>
<td>34.7</td>
</tr>
<tr>
<td>120</td>
<td>34.4</td>
<td>34.4</td>
<td>34.1</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>34.5 ± 2.2</td>
<td>35.1 ± 0.7</td>
<td>34.4 ± 1.0</td>
</tr>
<tr>
<td><strong>Left chest</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>32.9</td>
<td>32.6</td>
<td>32.8</td>
</tr>
<tr>
<td>60</td>
<td>27.2*</td>
<td>28.2*</td>
<td>29.1*</td>
</tr>
<tr>
<td>120</td>
<td>27.5*</td>
<td>28.4*</td>
<td>29.5*</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>27.9 ± 2.8</td>
<td>28.7 ± 3.1</td>
<td>29.7 ± 1.8†</td>
</tr>
<tr>
<td><strong>Right upper arm</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>32.7</td>
<td>33.5</td>
<td>32.6</td>
</tr>
<tr>
<td>60</td>
<td>27.7*</td>
<td>29.0*</td>
<td>28.5*</td>
</tr>
<tr>
<td>120</td>
<td>27.2*</td>
<td>28.1*</td>
<td>27.4*</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>28.4 ± 2.0</td>
<td>29.6 ± 1.9</td>
<td>29.0 ± 1.7</td>
</tr>
<tr>
<td><strong>Forearm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>32.1</td>
<td>32.5</td>
<td>32.5</td>
</tr>
<tr>
<td>60</td>
<td>27.3*</td>
<td>27.6*</td>
<td>28.2*</td>
</tr>
<tr>
<td>120</td>
<td>26.2*</td>
<td>26.1*</td>
<td>26.9*</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>27.9 ± 2.3</td>
<td>28.1 ± 2.1</td>
<td>28.7 ± 2.0</td>
</tr>
<tr>
<td><strong>Left hand</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>27.0</td>
<td>28.0</td>
<td>28.3</td>
</tr>
<tr>
<td>60</td>
<td>22.2*</td>
<td>22.6*</td>
<td>23.7*</td>
</tr>
<tr>
<td>120</td>
<td>21.3*</td>
<td>20.8*</td>
<td>22.6*</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>22.8 ± 3.2</td>
<td>22.9 ± 2.4</td>
<td>24.2 ± 3.3</td>
</tr>
<tr>
<td><strong>Right thigh</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>32.3</td>
<td>33.2</td>
<td>33.0</td>
</tr>
<tr>
<td>60</td>
<td>29.9*</td>
<td>30.3*</td>
<td>30.3*</td>
</tr>
<tr>
<td>120</td>
<td>29.2*</td>
<td>29.9*</td>
<td>29.8*</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>30.1 ± 1.9</td>
<td>30.6 ± 1.7</td>
<td>30.6 ± 1.9</td>
</tr>
<tr>
<td><strong>Left calf</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>32.1</td>
<td>31.9</td>
<td>32.1</td>
</tr>
<tr>
<td>60</td>
<td>31.9</td>
<td>30.3*</td>
<td>30.9*</td>
</tr>
<tr>
<td>120</td>
<td>30.9*</td>
<td>29.7*</td>
<td>29.8*</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>31.9 ± 1.2</td>
<td>30.6 ± 2.2</td>
<td>31.0 ± 1.9</td>
</tr>
</tbody>
</table>

Values are mean ± SD. * significant difference vs. 0 † significant difference vs. SL (P < 0.05).
Table 4.2. The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level (SL) and at 4000 m before (UN) and after (PEX) acclimatisation on fingernail and toenail temperature (°C).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Fingernail</th>
<th>Toenail</th>
<th>Fingernail</th>
<th>Toenail</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SL</td>
<td>UN</td>
<td>PEX</td>
<td>SL</td>
</tr>
<tr>
<td>0</td>
<td>23.2 ± 3.3</td>
<td>24.2 ± 3.4</td>
<td>24.3 ± 3.8</td>
<td>20.6 ± 1.2</td>
</tr>
<tr>
<td>60</td>
<td>20.3 ± 3.7*</td>
<td>20.3 ± 3.7*</td>
<td>20.7 ± 3.4*</td>
<td>17.8 ± 0.6*</td>
</tr>
<tr>
<td>120</td>
<td>18.3 ± 1.4*</td>
<td>17.5 ± 1.1*</td>
<td>18.2 ± 1.6*</td>
<td>16.9 ± 1.1*</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>20.4 ± 3.5</td>
<td>20.5 ± 3.9</td>
<td>20.6 ± 3.6</td>
<td>18.1 ± 0.9</td>
</tr>
</tbody>
</table>

Values are mean ± SD. * significant difference vs. 0, † significant difference vs. SL (P < 0.05).

4.4.3 Metabolic responses

Metabolic heat production was similar at rest in all trials during exposure to a thermo-neutral, normoxic environment (F2, 18 = 0.038, P = 0.963, η² = 0.004). As expected, M increased in all trials during the CAT (F12, 108 = 12.071, P = 0.000, η² = 0.528). However, compared to SL, M was less after 20 minutes of CAT on UN and PEX (F2, 18 = 7.907, P = 0.003, η² = 0.431) (Figure 4.4).

During UN, RER was higher than SL and PEX (F2, 18 = 6.501, P = 0.008, η² = 0.327) (Table 4.3). This may have been a direct result of the hypoxic ventilatory response during UN, however the same RER response was not observed during PEX yet a similar pattern in ventilator response was.

During CAT, the percentage of kilocalories derived from carbohydrate oxidation, calculated using RER values, was greater during UN compared to SL and PEX (F2, 18 = 9.025, P = 0.002, η² = 0.416) (Figure 4.5).
**Figure 4.4.** The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level (♦) and at 4000 m before (■) and after (△) acclimatisation on metabolic heat production (W·m⁻²). Values are mean ± SD. * significant difference vs 0 mins, † significant difference vs. SL (P < 0.05).

**Table 4.3.** The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level (SL) and at 4000 m before (UN) and after (PEX) acclimatisation on the respiratory exchange ratio (RER).

<table>
<thead>
<tr>
<th>RER (respiratory exchange ratio)</th>
<th>SL</th>
<th>UN</th>
<th>PEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.84 ± 0.14</td>
<td>0.76 ± 0.05</td>
<td>0.74 ± 0.03</td>
</tr>
<tr>
<td>60</td>
<td>0.91 ± 0.3</td>
<td>1.04 ± 0.13*#</td>
<td>0.87 ± 0.13</td>
</tr>
<tr>
<td>120</td>
<td>0.81 ± 0.11</td>
<td>1.05 ± 0.14*†#</td>
<td>0.85 ± 0.09</td>
</tr>
<tr>
<td>Mean</td>
<td>0.9 ± 0.2</td>
<td>1.00 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>

Values are mean ± SD. * significant difference vs.0 mins, † significant difference vs. SL, # significant difference vs. PEX (P < 0.05).
Figure 4.5. The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level (■) and at 4000 m before (■) and after (■) acclimatisation on carbohydrate and lipid oxidation (%). Values are mean ± SD. † significant difference vs. SL, # significant difference vs. PEX (P < 0.05).

4.4.4 Observed shivering activity and EMG

After 80 minutes, observed shivering activity was lower PEX compared to SL and UN (F_2,18 = 6.322, P = 0.008, η^2 = 0.322) (Figure 4.6). After 110 minutes, maximum observed shivering activity was reached in all trials. However, shivering intensity at this time was higher during SL and UN (2 = Generalised but discontinuous shivering) compared to PEX (1 = Mild shivering bursts). While not significant, EMG traces displayed lower shivering activity during PEX compared to SL and UN, particularly toward the end (F_2,18 = 0.908, P = 0.421, η^2 = 0.087) (Figure 4.7).
Figure 4.6. The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level (■) and at 4000 m before (■) and after (■) acclimatisation on observed shivering activity. Values are mean ± SD. * significant difference vs. 0 mins, † significant difference vs. SL, ¥ significant difference vs. UN (P < 0.05).

Figure 4.7. The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level (♦) and at 4000 m before (■) and after (△) acclimatisation on EMG activity of the pectoralis major (µV). Values are mean ± SD.
4.4.5 Thermal comfort and pain sensation

Thermal comfort was increased PEX compared with SL and UN \((F_{1.284, 11.558} = 11.779, P = 0.004, \eta^2 = 0.542)\) (**Figure 4.8**). Perceived peripheral coldness was lower during PEX compared to SL and UN \((F_{2, 18} = 14.407, P = 0.000, \eta^2 = 0.572)\) (**Figure 4.9A**). An interaction established that perceived peripheral coldness depended on hypoxic acclimatisation \((F_{24, 216} = 3.441, P = 0.000, \eta^2 = 0.086)\). Perceived central coldness was also lower during PEX compared to SL and UN \((F_{2, 18} = 5.678, P = 0.012, \eta^2 = 0.365)\) (**Figure 4.9B**). Similarly, an interaction suggested perceived central coldness was also dependant on hypoxic acclimatisation \((F_{24, 216} = 2.018, P = 0.005, \eta^2 = 0.084)\). Perceived coldness at the periphery was higher than perceived central coldness throughout all trials \((P < 0.05)\).

**Figure 4.8.** The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level (♦) and at 4000 m before (■) and after (△) acclimatisation on thermal comfort (McGinnis’ thermal comfort scale). Values are mean ± SD. * significant difference vs. 0 mins, † significant difference vs. SL \((P < 0.05)\).
Figure 4.9. The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level (♦) and at 4000 m before (■) and after (△) acclimatisation on perceived central coldness (A) and Perceived peripheral coldness (B). Values are mean ± SD. * significant difference vs. 0 mins; † significant difference vs. SL, ¥ significant difference vs. UN (P < 0.05).

4.4.6 Heart rate and Arterial oxygen saturation

HR was higher UN compared to PEX during the initial 50 minutes (F_{2, 18} = 7.254, P = 0.005, η^2 = 0.385) (Table 4.4). Arterial oxygen saturation was lower during UN compared to SL (F_{1.082, 9.734} = 23.517, P = 0.001, η^2 = 0.694) (Table 4.4). Arterial oxygen saturation during PEX was not different to SL or UN.
Table 4.4. The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level SL and at 4000 m before (UN) and after (PEX) acclimatisation on heart rate (beats·min\(^{-1}\)) and arterial oxygen saturation (S\(_a\)O\(_2\) %).

<table>
<thead>
<tr>
<th></th>
<th>HR (beats·min(^{-1}))</th>
<th>S(_a)O(_2)(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SL UN PEX SL UN PEX</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>55 ± 9 60 ± 9†</td>
<td>54 ± 6 98 ± 2 85 ± 9†</td>
</tr>
<tr>
<td>60</td>
<td>57 ± 11 56 ± 10 54 ± 9</td>
<td>97 ± 2 85 ± 7† 90 ± 4</td>
</tr>
<tr>
<td>120</td>
<td>59 ± 10 65 ± 10 59 ± 9</td>
<td>97 ± 2.6 86 ± 6† 90 ± 4</td>
</tr>
<tr>
<td>Mean</td>
<td>57 ± 11 60 ± 10 54 ± 9</td>
<td>97 ± 3 85 ± 9 91 ± 5</td>
</tr>
</tbody>
</table>

Values are mean ± SD. † significant difference vs. SL, # significant difference vs. PEX (P < 0.05).

4.4.7 Results summary

The key findings of these results demonstrate an unaltered core and mean skin temperature during UN and PEX, compared with SL. However, metabolic heat production was significantly altered during UN and remained so PEX. RER and substrate oxidation were altered during UN as was shivering activity, which demonstrated a reduced capacity for non-shivering thermogenesis. Thermal comfort was increased PEX compared with SL and UN. Heart rate was higher UN compared with PEX during the initial 50 minutes. Arterial oxygen saturation was lower during UN compared with SL.
4.5 Discussion

The aim of this study was to examine the acute and chronic effects of hypoxia upon thermoregulation in mild cold. As anticipated, un-acclimatised men exposed to hypoxia in the cold demonstrated an impaired thermoregulatory and metabolic response compared with cold exposure at sea level. This was demonstrated by a reduced contribution of NST via an increased catabolism of CHO that initiated an earlier onset and increased intensity of shivering activity. Despite this, cold exposure in hypoxia regardless of acclimatisation did not impair core or mean skin temperature compared to cold alone. Following prolonged hypoxic exposure however, perceptions of thermal comfort reflected an improved cold tolerance and impaired thermal sensitivity that might lead to poor behavioural thermoregulation and greater risk of cold injury.

Contradictory to early reports (Bullard, 1961; Cipriano and Goldman, 1975; Kottke et al. 1948), un-acclimatised exposure to hypoxia in the cold did not increase the rate of heat loss from the core or periphery. Core temperature however, has been reported elsewhere as unaltered during both normobaric and hypobaric hypoxia in the cold (Blatteis and Lutherer, 1976; Brown et al. 1952; Robinson and Haymes, 1990). On the contrary, when individuals were un-acclimatised, reduced temperatures at the toenail bed were observed. Exposure to acute hypoxia in the cold has been associated with a reduction in CIVD at the extremities (Mathew et al. 1977; Takeoka et al. 1993) and an increased risk of local cold injury at altitude (Golja et al. 2004). Following the 18-day mountaineering expedition at altitude, core and mean skin temperatures during subsequent cold exposure in hypoxia were also unaltered compared with cold alone. Local temperature at the forehead and left chest however, were higher during acclimatised exposure to hypoxia compared to normoxia. Future studies should look to measure skin blood flow at these local areas to see if warming is repeated. Potentially, warmer skin temperature at these sites is reflecting an adaptation to hypoxia following a prolonged stay.
Thermal comfort and perception provides the basis for the initiation of behavioural thermoregulation (Golja et al. 2004). During unacclimatised hypoxic-cold exposure in the present study, men rated their thermal comfort the same as in cold alone. This has been previously reported (Golja et al. 2005). Core and skin temperature sensors transform thermal energy into neural coded information that give a sensation of either cold or warmth and a perception of pleasure or displeasure (Cabanic, 1969, 1981; Hensel 1976). Given that core and mean skin temperatures were the same in both trials it is likely the amount of thermal energy was also the same which suggests why no differences in thermal comfort occurred. This contradicts previous work where an inspired air content of 10% oxygen decreased cold sensation in humans (Golja et al. 2004). However, since it has been suggested oxygen limitations may only begin to implement thermoregulatory impairments when inspired oxygen is less than 11% (Gautier et al. 1997), the modest level of hypoxia that participants were exposed to in the present study may have induced milder impairments. It should be noted however, that arterial oxygen saturation levels varied substantially between participants during unacclimatised hypoxic exposure and conclusions from this study therefore should be generalised to wider populations with caution. Nonetheless, despite variations no participant rated themselves to be suffering AMS symptoms as highlighted by the Lake Louise scale.

Post-expedition, a decrease in cold sensation at the core and periphery was demonstrated. This suggests prolonged exposure to altitude stressors during mountaineering may affect core and skin temperature sensors so that neural coded information is impeded and nerve conduction impaired (Golja et al. 2004; Malanda et al. 2008). When oxygen supply diminishes below the metabolic demands of the nerve tissue, the energy requiring cell functions are reduced and membrane depolarisation causes complete loss of neural cell function (Astrup, 1982; Golja et al. 2004). Neural impairment by prolonged hypoxic exposure may affect both the central and peripheral nervous system. Acclimatised individuals at altitude may often feel warmer than their core temperature
would suggest. As a result, behavioural thermoregulation may be neglected and as altitude continues to increase and ambient temperatures reduce, impaired thermal sensation may increase the likelihood of cold injury (e.g. hypothermia and frostbite).

Metabolic heat production was reduced during the initial hypoxia and cold exposure, which was similar to earlier reports (Blatteis and Lutherer, 1976; Robinson and Haymes, 1990). A reduction in heat production coupled with an earlier onset and more intense shivering activity supports that hypoxia may suppress NST (Blatteis and Lutherer, 1976; Bullard, 1961; Gautier, 1996; Robinson and Haymes, 1990). Given that BAT is the primary energy source for NST and has been observed to be mainly activated during cold exposure (van Marken Lichtenbelt et al. 2009), suggests that hypoxia may indeed restrict the activity of BAT in the cold leading to an increased likelihood of a reduction in body temperature. This is supported by previous work which suggests the hypoxic-induced suppression of NST by an inhibition of the aerobic catabolism of lipid stores, when un-acclimatised, may occur through an increased hormonal response to hypoxic stress that decreases the lipolytic response of adipocytes and thus lowers lipid mobilisation (de Glisezinski et al. 1999; Masoro, 1966; Robinson and Haymes, 1990).

Carbohydrate oxidation increased by almost 100% when un-acclimatised men were exposed to hypoxia and cold compared with cold alone. However, this increase was reduced by 50% post-expedition. Increased carbohydrate oxidation occurs to fuel intense shivering thermogenesis (Martineau and Jacobs, 1988) which may explain how the body successfully maintained core and mean skin temperature given the reduction of NST. Increased shivering activity coupled with an increased rate of carbohydrate oxidation suggests a physiological response similar to moderate exercise (Roberts et al. 1996a). While muscle glycogen and blood glucose contribute equally to carbohydrate energy production, sources become depleted quickly compared to fatty acids and glycerol (Coyle, 1995). Therefore, un-acclimatised men with a diminished ability to oxidise lipids in hypoxic and cold environments risk muscle glycogen depletion, earlier onset of fatigue and
ultimately cold injury. Comparable with un-acclimatised exposure to cold and hypoxia, the metabolic response remained suppressed post-expedition. Nonetheless, shivering activity was reduced and was also less than normoxia. This finding supports that NST recovery may occur following a stay at altitude which may result from a small recovery in fuel selection.

Present results also suggest there may be an increase in thermal comfort following a stay at high altitude. Reduced cold sensitivity and increased thermal comfort accompanying acclimatisation may be related to the observed reductions in shivering activity post-expedition. Given peripheral thermo-receptors at the skin control the onset of shivering it would appear these are not readily stimulated upon exposure to mild cold in acclimatised individuals. In conclusion, following an 18 day mountaineering expedition, a decrease in shivering despite no changes in core or mean skin temperature is consistent with a greater reliance on NST. The alterations observed post-expedition in thermal comfort may suggest a greater thermal tolerance and poorer thermal sensation that may increase susceptibility to cold injury.
CHAPTER FIVE

Hypoxia and cold effects on Mucosal Immunity before and after an altitude stay

5.1 Abstract

The purpose of this study was to examine the effect of hypoxia and cold on mucosal immunity. Ten males completed four different trials. Three 2 hour CATs were performed in an environmental chamber (CAT 15°C, RH 44%): 1. sea-level (n = 15) (SL: F\textsubscript{1}O\textsubscript{2} = 0.209); 2. un-acclimatized in normobaric hypoxia (n = 15) (UN: F\textsubscript{1}O\textsubscript{2} = 0.125, ~4000m) and 3. post expedition in normobaric hypoxia (n = 10) (PEX: F\textsubscript{1}O\textsubscript{2} = 0.125, ~4000m). Participants also completed a thermo-neutral control trial (n = 10) (TC: F\textsubscript{1}O\textsubscript{2} = 0.209, 19.5°C and 45% RH). Un-stimulated whole saliva samples were collected before, at the end of CAT, immediately after, and one-hour after the CAT. On TC, saliva was collected at the same time of day as CAT trials. Cold exposure caused a progressive decline in T\textsubscript{rec} (~0.4°C) throughout all trials ($P = 0.001$) which was not further altered by altitude ($P = 0.097$). Hypoxia also caused a significant reduction in oxygen saturation during UN (SL: 98 ± 2, UN: 86 ± 6 PEX: 91 ± 5 %; $P = 0.000$). Saliva flow rate (µl·min\textsuperscript{-1}) was decreased by hypoxia in the cold during UN compared with SL and TC ($P = 0.047$; $P = 0.022$) respectively. No differences were observed in s-IgA concentration (µg·ml\textsuperscript{-1}) ($P = 0.642$) or s-IgA secretion rate (µg·min\textsuperscript{-1}) ($P = 0.335$). Cold exposure however, increased α-amylase concentration compared with TC ($P = 0.003$). In combination, a cold-induced increase in α-amylase concentration and a hypoxic-induced reduction in saliva flow rate might increase the risk of infection for un-acclimatised individuals at altitude since saliva is important for oral washing.
5.2 Introduction

Given unacclimatised exposure to cold and hypoxia denotes changes in thermoregulatory homeostasis (Chapter 4), it would be beneficial to understand how these correspond to immune function in the same conditions. This knowledge could better inform individuals regarding health risks involved when traveling to cold, hypoxic environments. A study investigating thermoregulation and immune function using controlled methodology has not been reported.

Sympathoadrenal responses associated with acute and chronic high altitude exposure can influence immune function which may increase the risk of illness and infection while individuals are a far distance from appropriate medical resources (Meehan et al. 1988; Mazzeo et al. 1994; Mazzeo et al. 1995; Mazzeo et al. 2001). For example, early research has found a higher prevalence of pneumonia among military troops stationed at high altitude (Singh et al. 1977). However, findings have limited application to new and un-acclimatised arrivals at altitude. Furthermore, individuals visiting high altitudes are often exposed to multiple stressors, including cold, dehydration, novel infectious agents and poor hygiene. It is incorrect to assume an increase in infection is exclusively due to hypoxia-induced immune suppression. Whether the risk of illness or infection is exacerbated in un-acclimatised expeditioners upon arrival to hypoxic and cold environments remains unknown. Furthermore, it has not been clarified whether such potential detriments to mucosal immunity are reduced following a period of altitude acclimatisation. Given the possibility of low oxygen conditions during space flight, this information may also have significant implications for spaceflight personnel during long term missions (Cogoli, 1981; Taylor and Dardano, 1983).

Cold exposure is associated with an increased incidence of bronchorrhea, sinusitis, and upper respiratory tract infections (URTI) (Clothier, 1974; Giesbrecht, 1995; Castellani et al. 2002; Lavoy et al. 2011). Much research to date however, has examined individuals undertaking the additional stress of physical activity which likely exacerbates the immune response to cold (Castellani et al. 2002). Since URTIs are the main cause of illness and missed practice in elite cross country skiers
(Berglund and Hemmingson, 1990), cold-induced decrements in immune-surveillance may be a problem for individuals at altitude. The additional stressor of cold in hypoxia-exposed humans may evoke a similar suppression on the mucosal immune system as that demonstrated with physical activity in the cold.

Short term hypoxia is reported to induce similar immunosuppression upon the mobilisation of T-cells and natural killer cells (Klokker et al. 1993; Klokker et al. 1995) as that demonstrated during surgery (Lennard et al. 1985) and exercise (Pedersen et al. 1990). The regulation of cytokine production and release has also been shown to be reduced in hypoxia (Pedersen and Steensberg, 2002). On the contrary, nasal mucosal immunity is suggested to be unchanged by hypoxia and any reduced secretory IgA and lysozyme levels may be caused by the combined stresses of cold, isolation and reduced humidity (Meehan et al. 1988; Muchmore et al. 1981). While cold exposure is often unavoidable at high altitude, the effect of hypoxia itself upon the normal mucosal immune response to cold has received little attention. Salivary IgA is often the mucosal secretion of choice for studies of the human mucosal immune system due to the ease and standardisation of collections. It is well established to characterise the effects of many stressors, for this reason however, it may not always reflect competencies in other aspects of immune defence and therefore it is of benefit to assess additional mucosal immune parameters. Secretion of amylase from saliva glands is controlled by autonomic nervous signals and substantial literature reveals that salivary amylase is correlated to sympathetic activity under conditions of stress. Specifically, α-amylase increased under a variety of physically (i.e. exposure to heat or cold) and psychologically (exams) stressful conditions (Chatterton et al. 1996). Furthermore, s-IgA acts with α-amylase to provide the first line of defence against pathogens and antigens at the mucosa. A study to determine the effect of acute and chronic hypoxia on s-IgA and α-amylase responses in the cold compared to cold alone will likely clarify any increased risk of infection upon and following ascent to high altitude.
The aim of the present study was to examine the effect of hypoxia on saliva responses to the cold before and after an 18 day altitude expedition. This was achieved by comparing saliva responses in thermo-neutral, mild cold, and mild cold with hypoxia. To determine the effect of prolonged exposure to a multi-stressor, mountainous environment, typically experienced by mountaineers, the same saliva responses were compared in the same mild cold with hypoxia. Given fluctuations in catecholamines and cortisol activate the SNS and effect s-IgA and amylase responses; it was unnecessary to measure these stress hormones if changes in mucosal immunity occurred. It was hypothesised that un-acclimatised exposure to hypoxia would evoke greater detrimental effects on mucosal immunity in the cold compared with normoxia. This might include an increase in α-amylase concentration and a reduction in s-IgA secretion rate. Following an altitude stay it was hypothesised that typical s-IgA and α-amylase responses to the cold would be resumed regardless of hypoxia.
5.3 Method

5.3.1 Participants

Fifteen healthy males (mean ± SD: age, 22 ± 3 years; height, 180 ± 6 cm; body mass, 66 ± 12 kg and body fat, 12 ± 6 %) were recruited from an Alpine expedition. Due to unexpected circumstances only ten participants were able to complete PEX and TC. Participants gave written consent and medical history before the study which received local Ethics Committee approval.

5.3.2 Preliminary measurements

Before each experimental trial participants arrived at the laboratory after an overnight fast having consumed no food, caffeine or alcohol within 10 h. Participants ingested water equal to at least 35 ml·kg$^{-1}$ body mass the day prior to each trial. After voiding bladder and bowels, nude body mass and height were measured. Daily energy requirements were determined by body composition and calculated from a mean of daily metabolic rate, hourly basal metabolic rate, eight hours of sleeping, eight hours of occupational activities (e.g. moving, sitting and standing) and eight hours of residual time (WHO, 2004). Energy requirements were provided and given as standardised meals (breakfast, lunch and dinner) in the 24 h period prior to each experimental trial.

5.3.3 Experimental Trials

Participants completed three experimental trials in a normobaric, temperature and humidity controlled chamber (Dry bulb temperature of the chamber remained at 15°C with a relative humidity (RH) 40% and 0.2 m.s$^{-1}$ wind velocity; Delta Environmental Systems, Chester, UK) over a 12 week period (Chapter 4). Trials were 1. sea-level (SL: $F_{I,O_2} = 0.209$); 2. un-acclimatised in normobaric hypoxia (UN: $F_{I,O_2} = 0.125$, ~4000m); and 3. post-expedition (n=10) in normobaric hypoxia (PEX: $F_{I,O_2} = 0.125$, ~4000m). To avoid an order effect SL and UN were performed before
the expedition in half of the participants and 2 months after the expedition in the remainder. Ten of the fifteen participants also completed a thermo-neutral control trial (TC) fully clothed in ambient conditions (19.5°C and 45% RH) to account for the diurnal variation in saliva responses. Participants completed each trial at the same time of day and consumed no food, caffeine or alcohol within 4 h of each trial. Throughout UN and PEX, inspired oxygen fraction (F_{I\text{O}_2}) was equal to 0.125 to simulate an altitude of approximately 4000 m. Following pre-trial measures and after 10 minutes supine rest in normothermia (ambient temperature: 21.3 ± 1.1°C; relative humidity: 52 ± 5.4%), baseline measures of T_{rec} and S_{a\text{O}_2}\% were recorded. Wearing shorts only, participants then entered the chamber and rested in a supine position on roll mats situated upon the floor for 120 minutes. Early removal from the chamber occurred if: 1. core temperature reduced to 36°C or fell 1.5°C below baseline, 2. Lake Louise scores went above 1 (UN and PEX only) or 3. participants chose to withdraw. Upon completion participants were re-warmed and monitored until core temperature was within 0.5°C of baseline.

5.3.4 Saliva collection and analysis

Un-stimulated whole saliva samples were collected using pre-weighed universal tubes (Chapter 3). Saliva samples were collected while the participant sat quietly in the laboratory (1: before CAT, 2: at the end of CAT – inside chamber conditions, 3: immediately after CAT – outside chamber (21.3 ± 1.1°C and rh: 52 ± 5.4%), 4: one-hour after CAT (21.3 ± 1.1°C and rh: 52 ± 5.4%). Saliva volume was estimated by weighing the universal container immediately after collection to the nearest mg. From this, saliva flow rate was determined by dividing the volume of saliva by the collection time. Saliva was aspirated into Eppendorfs and stored at -40°C for further analysis. After thawing s-IgA concentration was determined by ELISA (Salimetrics Europe Ltd, Suffolk, UK) using IgA
monomer from human serum as standard. The intra-assay CV was 1.4%. s-IgA secretion rate was calculated by multiplying the saliva flow rate by s-IgA concentration.

Alpha amylase concentration was determined by ELISA (Salimetrics Europe Ltd, Suffolk, UK) using a chromagenic substrate, 2-chloro-p-nitrophenol linked with maltotriose. The intra-assay CV was 2.5%.

5.3.5 Statistical Analysis

A sample size of ten participants was estimated using previous data examining the effects of environmental stress on immune indices (Costa et al. 2010; Laing et al. 2008; Oliver et al. 2007). One way fully repeated measures ANOVA were performed on pre-experimental body composition measures. Two-way fully repeated measures ANOVA were performed on saliva parameters (Chapter 3).
5.4 Results

5.4.1 Thermoregulatory (T_{rec} and T_{sk}) and metabolic responses

Core and mean skin temperature values were analysed using mean scores for n = 10 and n = 15 on SL and UN. Given n = 10 completed all four trials and significance scores were not different for the two trials completed by n = 15 and n = 10, n = 10 was used in order to be able to compare all trials (SL, UN, PEX and TC). Core and mean skin temperatures were lower after cold air tests than at baseline (T_{rec}, F (12, 168) = 8.955, P = 0.000, \eta^2 = 0.610; T_{sk}, F (12, 168) = 227.041, P = 0.000, \eta^2 = 0.610) (Chapter 4). At rest M was not different between either trial (SL: 49 ± 7, UN: 49 ± 11; PEX: 50 ± 10 W·m^{-2}; F (1, 14) = 0.033, P = 0.859, \eta^2 = 0.997). However, compared with SL, the cold-induced increase in M was reduced with hypoxic exposure (Table 5.1: F (1, 14) = 21.188, P = 0.000, \eta^2 = 0.398). RER was higher on UN (Table 5.1: F (1, 14) = 19.478, P = 0.001, \eta^2 = 0.418).

Table 5.1. The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level SL) and at 4000 m before (UN) and after (PEX) acclimatisation on rectal temperature, mean weighted skin temperature, metabolic heat production and respiratory exchange ratio.

<table>
<thead>
<tr>
<th></th>
<th>SL</th>
<th>UN</th>
<th>PEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_{rec} at 120 min CAT (°C)</td>
<td>36.9 ± 0.3</td>
<td>36.8 ± 0.3</td>
<td>36.7 ± 0.2</td>
</tr>
<tr>
<td>Mean weighted T_{sk} at 120 min CAT (°C)</td>
<td>29.3 ± 1.0</td>
<td>29.5 ± 1.1</td>
<td>29.8 ± 0.9</td>
</tr>
<tr>
<td>M at 120 min CAT (W·m^{-2})</td>
<td>118 ± 44</td>
<td>69 ± 13 †</td>
<td>83 ± 20 †</td>
</tr>
<tr>
<td>RER at 120 min CAT</td>
<td>0.81 ± 0.11</td>
<td>1.05 ± 0.14 †</td>
<td>0.85 ± 0.09</td>
</tr>
</tbody>
</table>

Values are mean ± SD. † significant difference vs. SL (P < 0.05).

5.4.2 Heart rate and arterial oxygen saturation

Heart rate was higher in UN compared to PEX but was not different to SL (SL: 57 ± 11, UN: 60 ± 910, PEX: 54 ± 9 beats·min^{-1}; F_{2, 18} = 7.254, P = 0.005, \eta^2 = 0.385). Arterial oxygen saturation however, was lower during UN compared to SL but was unchanged during PEX, albeit values on
PEX were still low (SL: 98 ± 2, UN: 86 ± 6 PEX: 91 ± 5 %; F (1, 14) = 71.704, P = 0.000, η² = 0.163).

5.4.3 Saliva IgA and amylase responses

A decline in s-IgA concentration was demonstrated following baseline measures (Figure 5.1: F (3, 42) = 9.540, P = 0.000, η² = 0.595). No interactions were observed for s-IgA concentration (F (3, 42) = 0.564, P = 0.642, η² = 0.961) or secretion rate (F (3, 42) = 1.164, P = 0.335, η² = 0.923). However, an interaction was found where saliva flow rate increased from baseline throughout cold exposure during SL but was suppressed with hypoxia in UN (Figure 5.1: F (3, 42) = 2.873, P = 0.047, η² = 0.118). There were no observed differences in any saliva parameter between UN and PEX or SL and PEX. Saliva flow rate was less during SL and UN compared with TC but PEX was not (Figure 5.1: F (3, 24) = 3.859, P = 0.022, η² = 0.325).

Hypoxia and cold exposure conferred no further increases in α-amylase concentration compared with cold only (Figure 5.2: F (2, 18) = 0.490, P = 0.621, η² = 0.052). Compared with TC however, cold exposure, with or without hypoxia, increased amylase concentration almost three-fold by the end of the CAT (Figure 5.2: F (3, 24) = 5.957, P = 0.003, η² = 0.427). Nonetheless, α-amylase secretion rate was not different between trials (Figure 5.2: F (3, 18) = 2.320, P = 0.110, η² = 0.278).

5.4.4 Results summary

Core and mean skin temperatures were lower after CATs. Compared with SL, the cold-induced increase in M was reduced with hypoxic exposure. Saliva flow rate increased from baseline throughout cold exposure during SL but was suppressed in UN. Hypoxia and cold exposure caused no further increases in α-amylase concentration compared with cold only. Compared with TC however, cold exposure, with or without hypoxia, increased amylase concentration almost three-fold by the end of the CAT. For hydration data see chapter 4.
Figure 5.1. The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level (♦), at 4000 m before (■) and after (▲) acclimatisation, and a thermo-neutral control (19.5°C) (●) on saliva flow rate (µl·min⁻¹) (A), s-IgA concentration (µg·ml⁻¹) (B) and s-IgA secretion rate (µg·min⁻¹) (C). Values are mean ± SD. * significant difference vs Baseline, # vs. TC (P < 0.05).
Figure 5.2. The effect of 120 min cold air test (CAT; 15°C) in un-acclimatised participants at sea level (♦), at 4000 m before (■) and after (▲) acclimatisation, and a thermo-neutral control (●) on α-amylase concentration (µg·ml⁻¹) (A) and secretion rate (µg·min⁻¹) (B). Values are mean ± SD. * significant difference vs Baseline, # vs. TC (P < 0.05).
5.5 Discussion

The aim of the present study was to examine the effect of hypoxia on saliva responses to the cold before and after an 18 day altitude expedition. Due to the potential for greater sympathetic nervous system activity, vasoconstriction of blood vessels at the salivary glands and reduced saliva secretion (Bishop, 2006), it was hypothesised that hypoxia in the cold, prior to acclimatisation, would evoke an increase in α-amylase concentration and reductions in saliva flow rate, s-IgA concentration and secretion rate of greater magnitude than when individuals were exposed to cold only or post-expedition. In direct contrast to the hypothesis, s-IgA concentration and secretion rate, regardless of acclimatisation, were not affected by cold or hypoxia and cold. Nonetheless, α-amylase concentration was increased during cold exposure but was not exacerbated by hypoxia. When un-acclimatised however, saliva flow rate was reduced in cold and hypoxia compared with normoxia.

Since the effect of acute hypoxia on leukocytes resembles the same effect as exercise, it has been suggested that exercise and hypoxia-induced changes in leukocyte subpopulations might be mediated by similar mechanisms involving neuroendocrinological factors (Pedersen and Steensberg, 2002). Although physiological mechanisms underlying the decline in s-IgA responses remain unclear, it is likely that both neural and endocrine factors also influence this mucosal immune response (Pedersen and Steensberg, 2002). Given arterial oxygen saturation was reduced during un-acclimatised hypoxic exposure in the present study, and remained low following acclimatisation, it is not unreasonable to suggest both the neural and endocrine systems were compensating to maintain homeostasis (Mazzeo, 2005), thus explaining the difference in saliva flow rate compared with normoxia. Temporal skin temperature was higher during hypoxic exposure (Chapter 4) suggesting cerebral blood flow may have been increased. This may have been a subsequent effect of increased neural and endocrine activity in an environment of reduced oxygen. Future research should look to examine cerebral blood flow in hypoxia to help further understand mechanisms to maintain homeostasis.
The absence of hypoxia-induced s-IgA and α-amylase alterations to cold suggests the mucosal immune system is robust to a combination of environmental stressors, and as such common URTI complaints are not likely to be related to the mucosal system upon arrival to high altitude or following a prolonged stay. Nonetheless, given α-amylase concentration and secretion rate were affected by cold, regardless of hypoxia, and saliva flow rate was only affected by hypoxia when un-acclimatised, it is possible there is an increased risk of URTI for new arrivals at altitude if temperatures are particularly low.

A study to examine the effects of a live high – train low (LHTL) training camp on mucosal immunity reported similar s-IgA changes from baseline after the first 12 days of training in both the control (i.e. lived and trained at 1200 m) and LHTL (groups lived at 2500 m or 3000 m and trained at 1200 m) groups (Tiollier et al. 2005). However, after the last 6-day phase (when living was at an altitude of 3500 m) s-IgA concentration decreased more in the LHTL group compared with the 2500 and 3000 m phases. It has been suggested that an altitude of 3500 m corresponds to a threshold that physiological changes intended to tolerate the drop in the partial pressure of oxygen fail to fully compensate the effect of hypoxia (Colin et al. 1999). Thus, it was speculated that at or above this altitude the adverse effect of hypoxia on s-IgA responses is more substantial (Tiollier et al. 2005). In the present study, participants were exposed to an altitude equal to 4000 m while at rest and displayed no alterations in s-IgA regardless of acclimatisation status. Therefore, it is more likely the observed changes in s-IgA during the last phase of an 18 day LHTL training camp were not due to the level of hypoxia per se, or as a result of chronic exposure, but more likely a cumulative effect of intense physical training at altitude. Had there been an exercise element during cold air tests of the present study, participants may have demonstrated greater changes in s-IgA and α-amylase. Further, it might have been clearer to see whether acclimatisation reduced such changes. Additionally, the normobaric nature of the altitude exposures discussed here does not fully replicate typical high altitude conditions. It is plausible to suggest that if barometric pressure was also
reduced to reflect the real value coinciding with that level of altitude (i.e. 661 mmHg at 4000 m), the impact on homeostasis and subsequent mucosal immune status may be greater during cold exposure.

Given that salivary secretion is under neural control (Chicharro et al. 1998) and chronic hypoxia has been shown to cause marked activation of the sympathetic nervous system (Chicharro et al. 1998; Calbet, 2003; Zaccaria et al. 2004; Tiollier et al. 2005), this provides the physiological basis for a potential involvement of neural factors in the s-IgA changes related to hypoxia. Nonetheless, since mucosal responses to cold were unchanged in hypoxia compared with normoxia it is concluded that altitude exposure while at rest, before and following an altitude stay, does not cause disruption to either sympathetic nervous system activity (Mazzeo et al. 2003) or alpha-adrenergic mechanisms (Ring et al. 2000). Secondly, the reduction in arterial oxygen saturation accompanying exposure to acute hypoxia unlikely evokes a physiological or metabolic stress great enough to disrupt mucosal immune responses to cold at rest. On the contrary, in combination, a cold-induced increase in α-amylase concentration and a hypoxic-induced reduction in saliva flow rate might increase the risk of infection for illness-prone, un-acclimatised individuals at higher altitudes (e.g. > 4500m) since saliva is important for oral washing. With regard to the number of mucosal parameters available for the assessment of potentially stressful situations, future studies would do well to analyse a wider variety of such parameters in order to provide clarity and help build a framework of likely mucosal immune responses in extreme environments (i.e. cold and hypoxia).
CHAPTER SIX

Practical Field Methods for the Pre-hospital Management of Hypothermia: Shivering Cold Casualties

6.1 Abstract

The aim of this study was to examine thermoregulatory responses of shivering men to four rewarming treatments. Seven males completed five randomised trials. These included a thermo-neutral control trial (TC) and four cold trials. On each cold trial, participants were cooled to 36°C core temperature. They then completed a 3-hour cold air test (CAT, 0°C) in one of four field methods; 1. Single layer polythene survival bag (PB), 2. PB with 70°C hot drink in insulated contatiner (PB+HD), 3. triple-layered metallised Blizzard survival bag with cells to trap and reflect heat (BB), and 4. BB with chemical heat pads (BB+HP). Core and mean skin temperature, metabolic heat production and thermal comfort were assessed. Prior to CAT, time to 36°C was not different ($P = 0.50$). Compared with PB and PB+HD, skin temperature was greater (PB $25 \pm 1$, PB+HD $25 \pm 1$, BB $27 \pm 2$, BB+HP $28 \pm 2$, TC $32 \pm 1 ^\circ C$, $P < 0.01$) and metabolic heat production lower during BB and BB+HP (PB $133 \pm 73$, PB+HD $149 \pm 74$, BB $112 \pm 73$, BB+HP $96 \pm 62$, TC $57 \pm 16 \, W\cdot m^{-2}$, $P < 0.01$). Thermal comfort was higher in BB+HP (cold) compared with PB+HD (very cold) ($P < 0.05$). Despite cold exposure, all methods supported re-warming to resting core temperatures in shivering casualties. However, less metabolic heat production was required to promote core temperature in triple-layered metallized survival bags compared with polythene survival bags. Hot drinks had no thermoregulatory or thermal comfort benefit.
6.2 Introduction

It is known that exposure to cold with hypoxia may induce greater health risks with regard to thermoregulatory homeostasis (Chapter 4). However, hypoxia aside, it is not clear whether these changes would be more severe with harsher conditions that induce mild hypothermia, and whether they can be treated appropriately with simple cold protection methods available for use in the field.

Accidental hypothermia affects approximately 10% of casualties reported in mountainous environments and is a significant contributor to fatalities (Sallis and Chassay, 1999; Sharp, 2007). Although mortality rates are relatively low with severe non-trauma related hypothermia (<28°C), they rise alarmingly with traumatic injury where mortality is 40, 69 and 100% at 34, 33 and 32°C, respectively (Moss, 1986; Jurkovich, 1987). Trauma is a frequent medical problem observed in mountainous environments, accounting for approximately 40% of all emergency call outs and 80% of all rescues (Hearns, 2003). Rescue times for casualties depend on global location and weather conditions. Yet even where distances are relatively short between emergency services and a casualty (e.g. Scottish Highlands) the reported times for evacuation are 2.25 hours by helicopter and approximately 3.5 hours when individuals are evacuated by others means (e.g. on foot) (Crocket, 1995; Hindsholm et al. 1992). Simple methods that prevent heat loss and promote re-warming that casualties or first-responders can administer instantly whilst they wait for evacuation will likely reduce hypothermia related fatalities.

Vapour proof (e.g. polythene) survival bags and metalized plastic sheeting are produced and carried for emergency use in cold and mountainous environments. These single layer products are also associated with lower shivering and higher core temperatures in anesthetized patients undergoing surgery (Buggy and Hughes, 1994; Allen et al. 2010). Contradictory to investigations with human subjects, where results showed single-layered devices to provide similar thermal protection as multi-layered devices (Appendix I), an in vitro study where cooling times of pre-heated fluid bags were compared between different field methods, a multi-layered survival bag (Blizzard Survival™)

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was shown to be superior at preventing heat loss from fluid filled bags compared with a polythene survival bag, single layer metalized plastic sheeting or wool blankets (Allen et al. 2010). Further, a combination of the multi-layered metalized sheeting survival bag (Blizzard Reflexcell™) with chemical heat pads was shown to be superior to all other methods tested (Allen et al. 2010). Given the limitations of a fluid filled bag model to simulate the complex thermoregulatory control of humans, in vivo studies are required to confirm the beneficial effects of a light-weight multi-layer metalized sheeting survival bag combined with chemical heat pads.

In the field sources of heat are limited to exercise, body-to-body contact, portable warming devices such as the charcoal burning ‘Heat Pac’, heat pads and the ingestion of warm drinks. Exercise has been shown to re-warm mildly cold casualties more quickly than shivering alone (Giesbrecht et al. 1987); however, it has obvious limited use for those forced to remain sedentary because of injury, unconscious state or poor weather. The administration of hot drinks is recommended practice in shivering cold casualties by the International Commission for Mountain Emergency Medicine (Durrer et al. 1998); however, to the best of the author’s knowledge, no study has investigated the efficacy of this practice. Studies investigating the effect of cold beverages indicate core temperature may be lowered by approximately 0.7°C with the ingestion of one litre of cold fluid (4-7°C) (Imms & Lighten, 1989; Lee et al. 2008). It is therefore hypothesized that the ingestion of a similar volume of fluid at 70°C could raise core temperature at least transiently.

Numerous studies have investigated re-warming efficacy of external heat application with results varying dependent on the amount of heat delivered and the surface area to which the heat is applied (Daanen et al. 1992; Giesbrecht et al. 1994). In mildly hypothermic casualties capable of normal shivering, externally applied heat sources (i.e. body-to-body contact and ‘Heat Pac’) have been shown to be either no better than shivering alone or detrimental to re-warming as they decrease shivering heat production (Giesbrecht et al. 1987, Giesbrecht et al. 1994). Studies examining these field methods have completed experiments in thermo-neutral conditions over short periods (<2
hours) as opposed to longer exposures in the cold which casualties typically experience awaiting rescue. It is plausible that in colder conditions, with modest heat sources not applied directly to the skin, a survival bag containing chemical heat pads (e.g. Blizzard Heat™) may confer a significant advantage for thermoregulation compared with polythene survival bags.

The aim of this investigation was to examine the effectiveness of four practical field methods for the treatment of cold casualties with a normal shivering response. A secondary aim was to examine the effect of each field method on long-term energy expenditure and thermal comfort. To simulate real-life awaiting-rescue events these methods were studied in a cold environment (0°C) following the reduction of core temperature with cold water immersion to 36°C. It was hypothesised that the core re-warming rate (°C/h) would be greatest in the Blizzard Heat bag with the chemical heat pads, followed by the Blizzard survival and polythene survival bag with hot drink. Polythene survival bag alone was hypothesised to be the least effective method of re-warming.
6.3 Method

6.3.1 Participants
Seven healthy males (mean ± SD: age, 21.4 ± 2.6 years; height, 177.8 ± 5.1 cm; nude body mass, 70.5 ± 5.2 kg; body surface area, 1.87 ± 0.10 m$^2$; body fat, 9.9 ± 3.0 %) volunteered to participate in this study which received local Ethics Committee approval. Prior to commencing trials participants completed weekly medical reports (Appendix H) to ensure the absence of illness prior to and during the experimental period and informed written consent.

6.3.2 Study design
Separated by 7 days, participants completed five randomised experimental trials (Research Randomiser; www.randomizer.org). The field re-warming interventions were a polythene bag (PB) (Giesbrecht et al. 1987), a polythene bag with a hot drink (PB+HD), Blizzard survival bag (BB) and Blizzard survival heat bag (BB+HP). The fifth trial was a thermo-neutral control (TC) where participants remained seated in an ambient temperature 20.2 ± 1.7°C.

6.3.3 Experimental procedures
The day prior to each experimental trial participants ingested water equal to 35 ml·kg$^{-1}$ body mass and the same meals. After an overnight fast and having consumed no caffeine or alcohol within 10 hours participants arrived to the laboratory, euhydrated at 0800 hours. After voiding bladder and bowels anthropometric measures of height and body mass were obtained. Body composition was also assessed (Chapter 3).

Following pre-trial measures, participants began a 30 minute seated rest in a thermo-neutral (TN) environment (ambient temperature: 19.2 ± 1.1°C; relative humidity (RH): 41 ± 6%). Core temperature ($T_{rec}$) was measured continuously and recorded every 5 minutes. Metabolic heat production (M) was assessed every 10 minutes and calculated via indirect calorimetry methods.
Thermal comfort (Hollies and Goldman, 1977) and pain sensation (Chen et al. 1998) were also recorded every 5 minutes. During TC the initial 30 minute rest period was followed by an additional 30 minute rest period.

After the 30 minute seated rest on cooling trials participants were immersed up to the axilla in 13.0 ± 0.1°C stirred water wearing swim shorts until core temperature reduced to 36°C. During cold water immersion $T_{rec}$, $M$, thermal comfort and pain were recorded every 5 minutes. Upon removal from water participants dressed in dry shorts, socks and gloves prior to entering the environmental chamber (0°C, RH 40% and wind velocity 0.2 m.s$^{-1}$: Delta Environmental Systems, Chester, UK). Socks and gloves were prescribed based upon results of pilot work which demonstrated pain at the extremities may cause necessary withdrawal. After 5 minutes seated, participants were given one of four interventions which covered whole body and head, then remained seated for the 3 hour CAT or until core temperature reached 35°C. During Blizzard Heat trials heat pads were placed evenly to the bag with Velcro adhesive and not placed directly onto skin to avoid contact with skin thermistors. Unlike supine rest in Chapter 4, a seated position was adopted in order for blood and saliva samples to be collected most efficiently. During field interventions participants consumed flavoured water of trace nutritional value in volumes equal to 6 ml·kg$^{-1}$ body mass at 0, 60 and 120 minutes. Participants consumed beverages within 15 minutes which were served in insulated drinks containers to minimise heat loss. During PB+HD beverages were served at 80°C whilst on other trials were 36°C. No food was provided during CATs. During the CAT $T_{rec}$, skin temperature ($T_{sk}$), thermal comfort and pain sensation were recorded every 5 minutes. Skin temperature was recorded at eight sites using skin thermistors. Weighted mean skin temperature was calculated using an area weighted formula (ISO 9886, 2004). Expired gases were collected every 10 minutes for the determination of M. Afterdrop period was measured in minutes as the total time taken for an increase in core temperature following exit of the cold water and entrance to the chamber. Urine was collected ad libitum from the onset of cold stress until re-warming had commenced. Samples
were stored at -40°C and later thawed for analysis of urinary urea nitrogen content (Randox Laboratories, Co. Antrim, UK). Following CATs participants were immersed up to the axilla in 40°C water until core temperature reached 36°C or 1 hour passed. Participants re-clothed and consumed a meal (1234 Kcal; CHO 58%, Fat 28%; Protein 14%) (Costa et al. 2010). Following 2 hours seated rest and monitoring, participants left the laboratory. On TC participants sat for the same time period as in the CATs but freely choose their attire. They were not permitted to eat food during this period and were required to consume fluids (36°C) equal to 6 ml·kg⁻¹ body mass at 0, 60 and 120 minutes.

### 6.3.4 Calculations

The quantity of urinary nitrogen excreted and collected during the entire cold stress period was used as an index of protein oxidation (Ravussin et al. 1985). The oxidation of carbohydrate and lipid were assessed by the amount of oxygen consumed per gram of substrate oxidized:

\[
\text{Carbohydrate Oxidation (g·4h}^{-1}) = 4.21 \cdot \text{VCO}_2 - 2.96 \cdot \text{VO}_2 - 2.37 \cdot N \\
\text{Lipid Oxidation (g·4h}^{-1}) = 1.7 \cdot \text{VO}_2 - 1.7 \cdot \text{VCO}_2 - 1.77 \cdot N \\
\text{Protein Oxidation (g·4h}^{-1}) = \text{urinary urea nitrogen} \cdot 6.25 \\
\text{Energy Expenditure (kJ) = 16.18 \cdot VO}_2 + 5.02 \cdot \text{VCO}_2 - 5.99 \cdot N}
\]

\[
\text{Nitrogen (N) = urinary nitrogen in grams}
\]

### 6.3.5 Statistical analyses

A sample of six was estimated using rectal temperature data from a previous investigation (Giesbrecht et al. 1987). To allow for subject incompletion seven participants were recruited. A one-way fully repeated measures ANOVA was performed on body composition data and on re-warming parameters. Two-way fully repeated-measures ANOVA (time x trial) were performed on thermoregulatory and metabolic variables.
6.4 Results

6.4.1 Preliminary and cold water immersion measures

Throughout the experimental period body mass and composition did not change (Table 6.1: Body mass: \( P = 0.496 \); Body fat: \( P = 0.631 \)). Participants were always hydrated upon arrival to the laboratory (Chapter 3). Core temperature prior to cold water immersion was similar in each trial (Table 6.1: \( P = 0.881 \)). No trial differences occurred between oxygen consumption or time required for individuals to reach 36°C (Table 6.1 and Figure 6.3: \( P = 0.347 \) and \( P = 0.496 \)). Oxygen consumption was greater during cold water immersion compared with seated rest (\( P = 0.000 \)). When analysed for order of trial (e.g. trial 1 vs. 4) there were no differences \( T_{\text{rec}} \) (\( P = 0.917 \)) or time to 36°C (\( P = 0.660 \)) suggesting that a single weekly cold water immersion evokes no cold acclimatisation.

**Table 6.1.** Body composition and resting core temperature (\( T_{\text{rec}} \)) immediately prior to cold water immersion and time taken to reach 36°C during the cold water immersion.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Body Mass (Kg)</th>
<th>Body Fat (%)</th>
<th>Mean ( T_{\text{rec}} ) during 30min rest (°C)</th>
<th>Time to 36°C (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB</td>
<td>70.4 ± 5.5</td>
<td>9.5 ± 3.3</td>
<td>36.7 ± 0.2</td>
<td>28 ± 11</td>
</tr>
<tr>
<td>PB+HD</td>
<td>70.4 ± 5.8</td>
<td>9.6 ± 2.4</td>
<td>36.6 ± 0.2</td>
<td>28 ± 11</td>
</tr>
<tr>
<td>BB</td>
<td>70.2 ± 6.0</td>
<td>10.1 ± 3.8</td>
<td>36.7 ± 0.2</td>
<td>35 ± 19</td>
</tr>
<tr>
<td>BB+HP</td>
<td>71.0 ± 5.4</td>
<td>10.4 ± 3.0</td>
<td>36.7 ± 0.3</td>
<td>36 ± 18</td>
</tr>
<tr>
<td>TC</td>
<td>70.5 ± 5.1</td>
<td>9.8 ± 3.1</td>
<td>36.7 ± 0.2</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Results are means ± SD, \( n = 7 \). Abbreviations: PB, polythene survival bag; PP+HD, hot drink plus polythene survival bag; BB, Blizzard™ Survival bag; BB+HP, Blizzard Heat™ Survival bag; TC, Thermo-neutral control; N/A, not applicable.
6.4.2 Cold Air Test Results

Initial re-warming (0–30 mins post cold water immersion)

There were no significant differences in re-warming parameters between the four interventions (Table 6.2). Skin temperature was however higher during the first hour on BB and BB+HP compared with PB and PB+HD (Figure 6.2: \( P = 0.001 \)). Additionally in the first hour, metabolic heat production was similar in BB and BB+HP compared with TC; whereas it was higher in PB and PB+HD (Figure 6.3: \( P = 0.028 \)).

Table 6.2. A summary of re-warming parameters

<table>
<thead>
<tr>
<th>Trial</th>
<th>Afterdrop magnitude (°C)</th>
<th>Time to afterdrop nadir (mins)</th>
<th>Afterdrop period (mins)</th>
<th>Re-warming rate (°C·h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB</td>
<td>0.55 ± 0.28</td>
<td>20 ± 13</td>
<td>76 ± 61</td>
<td>0.39 ± 0.11</td>
</tr>
<tr>
<td>PB+HD</td>
<td>0.61 ± 0.21</td>
<td>21 ± 12</td>
<td>93 ± 63</td>
<td>0.37 ± 0.14</td>
</tr>
<tr>
<td>BB</td>
<td>0.51 ± 0.13</td>
<td>24 ± 19</td>
<td>74 ± 41</td>
<td>0.34 ± 0.10</td>
</tr>
<tr>
<td>BB+HP</td>
<td>0.62 ± 0.26</td>
<td>19 ± 18</td>
<td>84 ± 58</td>
<td>0.37 ± 0.12</td>
</tr>
</tbody>
</table>

Results are means ± SD, \( n = 7 \). Statistics: Afterdrop magnitude, \( F_{(3,18)} = 0.610, P = 0.617, \eta^2 = 0.092 \); Time to afterdrop nadir, \( F_{(3,18)} = 0.311, P = 0.817, \eta^2 = 0.045 \); Afterdrop period, \( F_{(3,18)} = 0.439, P = 0.728, \eta^2 = 0.068 \); Re-warming rate, \( F_{(3,18)} = 0.522, P = 0.673, \eta^2 = 0.082 \). Effect sizes (\( \eta^2 \)) interpreted as small (0.01), medium (0.06) and large (0.14) (Cohen, 1988).

6.4.3 Longer Term Cold Protection (30 – 180 mins): Core and skin temperature

Compared with the TC, \( T_{rec} \) was lower in all cold trials (\( F_{(32,192)} = 7.936, P = 0.000, \eta^2 = 0.112 \)). However there were no \( T_{rec} \) differences during cold exposure between the four interventions. All methods returned \( T_{rec} \) to within normal resting core temperature by 180 min. Mean weighted skin temperature (\( T_{sk} \)) was higher TC compared to re-warming trials (Figure 6.2: \( F_{(24,144)} = 10.039, P = \).
0.000, $\eta^2 = 0.044$). By 10 minutes into the cold air test all practical field methods had increased $T_{sk}$ compared to 0 minutes ($P = 0.000$). However compared with PB and PB+HD, $T_{sk}$ was higher from 30 minutes on BB and BB+HP. There were no differences between BB and BB+HP or PB and PB+HD.

**Figure 6.1.** The effect of 180 minute cold air test (CAT; 0°C) on core temperature in four field cold casualty interventions and a thermo-neutral control. TN; thermo-neutral, End CWI; end of cold water immersion. Values are Means ± SD. * significant difference vs. TC ($P < 0.05$).
**Figure 6.2.** The effect of 180 minute cold air test (CAT; 0°C) on mean weighted skin temperature in four field cold casualty and a thermo-neutral control. Values are Means ± SD. * significant difference vs. TC, † significant difference vs. PB, ‡ significant difference vs. PB+HD ($P < 0.05$).

### 6.4.4 Metabolic Responses

Metabolic heat production was higher than TC on all cold trials at the end of the cold water immersion and at 0 min of the cold air test (Figure 6.3: $F_{(32,192)} = 10.599, P = 0.000, \eta^2 = 0.158$). Metabolic heat production remained higher than TC in PB (10-30 min) and PB+HD (10-180 min).

In contrast, no differences occurred between TC and BB or BB+HP from 10-180 min. However, cold exposure increased the oxidation of carbohydrates 3 fold during PB, PB+HD and BB+HP above TC. Despite this, carbohydrate oxidation during BB was not different to other trials (Figure 6.4: $F_{(4, 24)} = 3.809, P = 0.016, \eta^2 = 0.388$). Regardless of cold exposure, the oxidation rate of lipids
was not different between any trial (Figure 6.4: $F_{(4, 24)} = 0.992, P = 0.432, \eta^2 = 0.142$). However, while not statistically significant, protein oxidation in the cold tended to be greater than TC (Figure 6.4: $F_{(4, 24)} = 2.690, P = 0.055, \eta^2 = 0.310$). Energy expenditure from the onset of cold stress was greater PB+HD compared to all other trials (Figure 6.5: $F_{(4, 24)} = 52.240, P = 0.000, \eta^2 = 0.310$). Energy expenditure during BB and BB+HP were not different ($P = 0.079$), however values were less than PB ($P = 0.025$) yet greater than TC ($P = 0.001$).

**Figure 6.3.** The effect of 180 minute cold air test (CAT; 0°C) on metabolic heat production in four field cold casualty interventions and a thermo-neutral control. TN; thermo-neutral, End CWI; end of cold water immersion. Values are Means ± SD. * significant difference vs. TC, † significant difference vs. PB, ‡ significant difference vs. PB+HD ($P < 0.05$).
Figure 6.4. The effect of 180 minute cold air test (CAT; 0°C) on substrate oxidation in four field cold casualty and thermo-neutral control. Values are Means ± SD. * significant difference vs. TC ($P < 0.05$).
Figure 6.5. The effect of 180 minute cold air test (CAT; 0°C) on energy expenditure in four field cold casualty interventions and thermo-neutral control. Values are Means ± SD. * significant difference vs. TC, † significant difference vs. PB, ‡ significant difference vs. PB+HD ($P < 0.05$).

6.4.5 Thermal comfort and pain sensation

Thermal comfort was higher throughout TC compared to the cold trials (Figure 6.6. $F_{(32,192)} = 3.734, P = 0.000, \eta^2 = 0.071$). Following 30 minutes of cold exposure thermal comfort was higher during BB+HP compared with PB+HD ($P < 0.05$). Pain sensation was lower during TC compared to all cold trials (Figure 6.5. $F_{(32,192)} = 3.880, P = 0.000, \eta^2 = 0.077$).
Figure 6.6. The effect of 180 minute cold air test (CAT; 0°C) on thermal comfort in four field cold casualty interventions and a thermo-neutral control. Values are Means ± SD. * significant difference vs. TC, ‡ significant difference vs. PB+HD ($P < 0.05$).
Figure 6.7. The effect of 180 minute cold air test (CAT; 0°C) on pain sensation in four field cold casualty interventions and a thermo-neutral control. Values are Means ± SD. * significant difference vs. TC ($P < 0.05$).

6.4.6 Results summary

Core temperature returned toward normal following three hours in all field treatments. Mean skin temperature however increased faster and was maintained at a higher level during both Blizzard trials. Metabolic heat production, thermal comfort and pain sensation were most similar to TC during BB+HP. Energy expenditure was less during BB+HP compared with any other treatment.
6.5 Discussion

The results of this investigation imply that the four interventions supported rewarming to near normal resting core temperatures of shivering cold casualties within three hours of cold exposure. Although typical rewarming parameters were not different between either intervention metabolic heat production was greater in polythene compared with the Blizzard survival bags during the first hour of cold exposure. Furthermore, re-warming in the Blizzard survival bags was achieved with lower total energy expenditure which may be beneficial for long term survival (Tikuisis, 1995; Tikuisis et al. 2002).

As anticipated multi-layer, metalized sheeting survival bags led to thermoregulatory advantages compared with the polythene survival bag with or without a hot drink. The administration of hourly hot drinks (~430 ml) seemed to confer no advantage in re-warming or the maintenance of core or skin temperature during cold exposure when compared with polythene survival bags alone. It has been reported that provided shivering is not contraindicated, core re-warming rate is not different in cold individuals using either active or passive rewarming devices (Williams et al. 2005). In contrast, Blizzard survival bags maintained a higher skin temperature throughout the cold air test which is indicative of superior cold protection and may be speculated to indicate a lower likelihood of peripheral cold injury. On the contrary, while vasoconstriction reduces heat loss from the skin, the reduced peripheral blood flow also increases tissue insulation helping to maintain core temperature (van Ooijen et al. 2005; Toner and McArdle, 1986). The application of external heat sources to the skin of an individual in the cold may blunt the vasoconstrictive response forcing heat to be transferred from the core to the skin (Grant et al. 2002). Furthermore, cutaneous temperatures greater than 29°C reduce peripheral thermo-receptor activity resulting with inadequate stimuli to activate shivering (Vokac and Hjeltnes, 1981; Rennie, 1988; Mercer. 1991). When shivering is blunted through peripheral warming in a severe cold environment, heat loss may exceed heat production (Giesbrecht et al. 1994; Grant et al. 2002). In the present study however, cutaneous
temperatures of participants never exceeded this threshold compared with normothermic individuals in earlier work (Grant et al. 2002). As a result, vasoconstriction was not blunted and as such, large amounts of heat were not transferred from the core to the periphery, thus these methods provided a more efficient heat source by conserving more heat.

Upon initial exposure to the cold, metabolic heat production was increased compared to thermo-neutral in all rewarming trials. By 90 minutes however, metabolic heat production had decreased on the Blizzard Heat trial to within thermo-neutral control values that were approximately 40% lower than those recorded during the polythene survival bag trials. Co-incidentally, heat production during polythene survival bag trials fluctuated throughout, that may reflect greater shivering thermogenesis (van Ooijen et al. 2005). Since warming of peripheral thermo-receptors reduces shivering and blunts metabolic heat production (Giesbrecht et al. 1987), cooler skin temperatures demonstrated during both polythene survival bag trials may have stimulated a greater thermo-receptor response and thus, shivering activity. Comparatively, during Blizzard Heat the warmer skin temperatures provided less stimulation to activate shivering and therefore produced less metabolic heat production. This has been considered detrimental for maintaining heat balance in a cold casualty (Giesbrecht et al. 1987; Giesbrecht et al. 1994). However, if cold exposure is not recognised by the body as severe shivering will not begin (Vybiral et al. 2000). Cold protection provided by the Blizzard Heat survival bag may therefore reduce the amount of heat an individual needs to produce through shivering and non-shivering thermogenesis. Given the amount of noise produced by survival bags in contact with the skin, it was not ecological to measure shivering via electromyography in this study. Nonetheless, metabolic heat production provides a clear indication of how much extra the body needed to work in order to maintain core temperature. Additionally, participants subjectively rated the amount of cold induced pain they felt in each bag which gave a representation of their perception of physical output needed to keep warm.
While survival depends on the capacity to counterbalance the rate of heat loss by increasing thermogenic rate (Haman, 2006), Blizzard survival bags may have significant benefit in longer term survival where food sources might be restricted and finite. Specifically, the lower cold-induced energy expenditure during the Blizzard Heat trial coupled with a continuous increase in core temperature likely provides a more efficient re-warming effect whereby energy is preserved and fatigue from excessive shivering is reduced (Martineau and Jacobs, 1988; Vallerand and Jacobs 1989; Vallerand et al. 1993). As a likely consequence of the superior cold protection provided by Blizzard Heat, perceptual measures (i.e. thermal comfort and pain) tended to be better during the cold air test compared with when individuals were in the polythene survival bag. In combination with previous *in vitro* results (Allen *et al*. 2010) it is speculated that where cold casualties have impaired shivering, Blizzard survival bags would also provide superior cold protection including re-warming capacity compared to polythene survival bags. Future studies where shivering activity is blunted either medically, or through the use of thermal manikins would help determine this superiority. An investigation with the superior rewarming interventions compared to no protection in more severe conditions would also identify any further strengths or weaknesses. To conclude, in contradiction to the hypothesis, all four of the cold treatment methods tested in 0°C, assisted shivering cold casualties to increase core temperature to normothermia within the same 3 hour period. Nonetheless, of the four methods the Blizzard Heat survival bag was the most efficient for field re-warming given a reduced metabolic heat production was required to increase core temperature.
CHAPTER SEVEN

Salivary IgA and Amylase Responses to Mild Hypothermia and Prolonged Cold Exposure

7.1 Abstract

The aim of this study was to examine the effect of mild hypothermia and prolonged cold exposure upon s-IgA and α-amylase responses. Twelve males completed two experimental trials which included a thermo-neutral control (TC) and a cold trial (COLD). On COLD, participants wore shorts only and were cooled to 36°C core temperature ($\text{T}_{\text{rec}}$) in 13°C water. They then completed a 3-hour cold air test (CAT, 0°C) with a polythene survival bag to prevent core temperature decreasing beyond ethical guidelines. Unstimulated whole saliva samples were collected at baseline, lowest $\text{T}_{\text{rec}}$, 1 h CAT, 2 h CAT and immediately after, 1 h and 3 h after CAT. An interaction was observed for saliva flow rate and s-IgA secretion rate ($F(6,66) = 3.379, P = 0.006; F_{(3.086, 33.947)} = 3.697, P = 0.020$, respectively). Saliva s-IgA secretion rate was significantly lower during COLD compared to TC ($P < 0.05$). Compared with lowest $\text{T}_{\text{rec}}$, saliva s-IgA secretion rate was higher at 1 and 3 h post CAT ($P < 0.05$) during COLD, but no differences occurred throughout TC. Saliva flow rate was significantly lower during the first hour of CAT during COLD compared with TC ($P < 0.05$) and remained lower until 3 h post. Saliva s-IgA concentration was significantly reduced following baseline measures on both cold and thermo-neutral trials ($P < 0.01$), whereas α-amylase concentration was greater throughout CAT compared to TC until re-warming had occurred ($P < 0.05$). These results suggest a reduction in core temperature ($\geq 1.5^\circ \text{C}$) resulting from cold water immersion and subsequent cold air exposure alters the usual daily saliva responses which may increase susceptibility to illness and infection (i.e. URTI, common colds, influenza) if re-warming is not initiated immediately.
7.2 Introduction

It is known that exposure to cold with hypoxia may induce greater health risks with regard to mucosal immunity compared to exposure to cold alone (Chapter 5). However, it is not clear whether these changes would be more severe in colder conditions that induce mild hypothermia. Furthermore, current knowledge in this area has not been verified in accordance with diurnal fluctuations and thermal-neutral control trials for comparison.

It is the popular belief that most common colds occur because the host is exposed to a cold environment beforehand (Dowling et al. 1958; Biggar et al. 1984). However, a direct causal relation between getting cold and developing an infection remains to be clarified (Lavoy et al. 2011). Cold exposure has been associated with an increased incidence of bronchorrhea, sinusitis, and upper respiratory tract infections (URTIs) (Clothier, 1974; Giesbrecht, 1995; Castellani et al. 2002; Lavoy et al. 2011). However, much of this research has examined individuals undertaking intense physical activity in the cold (i.e. athletes and military personnel). Cold-induced decrements in immune-surveillance are a particular problem for physically active individuals (Brenner et al. 1999). For example, URTIs are the main cause of illness and missed practice in elite cross country skiers (Berglund and Hemmingson, 1990). Nonetheless, the risk of illness or infection for those forced or required to remain sedentary in the cold, due to injury or adverse weather, for a prolonged period of time is unknown.

It has been suggested that a continuum exists for the effects of body core cooling on immune function whereby mild decreases in body core temperature have little stimulatory effects on immune function, but more severe decreases have depressive effects (Costa et al. 2010). However, it remains to be shown whether large ($\geq 1.5^\circ$C) reductions in body core temperature suppress mucosal immunity and increase the likelihood of infection (Costa et al. 2010).
A decrease in the saliva concentration of s-IgA accompanies an increased susceptibility to URTI (Gleeson et al. 1999; Hanson et al. 1983; Nieman and Nehlsen-Cannarella, 1991). However, breathing cold dry air itself can elicit symptoms similar to these infections, such as a persistent dry cough and nasal mucosal drying (Silvers, 1991; Giesbrecht, 1995). Thus it may be that cold weather negatively impacts the respiratory system independently of changes in immunity. For example, performing prolonged exercise in freezing cold conditions did not influence saliva flow rate or s-IgA secretion rate responses (Walsh et al. 2002). However, modest whole body cooling (T_{rec} 35.9°C) at rest showed reduced s-IgA responses (Costa et al. 2010). Unfortunately, a lack of a thermo-neutral control trial for comparison means changes may reflect diurnal variation of saliva concentrations (Walsh et al. 2002). The influence of prolonged cold exposure upon s-IgA and α-amylase responses in clinically hypothermic individuals (T_{rec} 35°C) has received little attention. In the field, cold stress may evoke suboptimal host defence that subsequently may increase the risk of illness and infection (Johnson et al. 1977; Houben et al. 1982; Hiramatsu et al. 1984).

Evidence to date indicates that a mild decrease in core body temperature (T_{rec} decrease ~ 0.5°C) during short (30 min) (Lackovic et al. 1988) or prolonged (2 h) (Brenner et al. 1999) cold air exposure can have immuno-stimulatory effects. During short cold air exposures (4°C) natural killer cell activity was found to increase (Lackovic et al. 1988). The immersion of healthy males in cold water (14°C) has been shown to induce a leukocytosis (Jansky et al. 1996). However, it is unknown what core temperature participants presented with during and following these immersions. Therefore, little information is available from controlled laboratory studies regarding any immuno-stimulatory effects of a decrease in core temperature ≥1.5°C. Further, it remains unclear how prolonged (3 h) cold air exposure in combination with such a decline in core temperature effects s-IgA and α-amylase responses. Previous explanations for the disruption to mucosal immunity observed with modest whole-body cooling at rest have included: cold air decreasing the temperature of mucosal membranes, the subsequent drying effect which may reduce mucociliary clearance and
phagocytic activity (Eccles, 2002), and a cooling-evoked neuro-endocrine regulation of trans-epithelial s-IgA translocation (Giesbrecht, 1995; Proctor and Carpenter, 2007).

The aim of the present study, therefore, was to examine the effect of mild hypothermia and prolonged cold exposure on s-IgA and α-amylase responses. It was hypothesised that mild hypothermia and cold exposure would lower s-IgA and amylase secretion in comparison with normal responses in a thermo-neutral environment. Given it would be unethical to expose participants to cold without some form of thermal protection, the polythene bag was chosen as it offers minimal warmth to the body inside compared with other devices (Chapter 6) and therefore would prevent a blinding of the effects of cold on immune function.
7.3 Method

7.3.1 Participants

Twelve healthy males (mean ± SD: age, 20.7 ± 2.2 years; height, 177.5 ± 6.6 cm; nude body mass, 68.6 ± 6.4 kg; body fat, 10.9 ± 3.7%) participated in this study. Participants gave written consent and medical history before the study, which received local Ethics Committee approval. Participants also completed the Wisconsin Upper Respiratory Symptom Survey (Appendix H) two weeks prior to and during the experimental period.

7.3.2 Preliminary measurements

Before each experimental trial participants arrived at the laboratory after an overnight fast having consumed no food, caffeine or alcohol within 10 h. Participants ingested water equal to at least 35 ml·kg\(^{-1}\) body mass the day prior to each trial. After voiding bladder and bowels, nude body mass and height were measured. Using segmental multiple frequency bioelectrical impedance measures of body fat were obtained.

7.3.3 Experimental Trials

Separated by 7 d, participants completed two randomised experimental trials (Research Randomiser; www.researchrandomizer.org). The two trials were: cold exposure with a polythene survival bag (COLD) and a thermo-neutral control trial (TC). A polythene survival bag was necessary to prevent core temperature falling to below mild hypothermic levels. Following pre-trial measures participants began each experimental trial at 0800. Following 30 minutes seated rest in a thermo-neutral environment (ambient temperature: 19.2 ± 1.1°C; relative humidity: 40.9 ± 6.0%), the first saliva sample was collected.
For the cold trial participants were immersed in 13.0 ± 0.1°C stirred water up to the axilla wearing swim shorts only until core temperature reduced 36°C. Upon removal from the water and after drying, participants entered the chamber where they remained seated for a 3 h cold air test (Dry bulb temperature ($T_{db}$) of the chamber remained constant at 0°C with a relative humidity (rh) 40% and 0.2 m.s$^{-1}$ wind velocity; Delta Environmental Systems, Chester, UK). Once in the chamber, participants sat wearing dry shorts only for five minutes before being given the survival bag. Expired gases were collected every 10 minutes for the determination of $M$. Saliva was collected at baseline, lowest $T_{rec}$, 1 h, 2 h and 3 h during cold exposure. The lowest $T_{rec}$ sample was collected in the chamber once core temperature showed the first sign of increasing following a distinct afterdrop phase. Early removal from the chamber occurred if core temperature reduced to 35°C. Following the cold air test participants were immersed in 40°C water to increase core temperature. After 1 h participants gave another saliva sample, re-clothed and consumed a meal (1234 Kcal; CHO 58%, Fat 28%; Protein 14%) (Costa et al. 2010). Following a further 2 h recovery, participants gave a final saliva sample and were free to leave the laboratory.

All participants completed a control trial to account for diurnal fluctuations in s-IgA concentration (Gleeson et al. 2001; Walsh et al. 2002). During the control trial participants remained seated in an ambient temperature of 20.2 ± 1.7°C and wore normal clothing (0.8-1.0 clo / 1.24-1.55 togs). Saliva samples were collected in the same manner as those in the cold trial.

7.3.4 Saliva collection and analysis

Un-stimulated whole saliva samples were collected using pre-weighed universal tubes (Chapter 3). Saliva samples were collected while the participant sat quietly in the laboratory. Saliva volume was estimated by weighing the universal container immediately after collection to the nearest mg. From this, saliva flow rate was determined by dividing the volume of saliva by the collection time. Saliva
was aspirated into Eppendorf tubes and stored at -40°C for further analysis. After thawing s-IgA concentration was determined by ELISA (Salimetrics Europe Ltd, Suffolk, UK) using IgA monomer from human serum as standard. The intra-assay CV was 2.6%. s-IgA secretion rate was calculated by multiplying the saliva flow rate by s-IgA concentration.

Alpha amylase concentration was determined by ELISA (Salimetrics Europe Ltd, Suffolk, UK) using a chromagenic substrate, 2-chloro-p-nitrophenol linked with maltotriose. The intra-assay CV was 2.1%.

### 7.3.5 Statistical analyses

A sample size of ten participants was estimated (http://www.dssresearch.com/toolkit/sscalc) using previous data examining the effects of stress on immune indices (Costa et al. 2010; Laing et al. 2008; Oliver et al. 2007). One way fully repeated measures ANOVA were performed on pre-experimental body composition measures. Two-way fully repeated measures ANOVA were performed on saliva parameters.
7.4 Results

7.4.1 Body mass and composition

Throughout the experimental period body mass and composition did not change (Table 7.1: Body mass: $P = 0.733$; Body fat: $P = 0.097$). Core temperature prior to cold water immersion was similar in each trial (Table 7.1: $P = 0.545$).

Table 7.1. Resting core temperature ($T_{\text{rec}}$) immediately prior to cold water immersion and time taken to reach 36°C during the cold water immersion

<table>
<thead>
<tr>
<th>Trial</th>
<th>Body mass (Kg)</th>
<th>Body fat (%)</th>
<th>Resting $T_{\text{rec}}$ (°C)</th>
<th>Time to 36°C (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold</td>
<td>68.6 ± 6.4</td>
<td>10.9 ± 3.7</td>
<td>36.8 ± 0.3</td>
<td>29 ± 14</td>
</tr>
<tr>
<td>TC</td>
<td>68.7 ± 6.2</td>
<td>11.4 ± 3.7</td>
<td>36.8 ± 0.2</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Results are means ± SD, n = 12. Abbreviations: TC, Thermo-neutral control; N/A, not applicable.

7.4.2 Thermoregulatory and metabolic responses

Compared with the thermo-neutral control, $T_{\text{rec}}$ and mean weighted skin temperature ($T_{\text{sk}}$) were lower during cold exposure (Table 7.2: $F_{(1.877,15.012)} = 4.192$, $P = 0.038$, $\eta^2 = 0.113$ and $F_{(24,144)} = 10.039$, $P = 0.000$, $\eta^2 = 0.044$, respectively). During the first hour of the cold air test, metabolic heat production was higher in the cold compared with thermo-neutral (Table 7.2: $F_{(1.073,8.583)} = 11.531$, $P = 0.008$, $\eta^2 = 0.083$).

Table 7.2. Summary of mean thermoregulatory parameters

<table>
<thead>
<tr>
<th>Trial</th>
<th>Lowest Core</th>
<th>Mean Weighted Skin</th>
<th>Metabolic Heat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature (°C)</td>
<td>Temperature (°C)</td>
<td>Production (W·m²)</td>
</tr>
<tr>
<td>COLD</td>
<td>35.4 ± 0.3</td>
<td>25.0 ± 1.3</td>
<td>150 ± 64</td>
</tr>
<tr>
<td>TC</td>
<td>36.6 ± 0.3</td>
<td>31.6 ± 1.0</td>
<td>49 ± 11</td>
</tr>
</tbody>
</table>

Results are means ± SD, n = 12.
7.4.3 Salivary secretory IgA and alpha amylase concentration

Saliva s-IgA secretion rate was lower during COLD at baseline and throughout CAT compared to TC (Figure 7.1C: \( P = 0.05 \)). Saliva s-IgA secretion rate however, was similar between the trials following one hour of recovery from cold exposure. Compared with lowest core temperature, saliva s-IgA secretion rate was higher at one and three hours post CAT during the cold trial, but no differences occurred throughout the thermo-neutral control. An interaction was observed for both saliva flow rate and s-IgA secretion rate (Figure 7.1A: \( F_{(6, 66)} = 3.379, P = 0.006, \eta^2 = 0.280 \); Figure 7.1C: \( F_{(3.086, 33.947)} = 3.697, P = 0.020, \eta^2 = 0.417 \) respectively). A main effect of time was observed for saliva s-IgA concentration (Figure 7.1B: \( F_{(2.93, 32.262)} = 7.696, P = 0.001, \eta^2 = 0.386 \)). Saliva flow rate was lower during the first hour of the CAT compared with thermo-neutral and remained lower until three hours post CAT. Saliva flow rate was higher three hours post-CAT compared with lowest core temperature. Following the initial morning sample there were no further changes in saliva flow rate during the thermo-neutral control. Saliva s-IgA concentration was reduced following baseline measures on both cold and thermo-neutral trials (\( P = 0.01 \)).

Saliva \( \alpha \)-amylase concentration was greater during COLD throughout CAT compared to TC (Figure 7.2A: \( F_{(1, 8)} = 8.506, P = 0.019, \eta^2 = 0.5150 \)). When core temperature was at its lowest during COLD, \( \alpha \)-amylase concentration was at its greatest. At 1h post CAT and 3h post CAT \( \alpha \)-amylase concentration in COLD and TC was the same (\( P = 0.05 \)). Despite a small increase when core temperature was at its lowest, saliva secretion rate remained unchanged between TC and COLD (Figure 7.2A: \( F_{(1, 8)} = 0.547, P = 0.481, \eta^2 = 0.063 \)).
**Figure 7.1.** The effect of 180 min cold air test (CAT; 0°C) on saliva flow rate (µl·min⁻¹) (A), s-IgA concentration (µg·ml⁻¹) (B) and secretion rate (µg·min⁻¹) (C) in comparison to a thermo-neutral control. Values are means ± SD. * significant difference vs. thermo-neutral, † significant difference vs. lowest Trec, ‡ significant difference vs. baseline (P < 0.05).
Figure 7.2. The effect of 180 min cold air test (CAT; 0°C) on saliva alpha amylase concentration (µg·ml⁻¹) (A) and amylase secretion rate (µg·ml⁻¹) (B) in comparison to a thermo-neutral control. Values are means ± SD. * significant difference vs. thermo-neutral (P < 0.05).

7.4.4 Results summary

Saliva s-IgA secretion rate was lower during COLD compared to TC. Saliva flow rate was lower during the first hour of the CAT compared with TC and remained lower until three hours post CAT. Saliva α-amylase concentration was greater during COLD throughout CAT compared to TC.
7.5 Discussion

The aim of the present study was to examine the effect of mild hypothermia and prolonged cold exposure on mucosal immunity in comparison to a thermo-neutral control trial. This novel design allowed for the diurnal variation in mucosal parameters to be accounted for. In support of the hypothesis, mild hypothermia evoked a reduction in saliva flow rate and an increase in α-amylase concentration which was not due to the time of day samples were collected. Saliva-IgA secretion rate was lower and α-amylase concentration was higher throughout the cold exposure until re-warming to within normal resting core temperature values had occurred.

Given the presence of a thermo-neutral control trial, these results extend previous research that showed whole body cooling (T_{rec} 35.9°C) via cold air exposure (0°C) decreased s-IgA secretion rate compared to baseline (Costa et al. 2010). Explanations included the effects of cold air reducing the temperature of the mucosal membranes, a possible drying effect of cold air and whole body cooling-evoked neuro-endocrine regulation of trans-epithelial s-IgA translocation (Giesbrecht, 1995; Proctor and Carpenter, 2007). The time course of the response suggested that whole body cooling decreases s-IgA translocation because s-IgA synthesis usually takes many hours to days (Costa et al. 2010; Hucklebridge et al. 1998). However, such explanations could only have been speculative owing to an absent control trial. During the present study a more intense cooling procedure induced mild hypothermia (T_{rec} 35°C) in almost all individuals. The diurnal variation pattern for s-IgA responses was similar throughout both cold and thermo-neutral conditions. However, both saliva flow rate and s-IgA secretion rate were markedly suppressed by mild hypothermia and cold exposure.

Early experiments (Proetz, 1953; Baetjer, 1967) examining beat frequency of cilia in the paranasal sinuses of rabbits and the trachea of chicks indicated that exposure to cold air decreases the temperature of the nasal respiratory epithelium and causes a decrease in mucociliary clearance (Eccles, 2002). A decrease in mucociliary clearance associated with cold exposure has been
suggested to increase the predisposition to respiratory infection (Diesel et al. 1991). With this in mind, throughout each trial, temperature controlled fluid (36°C) was consumed hourly in order to be able to combine findings with chapter 4. It is plausible that this fluid, similar to body temperature, may have reduced the drying effect of cold air and maintained the temperature of the mucosal membranes. It is speculated therefore that these earlier explanations for changes in mucosal immunity are less likely within the present study and an alternative factor is responsible for changes in saliva flow rate, s-IgA secretion rate and α-amylase concentration.

A body of literature exists, detailing saliva responses during exercise in the cold (Mylona et al. 2002; Walsh et al. 2002; Costa et al. 2010). However, the present study is the first to observe a reduction in the saliva flow rate and s-IgA secretion rate but an increase in the α-amylase concentration of mildly hypothermic, sedentary individuals exposed to the cold. Saliva flow rate, s-IgA secretion rate and α-amylase concentration were only similar to those levels observed during thermo-neutral control after three hours of re-warming and consumption of a hot meal. Despite this, that cold exposure and mild hypothermia disrupt mucosal immunity should be suggested with caution. This is because the s-IgA secretion rate recorded at rest, prior to cold exposure, was lower than at that same time during thermo-neutral control. It is possible this was due to an anticipatory effect of the forthcoming cold water immersion. Psychological stress, defined as the experience of negative events or the perception of distress, has been shown to alter saliva total protein concentration, α–amylase activity and IgA concentration (Bosch et al. 1996; Cohen et al. 2001). Such as, an increase in saliva total protein concentration but a decrease in saliva α-amylase activity was observed in patients awaiting dental treatment compared with a non-stress situation (Morse et al. 1983). It is possible that participants in the present study were more anxious upon arrival to the laboratory on the day they were going to be exposed to the cold compared with when they remained warm. In future, blinding participants to the experimental trial prior to baseline samples may be beneficial. On the contrary, after re-warming and a meal, s-IgA secretion rate was greater and α-
amylase concentration lower than when participants displayed their lowest core temperature, whereas no changes occurred throughout the thermo-neutral trial. This suggests that until re-warming is initiated, cold, sedentary individuals are at risk from increased mucosal immune disturbance.

In conclusion, a reduction in core temperature resulting from cold water immersion and cold air exposure alters the usual daily mucosal response which may increase a person’s susceptibility to illness and infection (i.e. URTI, common colds, influenza), particularly if they are not rewarmed immediately. In combination with earlier findings (Chapter 5), exposure to severe cold environments with hypoxia create an even greater risk for un-acclimatised individuals travelling to high altitudes. It is necessary for such individuals to practise a programme of constant monitoring to ensure health is maintained during exposure to extreme environments.
CHAPTER EIGHT

Practical Field Methods For Cold Protection of Non-shivering Cold Casualties: An in Vitro Model

8.1 Abstract

The aim of this investigation was to examine the effectiveness of three field methods for non-shivering cold casualty protection and re-warming. To achieve this, an in vitro fluid bag model was used to simulate a non-shivering cold casualty. Three field methods were compared with a control trial (CON). Trials were a polythene bag (PB), a triple-layered, metalised plastic sheeting survival bag (Blizzard Survival bag (BB)), a triple-layered, metalised plastic sheeting survival bag with heat pads (Blizzard Heat survival bag (BB+HP)). Cold protection methods were assessed for four hours in a range of environmental conditions (-18.5°C, 0°C and 18.5°C). Trials began once fluid bag core temperature had reached 37°C. The effectiveness of the survival bags was assessed by the amount of core temperature decline in each environmental condition. After exposure to 18.5°C there were no differences in final $T_{rec}$ between the cold protection methods ($P > 0.05$). In 0°C, final $T_{rec}$ during BB+HP was significantly greater than CON ($P = 0.004$). After 240 minutes in -18.5°C final $T_{rec}$ in BB+HP was significantly greater than PB ($P = 0.019$). At 60 minutes of exposure to 18.5°C the reduction in $T_{rec}$ was significantly greater in PB compared to BB+HP ($P = 0.036$). Following 120 minutes, the amount of heat lost was significantly greater in PB and BB compared to BB+HP ($P < 0.05$). The effectiveness of chemical heat pads in combination with a triple layered, metallised survival blanket may counteract the decline in thermogenesis accompanying the onset of severe hypothermia and reduce hypothermia related fatalities.
8.2 Introduction

When a human is hypothermic but has the ability to shiver, core temperature can recover to normal provided a protective layer is supplied (Chapter 6). Ethical guidelines surrounding hypothermia research often limit how cold a human can be made. This creates a grey area with regard to knowledge about how the human body can cope once shivering has ceased and there is no longer any form of internal heat production. It is pertinent to understand, even if only by the use of a fluid model, how well an external heat source that can be easily administered in the field, will treat a body with a core temperature so low that heat production via shivering has ceased.

During cold exposure vasoconstriction and shivering are the main defence against temperature loss (Holcomb, 2005). However, as core body temperature decreases the adrenergic, cardiovascular and metabolic mechanisms begin to fail (Farkash et al. 2002). When core temperature reaches 32°C shivering is terminated (Giesbrecht et al. 1997). Although mortality rates are relatively low with severe non-trauma related hypothermia (<28°C), they rise alarmingly with traumatic injury where mortality is 40, 69 and 100% at 34, 33 and 32°C (Moss, 1986; Jurkovic et al. 1987) (Chapter 6). In military environments, including wartime countries such as Afghanistan, where temperatures remain fairly mild in the winter, hypothermia is commonly encountered during combat operations (Marshall, 2005; Moran et al. 2003). Casualty’s air lifted by helicopter experience ambient temperature drops up to -15°C per 1000 feet of altitude gain (Johnson et al. 2010). This, in combination with a four hour evacuation flight will likely accelerate the onset of hypothermia. Additionally, drugs administered to the casualty such as sedatives and opioids decrease the thermogenic effect of shivering (Kurz et al. 1995; Giesbrecht and Bristow, 1997). Given a non-shivering casualty presents a more dangerous condition than one who is shivering (Hamilton and Paton, 1996), simple methods that prevent further heat loss in non-shivering casualties that can be administered instantly whilst waiting for evacuation will likely reduce hypothermia related fatalities (Arthurs et al. 2006).
Studies examining heat loss and field cold protection methods in non-shivering casualties are limited because of ethical limitations with cooling humans to a state of non-shivering. A recent in vitro study where cooling times of fluid bags were compared between different field methods showed a multi-layered survival bag (Blizzard Survival™) to be superior at preventing heat loss compared with a polythene survival bag, single layer metalized plastic sheeting and wool blanket (Allen et al. 2010). Further, a combination of the multi-layered metalized sheeting survival bag (Blizzard Reflexcell™) with chemical heat pads was shown to be superior to all other methods tested. Nonetheless, given that the exposure conditions did not reflect typical cold temperatures experienced during outdoor winter pursuits, it remains unknown how well these devices protect non-shivering, sedentary humans in the cold. Furthermore, trials were terminated before critical core temperatures were reached. Potentially, the amount of heat loss may increase as time in the cold continues. More specifically, when shivering is terminated as a result of severe hypothermia, body heat loss increases. Chemical pads inside a survival bag have obvious advantages in comparison to alternative active warming devices, such as durability, lightness and adaptability to many situations (Hamilton and Paton, 1996). However, rescue teams are unaware of an optimal field treatment method for non-shivering casualties.

The in depth study of severe hypothermia has been difficult given that the experimental study of humans has mostly been confined to mild hypothermia (Giesbrecht et al. 1997). However, in clinical situations, the narcotic drug meperidine is commonly used to inhibit post-operative shivering (Macintyre et al. 1987). With an immediate onset following intravenous administration and short duration of action, meperidine is particularly desirable for use in experimental research to effectively abolish shivering during mild hypothermia. Its use in cold exposed humans has resulted in a three-fold increase in core temperature after-drop and more than a four-fold increase in length of the after-drop period (Giesbrecht et al. 1997). However, since participants remained in a thermo-neutral environment following shivering inhibition, it remains unknown how severe the after-drop
would be in a cooler environment. The administration of narcotics has helped improve understanding of severe hypothermia in humans. It is of limited use however, because a medically trained practitioner must be present at all times. The use of a non-shivering fluid bag model allows research to be performed in a controlled, safe manner without risk to humans. Albeit, a human body has a far smaller heat capacity range than that of water in a fluid model, a number of varying environmental conditions can be applied using this design to gain a better understanding of cooling rates without requiring human participants.

The aim of this investigation was to examine field cold protection methods for the prevention of heat loss in a non-shivering casualty model in a range of environmental temperatures (18.5°C, 0°C and -18.5°C). To simulate real-life awaiting-rescue events, the field cold protection methods were studied for a four-hour period with thermo-neutral conditions (18.5°C). It was hypothesised that the active heating device (Blizzard Heat survival bag) would minimise the reduction in core temperature in all ambient conditions so that the final core temperature remained higher compared to the passive devices (Polythene and Blizzard survival bags).
8.3 Method

8.3.1 Fluid bag model

Two fluid bags were configured to represent a human adult male torso similar to those of human participants in chapters 4, 5, 6 and 7 (~60% of 70 Kg or 48.6 Kg (Allen et al. 2010)). Each torso model used one 60 L polyvinyl chloride bag with electrically welded seams and was filled with 48.6 L water pre-heated to 37.5°C. Water was used instead of saline given the ease of access, use and cost. Models had an indwelling thermistor that represented core temperature and two surface temperature probes situated on the anterior and posterior.

8.3.2 Study design

Separated by at least 24 hours, each fluid bag completed twelve randomised experimental trials (Research Randomiser; www.randomizer.org). Four of these trials were performed with three field cold protection methods that included a polythene bag, a Blizzard Survival bag and a Blizzard Survival heat bag. The fourth trial was a control where the fluid bag had no protection. Each method was assessed in -18.5°C, 0°C and 18.5°C.

8.3.3 Experimental procedures

Each fluid bag was filled with heated water to a starting core temperature of 37.5°C. At this temperature fluid bags were transported into the environmental chamber set to one of the three experimental temperatures (RH 40% and wind velocity 0.2 m.s⁻¹: Delta Environmental Systems, Chester, UK). During each trial, fluid bags were placed on a flexible plastic sheet to prevent direct contact with the floor. Core temperature was measured continuously and recorded every 5 minutes using a flexible thermistor inserted to the medial section of the fluid bag. Surface temperature was also recorded every 5 minutes at two sites using surface thermistors. Water was circulated in the
fluid models by manual rotation of the bag every 5 minutes to eliminate temperature gradients within the bag.

8.3.4 Statistical analyses

A two-way fully repeated-measures ANOVA (time x trial) was performed on thermoregulatory variables. Significance was accepted at $P < 0.05$. Where significant main effects and interactions occurred Post Hoc Tukey’s HSD was used. All data are presented as Mean ± SD.
8.4 Results

8.4.1 Final Core Temperature

After 240 minutes exposure to 18.5°C there were no differences between the final core temperatures in any of the trials. However, after 240 minutes in -18.5°C the final core temperature in BB+HP (26.0°C) was greater than PB (16.5°C) \((P = 0.019)\).

Final core temperatures on CON 0°C and CON -18.5°C were lower than PB 18.5°C \((P = 0.03\text{ and } P = 0.048, \text{ respectively})\), BB+HP 18.5°C \((P = 0.006\text{ and } P = 0.023, \text{ respectively})\) and BB+HP 0°C \((\text{Figure 8.1: } P = 0.033)\). Additionally, final core temperatures in PB -18.5°C and BB -18.5°C were also lower than PB 18.5°C \((P = 0.038\text{ and } P = 0.022, \text{ respectively})\). BB+HP in -18.5°C was not lower than any final core temperature in a warmer ambient condition except BB 18.5°C \((P = 0.038)\) which was also higher than PB in -18.5°C \((P = 0.026)\).
Figure 8.1. Final core temperature after 240-minute exposure to 18.5°C, 0°C and -18.5°C in three field cold casualty interventions and a control. Values are Means ± SD. * significant difference vs. CON 0°C; † significant difference vs. PB-18.5°C, a significant difference vs. CON 18.5°C, b significant difference vs. PB 18.5°C, c significant difference vs. BB+HP 18.5°C, d significant difference vs. BB+HP 0°C, e significant difference vs. BB 18.5°C (P < 0.05).

8.4.2 Hourly change in Core Temperature

At 60 minutes of exposure to thermo-neutral conditions (18.5°C) the reduction in core temperature was greater in PB compared to BB+HP and continued to increase throughout the remainder, with a final difference at 240 minutes of 2.9°C (Figure 8.2A: $F_{(3, 3)} = 11.890$, $P = 0.036$, $\eta^2 = 0.067$). The hourly change in core temperature within each thermo-neutral trial was greater following the initial core temperature at the onset of exposure (Figure 8.2A: $F_{(4, 4)} = 533.447$, $P = 0.000$, $\eta^2 = 0.002$). Similarly, a main effect of time in 0°C also established that the hourly change in each trial was
greater following the onset of exposure (Figure 8.2B: $F_{(4, 4)} = 1638.403, P = 0.000, \eta^2 = 0.001$). The amount of heat loss during CON in 0°C was greater each hour compared to the other trials in that environment (Figure 8.2B: $F_{(12, 12)} = 7.584, P = 0.001, \eta^2 = 0.116$). No differences occurred between PB and BB. However, following 120 minutes, the amount of heat lost was greater in PB and BB compared to BB+HP. The difference continued to increase over each hour so that by 240 minutes PB and BB had lost 14°C whereas BB+HP had only lost 7.6°C.

Following 60 minutes of exposure to -18°C, core temperature change was greater during CON compared to all trials ($P = 0.05$). Exposure to -18°C evoked an interaction whereby BB+HP had the smallest amount of heat loss at 60 minutes of exposure compared to CON and PB trials (Figure 8.2C: $F_{(12, 12)} = 15.364, P = 0.000, \eta^2 = 0.061$). The difference in core temperature between these trials continued to increase at each hour throughout. No difference occurred between BB and BB+HP until 180 minutes, yet a difference between PB and BB was established an hour earlier and continued to increase throughout the remainder of the exposure. With each hour following the onset of cold exposure core temperature heat loss increased in all trials (Figure 8.2C: $F_{(4, 4)} = 1314.03, P = 0.000, \eta^2 = 0.001$).
Figure 8.2 Hourly change in core temperature during 240 minute exposure to A, 18.5°C, B, 0°C and C, -18.5°C in three field cold casualty interventions and a control. Values are Means ± SD. # significant difference vs. 0; * significant difference vs. CON; † significant difference vs. PB, ‡ significant difference vs. BB (P < 0.05).
8.4.3 Predicted Survival for Non-Shivering Cold Casualty

Time to 32°C from 37°C was calculated as an estimate of time to mortality with traumatic injury (Moss, 1986; Jurkovic et al. 1987). During CON -18.5°C time to 32°C was lower (30 mins) compared to any other trial (Table 8.1). PB -18.5°C demonstrated a similar time to 32°C as CON 0°C, but was less than CON 18.5°C, PB 18.5°C and BB+HP 18.5°C. The longest time to 32°C from 37°C was observed during BB+HP 18.5°C (230 mins) which was longer than CON 0°C, PB 18.5°C and CON -18.5°C (P = 0.05) (Table 8.1). Time to 32°C during BB+HP 0°C was the same as PB 18.5°C. Time to 32°C did not differ between BB 0°C and BB -18.5°C. Time to 32°C during BB+HP -18.5°C was similar to CON 18.5°C. No differences occurred between PB and BB when exposed to the different ambient temperatures, yet time to 32°C was slightly longer during BB exposure to 0°C and -18.5°C (Table 8.1).

Following the reduction of core temperature to 32°C, the time to 24°C for each trial was calculated as an estimate of time from cessation of shivering (non-trauma related hypothermia) to apparent death (Durrer et al. 1998). Exposure to thermo-neutral conditions (18.5°C) did not cause a reduction in core temperature to 24°C in any trial (Table 8.1). However, time to 24°C from 32°C was less during CON in 0°C compared to PB (P = 0.033) and a trend occurred between CON and BB (P = 0.07) (Table 8.1: F(5, 5) = 25.293, P = 0.001, η² = 0.038). Core temperature did not reduce to 24°C in any of the temperature controlled environments for BB+HP.
Table 8.1. Time to 24°C from 32°C during 240 minute exposure to 0°C and -18.5°C.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Time from 32°C to 24°C (mins)</th>
<th>Time from 37°C to 32°C (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON 18.5°C</td>
<td>N/A</td>
<td>90 ± 1</td>
</tr>
<tr>
<td>PB 18.5°C</td>
<td>N/A</td>
<td>163 ± 11f</td>
</tr>
<tr>
<td>BB 18.5°C</td>
<td>N/A</td>
<td>180 ± 57</td>
</tr>
<tr>
<td>BB+HP 18.5°C</td>
<td>N/A</td>
<td>230 ± 1*bf</td>
</tr>
<tr>
<td>CON 0°C</td>
<td>93 ± 4</td>
<td>38 ± 11</td>
</tr>
<tr>
<td>PB 0°C</td>
<td>140 ± 1*</td>
<td>75 ± 28</td>
</tr>
<tr>
<td>BB 0°C</td>
<td>160 ± 7</td>
<td>68 ± 11cdf</td>
</tr>
<tr>
<td>BB+HP 0°C</td>
<td>N/A</td>
<td>163 ± 18*df</td>
</tr>
<tr>
<td>CON -18.5°C</td>
<td>53 ± 4</td>
<td>30 ± 7</td>
</tr>
<tr>
<td>PB -18.5°C</td>
<td>98 ± 18</td>
<td>38 ± 4abc</td>
</tr>
<tr>
<td>BB -18.5°C</td>
<td>115 ± 14</td>
<td>68 ± 4c</td>
</tr>
<tr>
<td>BB+HP -18.5°C</td>
<td>N/A</td>
<td>88 ± 4c</td>
</tr>
</tbody>
</table>

Values are mean ± SD. * significant difference vs. CON 18.5°C, * significant difference vs. CON 0°C, b significant difference vs. PB 18.5°C, c significant difference vs. BB+HP 18.5°C, d significant difference vs. BB+HP 0°C, f vs. CON -18.5°C (P < 0.05). Abbreviations: N/A, not applicable.

8.4.4 Results summary

After 240 minutes exposure to 18.5°C there were no differences between the final core temperatures in any of the trials. However, after 240 minutes in -18.5°C the final core temperature in BB+HP (26.0°C) was greater than PB (16.5°C). The longest time to 32°C from 37°C was observed during BB+HP 18.5°C (230 mins) which was longer than CON 0°C, PB 18.5°C and CON -18.5°C. Core temperature did not reduce to 24°C in any of the temperature controlled environments for BB+HP.
8.5 Discussion

The aim of the present investigation was to examine field cold protection methods in a non-shivering casualty model. To simulate real-life rescues these methods were studied over a four-hour period in a range of environmental temperatures. In support of the hypothesis the Blizzard Heat survival bag was superior compared to other devices in all environmental temperatures as indicated by a higher final core temperature. Additionally, the Blizzard Heat survival bag was the only method that prevented core temperature reaching 24°C, a temperature associated with death (Durrer et al. 1998). As reported previously, Blizzard survival was superior to polythene (Allen et al., 2010), yet neither could maintain a core temperature above 24°C during exposure to 0°C. These results imply that for a non-shivering casualty, lying sedentary and awaiting rescue in a cold environment, survival time may be increased by the immediate administration of a lightweight active warming survival device, such as the Blizzard Heat survival bag.

During exposure to 0°C, the hourly reduction in core temperature from the in vitro torso model protected with a Blizzard Heat survival bag was not only less than the passive devices exposed to the same conditions, but replicated that of both passive devices in a thermo-neutral environment (18.5°C). This novel finding supports a previous investigation where an active heating combination of a multi-layered metalized sheeting survival bag (Blizzard Reflexcell™) with chemical heat pads was superior to all other methods tested (Allen et al. 2010). Nonetheless, this previous investigation only exposed fluid models to a thermo-neutral ambient temperature which does not reflect the likely cold environments experienced by casualties. Exposure to -18.5°C evoked a similar outcome as 0°C so that the hourly reduction in core temperature from the model protected with a Blizzard Heat survival bag was again, not only less than both the polythene and Blizzard survival bags in the same conditions but similar to these passive devices in 0°C. Hourly heat loss was also similar between the in vitro torso model protected with a Blizzard Heat survival bag in -18.5°C and no protection in thermo-neutral conditions. These findings have implications for trauma casualties wounded in
warmer conditions, who, when air lifted by helicopter will experience ambient temperature drops up to -15°C per 1000 feet of altitude gain (Johnson et al. 2010). It is likely a Blizzard Heat survival bag will decrease the risk of accelerated heat loss during altitude gain and as such, reduce trauma related fatalities.

When exposed to 0°C and 18.5°C, the polythene bag and Reflexcell device without chemical heat pads (Blizzard survival bag) presented similar hourly heat loss and final core temperatures. This contradicts a recent investigation where the same multi-layered survival bag was found to be superior in comparison to single-layer polythene in thermo-neutral conditions (Allen et al. 2010). Nonetheless, when the fluid model was protected with the Blizzard survival bag, hourly heat loss in -18.5°C with still air (equivalent to -15°C with 6 km⋅h⁻¹ wind speed, -10°C with 20 km⋅h⁻¹ wind speed and -5°C with 95 km⋅h⁻¹ wind speed) was less compared with polythene. This also meant the final core temperature was higher with the Blizzard survival bag which in combination suggests as ambient temperatures decrease, multi-layered devices are better at reducing heat loss which may increase survival times and reduce the number of hypothermia related fatalities, particularly when time to 24°C from 32°C was greater in the Blizzard bag compared to polythene in 0°C and -18.5°C. However, as aforementioned, when protected with an active device, core temperature did not decrease to a critical level in any ambient condition.

When the fluid models were exposed to any temperature with no protection, a greater reduction in core temperature was observed compared to when a protective layer was available. This suggests for cold casualties unable to shiver, any extra layers that can be provided may indeed reduce the decline in core temperature (Hamilton and Paton, 1996). Active heating devices such as chemical heat pads are more beneficial for the immediate field treatment of hypothermic casualties prior to evacuation to superior methods because of the additional heat energy being put into the system, particularly when core temperature is critically low (i.e. < 32°C) (Durrer et al. 1998; Allen et al. 2010). Furthermore, of the devices tested in the present study, following the evacuation of
casualties from cold to thermo-neutral temperatures (e.g. 18.5°C), an active warming device may provide the most superior protection for a non-shivering casualty. This is because only a modest decline in core temperature occurred in 18.5°C during the four hour exposure in comparison to when fluid models were covered with passive devices.

Regarding surface temperature among all the devices examined, not one met the threshold temperature considered to cause thermal injury (Moritz and Henriques, 1947) which has also been shown with human subjects (Appendix J). Similar to previous research (Allen et al. 2010), given the fluid model in the present study did not consist of a biological organism and had no basal metabolic activity, extrapolation of this study to efficacy in humans may be limited. Despite this, the model was effective in detecting drops in temperature among the devices exposed to different ambient temperatures. Furthermore, the findings of this study reinforce those of a recent investigation by our group where the multi-layered metallised sheeting with chemical heat pads was the most superior device (Chapter 6). As such, re-warming of mildly hypothermic cold casualties to near resting core temperatures occurred with significantly less energy expenditure.

The present study suggests a continuum exists for the amount of heat lost, whereby the lower the ambient temperature is the greater the difference between heat lost from a passive device is compared with active heating. Given an increased risk of accidental hypothermia accompanies previous conclusions in earlier chapters, findings here have implications for those individuals climbing to high altitudes in cold conditions (Chapter 4 and 5) and those who by accident, have been immersed in cold water in cold ambient conditions (Chapter 6 and 7). In conclusion, a non-shivering fluid model exposed to different ambient environments suggested that the effectiveness of chemical heat pads in combination with a triple layered, metallised survival blanket may counteract the decline in thermogenesis accompanying the onset of severe hypothermia or administration of sedatives thus preventing hypothermia related fatalities including those combined with trauma.
CHAPTER NINE

General Discussion

9.1 Background

Military personnel, athletes and outdoor enthusiasts frequently travel to cold, mountainous regions for work, training and recreational activities. It is not uncommon therefore, for such individuals to be exposed to the extreme environmental conditions of cold and cold with hypoxia for prolonged periods of time. Additionally, arrival at high altitude predominantly occurs prior to hypoxic acclimatisation. High altitude environments have been suggested to alter thermoregulatory, metabolic, perceptual (Blatteis and Lutherer, 1976; Johnston et al. 1996; Golja et al. 2004) and immune responses (Pedersen and Steensberg, 2002) to the cold which may increase the susceptibility to cold injury (e.g. frostbite, hypothermia) and infection (e.g. URTI). Accidental hypothermia affects approximately 10% of casualties reported in mountainous environments and is a significant contributor to fatalities (Moss, 1986; Sharp, 2007). Cold exposure alone is associated with an increased incidence of bronchorrhea and URTI (Giesbrecht, 1995; Castellani et al. 2002). It has been suggested that a continuum exists for the effects of body core cooling on immune function whereby mild decreases in core temperature have little stimulatory effects, but more severe decreases have depressive effects (Costa et al. 2010). Given the cold and hypoxic environments that may be encountered during wilderness or mountainous pursuits and the subsequent risks to thermoregulation and immune function the main objectives of this thesis were to investigate: 1. the effects of acute and chronic exposure to high altitude on thermoregulation, metabolism (Chapter 4) and mucosal immune responses (Chapter 5) during cold exposure; 2. the effectiveness of four field methods for the treatment of cold casualties on thermoregulation and metabolism during an ‘awaiting rescue’ scenario in 0°C following cold water immersion (Chapter 6); 3. the mucosal immune response to prolonged cold exposure in cold casualties (Chapter 7); and 4. the efficacy of
three field protection methods for the treatment of non-shivering cold casualties using an *in vitro* torso model exposed to -18.5°C, 0°C and 18.5°C for four hours (*Chapter 8*).

Findings from the two threads of experiments in this study (i.e. thermoregulation and mucosal immunity) can be integrated together to help produce a clear representation of potential health risks for those individuals performing activities in extreme environments including cold and/or hypoxia. Such findings could be applied and used in practise by a wide spectrum of associations including, but not limited to, the medical industry, the military and outdoor pursuits companies. Knowledge gained from this thesis may help to reduce hypothermic related casualties in the field and reduce a potential increased susceptibility to illness and infection from pursuits in these extreme environments.

### 9.2 Summary of Main Findings

Un-acclimatised men exposed to hypoxia in the cold demonstrated a reduced capacity for NST via an increased catabolism of CHO substrates which may have been responsible for the earlier onset and increased intensity of shivering activity. Nonetheless, hypoxia, regardless of acclimatisation, did not impair typical core or mean skin temperature responses to the cold. Following an 18 day high altitude mountaineering expedition, a decrease in shivering despite no changes in core or mean skin temperature is consistent with a greater reliance on NST. The alterations observed post-expedition in thermal comfort may suggest greater thermal tolerance but increased susceptibility to cold injury (*Chapter 4*). Saliva flow rate was altered during un-acclimatised exposure to hypoxia in the cold compared with post-expedition, yet α-amylase concentration was increased in the cold with and without hypoxia in all conditions. However, since s-IgA concentration and secretion rate were unchanged regardless of hypoxia or cold, it is unlikely reductions in arterial oxygen saturation negatively effect the mucosal immune response to cold at the level employed in the study (*Chapter 5*). All four field re-warming methods supported re-warming of shivering cold casualties to near
normal resting core temperatures within three hours of cold exposure. Although typical re-warming parameters were not different between either intervention, metabolic heat production was greater in polythene bag trials compared with Blizzard during the first hour of cold exposure. Re-warming in the Blizzard survival bags was achieved with lower TEE which may be beneficial for long term survival (Chapter 6). While s-IgA concentration was unchanged during and following prolonged cold stress, saliva flow rate and s-IgA secretion rate were reduced and α-amylase concentration was increased until the return to normal resting core temperature. This supports that a reduction in core temperature (≥ 1.5°C) may increase susceptibility to illness and infection (i.e. URTI, influenza) (Chapter 7). A non-shivering torso model reinforced suggestions that the Blizzard Heat survival bag was the most superior of the field treatments investigated by demonstrating a reduced amount of core temperature heat loss and an increase in predicted survival time (Chapter 8).

9.3 Thermoregulation, metabolism and perception

Core and mean skin temperature were unaffected by exposure to hypoxia in the cold regardless of hypoxic acclimatisation. Similar decrements in core and mean skin temperature were observed during 2 hours supine rest in SL (F_iO_2: 20.9%), UN (F_iO_2: 12.5%) and PEX (F_iO_2: 12.5%) conditions (Chapter 4). These results suggest that underlying homeostatic mechanisms are robust to unaccustomed environmental conditions in order to maintain primary thermoregulation (i.e. core and skin temperature). Earlier reports are contradictory however, and compared with cold alone, un-acclimatised exposure to cold and hypoxic environments has been associated with a reduction in core temperature and an increase in skin temperature (Bullard, 1961; Cipriano and Goldman, 1975; Kottke et al. 1948). Nonetheless, core temperature has been reported elsewhere as unaltered during both normobaric and hypobaric hypoxia in the cold (Blatteis and Latherer, 1976; Brown et al. 1952; Robinson and Haymes, 1990). A limitation of chapter 4 is that the exposure temperature was 15°C which may be considered too mild to evoke such a thermoregulatory stress. However, in
combination with a simulated high altitude of 4000m it was regarded as an extreme and unaccustomed climate for the participants taking part. Additionally, this ambient temperature has been used previously in studies where differences did occur (Cipriano and Goldman, 1975). Given that the body of evidence surrounding the effect of hypoxia on thermoregulatory responses in the cold includes a wide spectrum of methodological differences, it would appear that thermoregulatory alterations cannot be predicted, and therefore suitable precautions, such as following acclimatisation protocols ahead of travelling, should be taken upon ascent to high altitude.

The metabolic response to cold was impaired during un-acclimatised exposure to hypoxia (Chapter 4). A reduction in heat production coupled with an earlier onset and more intense shivering activity supports that acute hypoxia suppresses NST (Blatteis and Lutherer, 1976; Bullard, 1961; Gautier, 1996; Robinson and Haymes, 1990). Additionally, significant alterations in lipid and carbohydrate oxidation were demonstrated prior to acclimatisation. It is plausible to suggest that the hypoxic-induced suppression of NST by an inhibition of the aerobic catabolism of lipid stores may occur through an increased hormonal response to hypoxic stress which decreases the lipolytic response of adipocytes and thus lowers lipid mobilisation (de Glisezinski et al. 1999; Masoro, 1966; Robinson and Haymes, 1990). As a result, carbohydrate oxidation is increased to fuel intense shivering thermogenesis (Martineau and Jacobs, 1988). In turn, this may explain how the body maintains core and mean skin temperature despite the absence of NST in hypoxia (Chapter 4). The severity of hypoxia-induced metabolic alterations, as a diagnostic marker of homeostatic disruption at altitude, appears to provide more information regarding the effects of hypoxia during cold exposure compared to core and mean skin temperature alone. Given that the metabolic response remained impaired following acclimatisation but shivering activity was reduced, supports that NST recovery may occur following a high altitude stay (Blatteis and Lutherer, 1976; Mathew et al. 1979). Since the work in this thesis (Chapter 4) was the first to quantify shivering using electromyography provides a particularly novel aspect that previous investigations do not.
Behavioural thermoregulation is initiated by the perception of thermal comfort (Golja et al. 2004). In support of a previous report (Golja et al. 2005), acute exposure to a reduced oxygen supply prior to acclimatisation did not alter sensitivity to the cold. Given core and mean skin temperatures were similar between trials it is likely the neural information transformed from peripheral sensors giving rise to the perception of thermal comfort was also similar (Chapter 4). However, following an 18 day altitude expedition the sensation of cold was decreased and thermal comfort was increased which suggests prolonged hypoxic exposure may impair nerve conduction so that thermal information is impeded (Golja et al. 2004; Malanda et al. 2008). Consequently, acclimatised individuals at altitude may often feel warmer than their core temperature would suggest. As a result, behavioural thermoregulation may be neglected. These alterations observed post-expedition may suggest a greater thermal tolerance but an increase in susceptibility to cold injury (Chapter 4).

Chapter 6 examined four field re-warming methods for the treatment of cold casualties. Core temperature re-warming rate was unaffected by the type of re-warming device administered and in all cases where cold individuals were able to adequately thermoregulate (e.g. shiver) the time taken to return to normal resting core temperature values was the same. This finding provides further support to a conclusion of Chapter 4, suggesting that homeostatic mechanisms ensure optimal core temperature control during exposure to environmental stressors where core temperature is compromised. Mean weighted skin temperature differed between the field methods however, whereby the triple-layered metallised sheeting survival bags maintained a higher skin temperature throughout the cold exposure compared to polythene trials. The administration of hourly hot drinks (~430 ml) seemed to confer no thermoregulatory advantage compared with polythene survival bags alone. It is plausible to suggest that these multi-layered devices may reduce the pre-disposition to peripheral cold injuries (e.g. frostbite) during more prolonged cold exposures (Chapter 6). As a likely result of the lower mean skin temperature during polythene trials, metabolic heat production was greater compared with the Blizzard survival bags during the first hour of cold exposure.
Furthermore, re-warming in the Blizzard Heat survival bag was achieved with lower TEE which may be beneficial for long term survival (Tikuisis, 1995; Tikuisis et al. 2002). As hypothesised, a consequence of the superior protection provided by the Blizzard Heat survival bag improved the perception of pain and thermal comfort.

That the Blizzard Heat survival bag offered superior thermal protection compared with single layer polythene (Chapter 6) was also suggested by the results of Chapter 8. An in vitro torso model exposed to the cold suggested that the Blizzard Heat survival device could maintain a higher core temperature throughout prolonged exposure to extreme conditions than other methods tested. This supports previous findings where an active heating combination of a multi-layered metalized sheeting survival bag (Blizzard Reflexcell™) with chemical heat pads was superior to all other methods tested (Allen et al. 2010). The findings of Chapter 8 however, offer more information regarding the depth of thermal protection provided by each device as a result of the number of different ambient conditions they were exposed to. Consequently, the active heating device was the only treatment able to prevent core temperature from becoming fatally low in all ambient conditions when shivering was absent (~24°C; Durrer et al. 1998). This may imply for a non-shivering casualty, lying sedentary and awaiting rescue in a cold environment, survival time may be increased by the immediate administration of a lightweight active warming survival device, such as the Blizzard Heat survival bag. While the results from this in vitro study have obvious limitations, the model was effective in detecting drops in temperature among the different devices exposed to different ambient temperatures.
9.4 Mucosal Immunity: Saliva flow rate, s-IgA concentration, s-IgA secretion rate, α-amylase concentration and α-amylase secretion rate

Cold exposure is associated with an increased incidence of bronchorrhea, sinusitis, and URTI (Clothier, 1974; Giesbrecht, 1995; Castellani et al. 2002; Lavoy et al. 2011). A decrease in s-IgA has been implicated as a possible causal factor in the increased susceptibility to URTI (Hanson et al. 1983; Nieman and Nehlsen-Cannarella, 1991; Gleeson et al. 1999; Cairns et al. 2002). Coincidentally, modest whole body cooling (T_{rec} 35.9°C) via cold air exposure (0°C) has demonstrated a decrease in s-IgA responses (Costa et al. 2010), nevertheless literature surrounding the effects of hypoxia on saliva responses are limited (Meehan et al. 1988; Muchmore et al. 1981). Further, whether the combination of hypoxia and cold exacerbates alterations in mucosal immunity, until now has not been examined.

Contradictory to the hypotheses in Chapter 5, hypoxia did not alter typical mucosal immune responses in the cold. However, following cold water immersion to reduce core temperature to 36°C in a normoxic environment, s-IgA secretion rate was reduced and α-amylase concentration was increased (Chapter 7). This suggests that when cold stress is mild (15°C) and core temperature remains un-compromised, hypoxia unlikely increases the predisposition to URTI. Nonetheless, given that s-IgA secretion rate was altered following a more severe cold stress in normoxia (Chapter 7), it is not unreasonable to suggest that colder conditions in hypoxia may indeed evoke s-IgA alterations, particularly as saliva flow rate was mildly suppressed in hypoxia in combination with a cold induced increase in α-amylase concentration (Chapter 5). Although physiological mechanisms underlying mucosal alterations remain unclear, it is likely that both neural and endocrine factors influence such responses (Pedersen and Steensberg, 2002). As arterial oxygen saturation was reduced during un-acclimatised hypoxic exposure in Chapter 5, both the neural and endocrine systems may have been compensating to maintain homeostasis (Mazzeo, 2005), thus explaining the difference in saliva flow rate. Nonetheless, the absence of hypoxia-induced s-IgA
alterations to cold suggests the mucosal immune system may be robust to a combination of environmental stressors and as such common URTI complaints may not resonate upon arrival to high altitude.

Following a prolonged stay at altitude, saliva flow rate, α-amylase and s-IgA responses remained unaltered during hypoxic exposure in the cold. This may be as a result of the observed recovery in arterial oxygen saturation to near normal sea level values following the 18 day expedition. Given an altitude of 3500m has been suggested to correspond to the threshold at which the physiological changes intended to tolerate the drop in partial pressure of oxygen fail to fully compensate the effect of hypoxia (Colin et al. 1999), it has been speculated that at or above this altitude the adverse effect of hypoxia on s-IgA is more substantial (Tiollier et al. 2005). However, in Chapter 5, both unacclimatised and acclimatised participants exposed to a simulated altitude of 4000m while at rest displayed no alterations in s-IgA. Therefore, it is more likely the previously observed s-IgA changes (Tiollier et al. 2005) were not due to the level of hypoxia per se or as a result of chronic exposure but more likely a cumulative effect of intense physical training. Additionally, the normobaric nature of the altitude exposure during Chapter 5 and other work (Tiollier et al. 2005) does not suitably replicate typical high altitude conditions. If barometric pressure was manipulated to reflect the value coinciding with that level of altitude (i.e. 661mmHg at 4000m) the impact on homeostasis and subsequent mucosal immune status may be greater during cold exposure. On the contrary, since s-IgA concentration and secretion rate were unchanged in both altitude exposures compared to normoxia (Chapter 5) suggests it is unlikely that altitude exposure while at rest, before and following an altitude stay, causes disruption to either sympathetic nervous system activity (Mazzeo et al. 2003) or alpha-adrenergic mechanisms (Ring et al. 2000). In conclusion, exposure of lowlanders to normobaric hypoxia, whether acute or following a prolonged stay, does not evoke a physiological or metabolic stress great enough to disrupt the typical mucosal immune response to cold. Very little research exists with regard to mucosal immunity at altitude so while the experiment
here (Chapter 5) does not offer masses of information it does provide the beginnings of what should be an area of continual investigation.

Chapter 7 identifies that saliva flow rate and s-IgA secretion rate are reduced and α-amylase concentration increased in mildly hypothermic, sedentary individuals exposed to the cold. These findings are consistent with previous work (Costa et al. 2010), yet the results of Chapter 7 are strengthened by the inclusion of a thermo-neutral control trial to account for the diurnal variations in saliva parameters. However, that cold exposure and mild hypothermia reduce s-IgA secretion rate should be suggested with caution. This is because s-IgA secretion rate at baseline, prior to cold exposure, was also lower than at that same time during thermo-neutral control. While psychological stress has been found to reduce s-IgA concentration in humans (Stone et al. 1987; Stone et al. 1994; Cohen et al. 2001), it is possible that participants were more anxious on the morning of their cold trial and as a result s-IgA responses were already altered. In the future, blinding participants to the trial may avoid this potential anticipatory effect. Nonetheless, following re-warming, s-IgA secretion rate was greater and α-amylase concentration lower than when participants displayed their lowest core temperature, whereas no significant changes occurred throughout the thermo-neutral control trial. This suggests that until re-warming is initiated, cold, sedentary individuals are at risk from increased mucosal immune disturbance which supports the popular belief that most common colds occur because the host is exposed to a cold environment (Dowling et al. 1958; Biggar et al. 1984). Furthermore, Chapter 7 provides some evidence that there may be a direct causal relation between an individual getting cold and subsequently developing an infection (Lavoy et al. 2011). To confirm this however, studies including larger subject numbers would be beneficial. In conclusion, a reduction in core temperature (≥1.5°C) resulting from cold water immersion and subsequent cold air exposure suppresses the usual daily s-IgA response which may increase susceptibility to illness and infection (i.e. URTI, common colds, influenza) if re-warming is not initiated immediately.
9.5 Future directions

Work in this area could continue to develop firstly by repeating the experiments outlined in this thesis in order to gain larger sample sizes and thus, more specific conclusions. Secondly, methods for each experiment could be performed in conditions that pose a greater strain on the human body (e.g. colder and more hypoxic) to demonstrate particular thresholds for normal human body function. It would be beneficial to examine a wider variety of immune parameters in order to provide a more complete template of the likely effects of exposure to extreme environments.
9.6 Conclusions

The major conclusions from this thesis are:

I. Demonstrated by a reduced capacity for NST via an increased catabolism of CHO, exposure to hypoxia in the cold impairs the thermoregulatory and metabolic response of un-acclimatised men.

II. Exposure to a simulated high altitude of 4000m in the cold following an 18 day mountaineering expedition evokes a decrease in shivering activity despite no changes in core or mean skin temperature which likely represents a greater reliance on NST for heat production.

III. Alterations observed post-expedition in thermal comfort during exposure to a simulated high altitude of 4000m in the cold may suggest a greater perception of thermal tolerance but an increase in susceptibility to cold injury.

IV. Two hours of exposure to a simulated high altitude of 4000m does not disrupt the typical mucosal immune response to cold regardless of hypoxic acclimatisation status. Common URS are not likely to be increased upon arrival to high altitude or following a prolonged stay.
V. Of four cold treatment methods tested in 0°C, with minimal wind, all were able to assist shivering cold casualties to increase core temperature to normothermia within three hours.

VI. Blizzard survival bags are a more efficient field re-warming device than polythene bags given complete core re-warming was achieved with lower total energy expenditure which may be beneficial for long term survival.

VII. A reduction in core temperature (≥ 1.5°C) resulting from cold water immersion and subsequent cold air exposure alters the usual daily mucosal immune responses which may increase susceptibility to illness and infection (i.e. URTI, common colds, influenza) if re-warming is not initiated immediately.

VIII. *In Vitro* torso models protected in various ambient temperatures with different cold protection field treatments highlighted that the Blizzard Heat survival bag was superior at reducing heat loss which resulted with higher final core temperatures compared to other devices. This active heating device was the only treatment able to prevent core temperature from becoming fatally low in all ambient conditions when shivering was absent

IX. The effectiveness of chemical heat pads in combination with a triple layered, metallised survival blanket may prevent the decline in thermogenesis accompanying the onset of severe hypothermia or administration of sedatives, thus preventing hypothermia related fatalities in the field, including those combined with trauma.
References


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http://www.llanberismountainrescue.co.uk/English/Previous.php


Appendix A:

Bangor University
School of Sport, Health and Exercise Sciences

Subject Information Sheet

Project Title: An Experimental Study of Practical Field Methods for Cold Casualty Protection and Treatment during Prolonged Cold Exposure

Research Co-ordinators: Dr Sam Oliver and Dr Neil Walsh

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Additional Investigators: Dr Matt Fortes, Dr Gavin Lawrence, Jenny Brierley, Alberto Dolci, Phil Heritage, Aaron Burdett, Robin Wilson, Beth Palmer and James Firman

Invitation to take part

You are being invited to take part in a research study. Before you decide to take part it is important for you to understand why the research is being conducted and what will be required of you should you agree to be involved. Please take time to read the following information carefully and discuss it with the investigators. Ask us if there is anything that is not clear or if you would like more information.

Do I have to take part?

This is entirely your decision. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without giving a reason. If you decide not to participate or withdraw during the study, your decision will not affect your relationship with the School of Sport, Health and Exercise Sciences, or any of the investigators involved in the study. All information collected during the study will be treated confidentially and if you choose to withdraw then your data will be deleted from our records.

Background

Prolonged cold exposure and becoming mildly cold is associated with impaired health and performance. Accidents in cold and wet environments can lead to hypothermia. This is especially the case when casualties remain stationary or are suffering from trauma with blood loss (for example, mountaineering or car accident). In the UK average mountain rescue times are typically at least 2 hours. It is therefore important that easily administrable and transportable cold protection methods are developed which can be used whilst casualties wait for emergency services rescue. Ultimately, mortality rates will be decreased if cold protection methods can be developed and shown to reduce or maintain a casualty’s core temperature. Current field cold protection methods have been shown to be relatively ineffective (e.g. polythene survival bag), or their effectiveness is presently unknown (e.g. hot drinks and Blizzard Survival bags). Therefore the present investigation aims to determine the effectiveness of polythene survival bags with hot drinks and Blizzard survival bags with and without heat pads.
Objective

To assess which field method offers optimal protection for casualties in a cold environment.

What will be expected of you?

If you decide to take part in this study there will be a number of constraints placed upon you. During the day prior to completing a trial you will be expected:

- To avoid drinking alcohol or caffeinated drinks (i.e. coffee, tea, coke and diet coke).
- To avoid participating in moderate to high intensity exercise.

Whilst completing the experimental trials in the laboratory you will be expected:

- To spend one whole day in the laboratory on four separate occasions.
- To eat and drink only what is prescribed to you.
- Arrive to the laboratory after an overnight fast (10-12 hours)
- To follow the study’s timetable.
- To be weighed nude (behind screens to maintain privacy).
- To have four blood samples taken during each one day trial (~65 ml) totaling 16 blood samples during the 4 week study period (~260 ml).
- To provide saliva and urine samples.
- To perform four cold water immersions (~13°C for a maximum of 60 minutes).
- To perform four 3 hour cold air tests in 0°C.
- To complete a health log 2 weeks prior, during and 2 weeks following the last trial.

You will be excluded if you are a smoker, asthmatic, diabetic, have a pacemaker or any heart condition or you are currently taking specific types of medication or supplements.

IN TOTAL, THE STUDY WILL REQUIRE YOU TO GIVE UP 34 HOURS OF YOUR TIME.

Summary of visits

Visit One: Project briefing and familiarisation (1 hour)

At this meeting you will be fully briefed about the requirements of the project. You will be talked through the subject information sheet by an investigator and given the opportunity to ask any questions. You will then leave with the information sheet so as to allow you time to discuss your possible involvement in the study with significant others. This will also allow you additional time to think of questions.
You will be asked to refrain from exercise and alcohol and caffeine consumption for 24 hours prior to each visit. At this visit you will be familiarised with the procedures for collection of saliva and venous blood. All blood samples will be collected by a qualified member of staff from a forearm vein using a small needle (~16 ml of blood at each collection). A saliva sample will be obtained by asking you to dribble into a plastic container for 5 minutes. You will also be familiarised with the cognitive function tests and also the health log which you will be asked to complete at the end of each day.

**Visit Two: Question and answer session (optional)**

Visit two is provided as an additional opportunity for you to ask questions. Once you are fully satisfied with the information and on agreement to take part in the study you will be asked to complete an informed consent form, a medical questionnaire and make arrangements for the following visits.

**Experimental trials:**

You will be required to complete four experimental trials in a random order each separated by 7 days. Each experimental trial will consist of an 8 hour period where you will be in the laboratory under treatment conditions (Visit three, four, five and six). Including the briefing meeting, familiarisation visit and all four experimental trials you will be required to visit the laboratory on seven occasions for a total of ~34 hours.

**Visit Three, Four, Five and Six: (8 hours)**

**Prior to arriving at the laboratory**

You will be instructed to consume your normal daily food intake and keep a food diary of what you ate the day before the trial. We will also provide you with your daily water requirements (approximately 2.5 L for a 70 kg person), which will help to ensure that you are hydrated prior to each trial. We ask that you arrive at the physiology laboratory with the appropriate clothing (i.e. swim shorts, a second pair of dry shorts and a towel) following an overnight fast at 07:00 hours.

**Experimental procedures**

On arrival to the laboratory and after completely emptying your bladder and bowels, your body composition will be estimated by a non-invasive bio-electrical impedance analysis; which requires you to stand on a set of weighing scales. On arrival at the laboratory you will be given a rectal probe to fit, and a universal container with which to collect a urine sample. The rectal probe will be used to monitor core temperature. You will also be asked to wear a chest strap so that we can monitor your heart rate. You will then sit quietly for 30 minutes in the laboratory (ambient temperature ~20°C) after which blood and saliva samples will be obtained and you will complete the cognitive function tests. You will then enter the cold water bath (~13°C) and be immersed up to your shoulders until your core temperature has decreased to 36°C (~40 minutes). At this point you will be withdrawn from the water. After drying and putting on your dry shorts you will enter the environmental chamber (0°C) with one of the four survival bags. You will then be asked to sit on a chair whilst inside the survival bag. You will be randomly assigned at each trial to one of the four practical cold protection methods: 1. Polythene survival bag; 2. Polythene survival bag with hot drink; 3. Blizzard Survival bag and 4. Blizzard Survival Heat bag. After getting into the survival bag and at 1 and 2 hours you will be provided with a flavoured drink. The quantity of this drink will depend on your body weight but will be
similar to a large mug of tea. On one trial (trial 2) this drink will be hot, i.e. similar to that temperature of regular tea or coffee. You will be asked to consume these drinks within 15 min. Regular measurements of thermoregulation will be made and core temperature will be continually monitored. Cognitive function tests and saliva samples will be obtained at one hour intervals. The experimental trials will end and you will be removed from the chamber at 3 hours or if core temperature decreases to 35°C. Following removal from the environmental chamber (0°C), a blood and saliva sample will be obtained and you will be wrapped in blankets. You may be immersed in warm water to aid rewarming. One and two hours following the cold exposure we will obtain further blood and saliva samples during which time you will sit in a comfortable ambient temperature ~20°C with blankets or dressed in your normal clothes. During this time, your core temperature will be monitored and you will be supervised by experimenters. In the second hour of this recovery you will be provided with a large meal and warm drink. Following the final blood and saliva sample you will be allowed to eat and drink freely and you will be permitted to leave when your core temperature returns to with 0.5°C of your initial core temperature. Transport home will be provided if required.

Advantages of taking part

A benefit of taking part in this study is that you will receive comprehensive feedback, with full explanations, of your body composition (e.g. body fat %) and blood measures (e.g. immune function). This feedback should help you with planning and monitoring your athletic training program. The feedback you will receive regarding your body composition is similar to that which many testing facilities provide as a fee-paying service. Advantages for Undergraduates from within SSHES are that you will gain a valuable insight into the procedures and work involved in a 3rd year project. Additionally participation in this project can be used for skills units of the Undergraduate portfolio.

Disadvantages of taking part

The disadvantages of taking part in this study, which you will probably be most concerned about, are: 1. cold exposure test 2. blood samples; and 3. time commitment.

1. Cold exposure: Cold tests lasting two to three hours have been safely used to stress the body’s ability to maintain core body temperature by our research group and many others. During these cold air tests you will likely experience some peripheral numbness and mild discomfort in your hands and feet. To minimise this discomfort, we will provide you with thermal gloves and socks.

A number of military studies have exposed individuals to freezing and/or wet conditions following periods of prolonged physical exercise, inadequate nutrition and sleep loss, and no medical complications were reported after rewarming in individuals whose core temperature decreased to 35°C). In these investigations no voluntary ‘drop out’ or medical withdrawal of subjects was reported. Additionally, in contrast to these previous investigations in which subjects sat uncovered, the provision of survival bags in the proposed study will provide insulation.
In the current investigation you will be removed from the climate chamber if your core temperature decreases to 35°C. It is important to note that 35°C is considered the upper limit of mild hypothermia (35-32°C), and following the removal of cold stress your thermoregulatory mechanisms are likely to steadily recover normal core temperature (~37°C). For example, in a study using cold water immersion to reduce core temperature to 33°C participants left to shiver post immersion were able to recover core temperature to 36°C within 1 hour.

Following removal from the chamber you will be wrapped in blankets. In addition, if your core temperature reduces below 35°C you will be immersed up to the axilla in a warm water bath (38-42°C). This technique has previously been used in a number of studies to rewarm participants in experiments where core temperature is decreased to between 33-34°C. For your safety your core temperature will be monitored by a rectal probe every minute throughout the cold exposure protocol and during the first hour of recovery period or until your core temperature returns to within 0.5°C of their starting core temperature.

The results of this study will ensure people can be better informed as to the most effective method of cold protection in extreme environmental conditions.

2. Blood samples: The blood samples will be taken using a smaller needle than is typically used by doctors in your local surgery or hospital. Therefore you will likely experience only very mild discomfort i.e. like a scratch. The quantity of blood we are taking in each visit is small (i.e. approximately one tenth of that which would be obtained when you give blood). Additionally, qualified phlebotomists experienced at performing this procedure will collect blood samples. To ensure you are completely happy with giving blood samples, we will familiarise you with the blood sampling procedure on your first visit to the laboratory.

3. Time commitment: To complete all aspects of the study we will require you to visit the laboratory on six occasions for a total of approximately 33 hours. Thus, participation in this project satisfies the requirement in hours for the Undergraduate portfolio skills unit.

At all times you will be closely supervised by an experimenter trained in First-Aid.

Any further questions will be happily answered by Dr Sam Oliver or any of the additional investigators.
Appendix B:

INFORMED CONSENT TO PARTICIPATE
IN A RESEARCH PROJECT OR EXPERIMENT

Title of Research Project:

The researcher conducting this project subscribes to the ethics conduct of research and to the protection at all times of the interests, comfort, and safety of participants. This form and the information sheet have been given to you for your own protection and full understanding of the procedures. Your signature on this form will signify that you have received information which describes the procedures, possible risks, and benefits of this research project, that you have received an adequate opportunity to consider the information, and that you voluntarily agree to participate in the project.

Having been asked by Jenny Brierley of the School of Sport, Health and Exercise Sciences at Bangor University, to participate in a research project titled:

Effect of hypoxia on the thermoregulatory responses to cold

I have received information regarding the procedures of the experiment.

I understand the procedures to be used in this experiment and any possible personal risks to me in taking part.

I understand that I may withdraw my participation in this experiment at any time.

I also understand that I may register any complaint I might have about this experiment to Professor Mike Khan, Head of the School of Sport Health and Exercise Sciences, and that I will be offered the opportunity of providing feedback on the experiment using standard report forms.

I may obtain copies of the results of this study, upon its completion, by contacting: Jenny Brierley (Tel: 07825841523 or Email: peu474@bangor.ac.uk)

I confirm that I have been given adequate opportunity to ask any questions and that these have been answered to my satisfaction.

I have been informed that the research material will be held confidential by the researcher.

I agree to participate in the study

NAME (please type or print legibly): ______________________________________________________

ADDRESS: (Optional)__________________________________________________________________

____________________________________________________________________________________

PARTICIPANT’S SIGNATURE: _______________ DATE: _______________ RESEARCHER’S SIGNATURE: _______________ DATE: _______________
### An Experimental Study of Practical Field Methods for Cold Casualty Protection and Treatment during Prolonged Cold Exposure

<table>
<thead>
<tr>
<th>1</th>
<th>Title of project</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>An Experimental Study of Practical Field Methods for Cold Casualty Protection and Treatment during Prolonged Cold Exposure</em></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2</th>
<th>Name and e-mail address(es) of all researcher(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sam Oliver - <a href="mailto:pes60b@bangor.ac.uk">pes60b@bangor.ac.uk</a></td>
</tr>
<tr>
<td></td>
<td>Neil Walsh – <a href="mailto:pes804@bangor.ac.uk">pes804@bangor.ac.uk</a></td>
</tr>
<tr>
<td></td>
<td>Gavin Lawrence - g.p.lawrence.ac.uk</td>
</tr>
<tr>
<td></td>
<td>Matt Fortes – <a href="mailto:pep006@bango.ac.uk">pep006@bango.ac.uk</a></td>
</tr>
<tr>
<td></td>
<td>Jenny Brierley – <a href="mailto:peu474@bangor.ac.uk">peu474@bangor.ac.uk</a></td>
</tr>
<tr>
<td></td>
<td>Alberto Dolci – <a href="mailto:dolcialberto@gmail.com">dolcialberto@gmail.com</a></td>
</tr>
<tr>
<td></td>
<td>Phil Heritage – <a href="mailto:p.heritage@bangor.ac.uk">p.heritage@bangor.ac.uk</a></td>
</tr>
<tr>
<td></td>
<td>Bethan Palmer – <a href="mailto:peu86e@bangor.ac.uk">peu86e@bangor.ac.uk</a></td>
</tr>
<tr>
<td></td>
<td>James Firman – <a href="mailto:peu81c@bangor.ac.uk">peu81c@bangor.ac.uk</a></td>
</tr>
<tr>
<td></td>
<td>Robin Wilson – <a href="mailto:peu833@bangor.ac.uk">peu833@bangor.ac.uk</a></td>
</tr>
<tr>
<td></td>
<td>Aaron Burdett – <a href="mailto:peu632@bangor.ac.uk">peu632@bangor.ac.uk</a></td>
</tr>
</tbody>
</table>

Please tick boxes

1. I confirm that I have read and understand the Information Sheet dated ……………………. for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason, without my medical care or legal rights being affected.

3. I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. If I do decide to withdraw I understand that it will have no influence on the marks I receive, the outcome of my period of study, or my standing with my supervisor, other staff members of with the School.

4. I understand that I may register any complaint I might have about this experiment with the Head of the School of Sport, Health and Exercise Sciences, and that I will be offered the opportunity of providing feedback on the experiment using the standard report forms.
5 I agree to take part in the above study.

Name of Participant ………………………………………………………………………..

Signature ……………………………. Date …………………………………………..

Name of Person taking consent…………………………………………………………

Signature ……………………………. Date …………………………………………..

WHEN COMPLETED – ONE COPY TO PARTICIPANT, ONE COPY TO RESEARCHER FILE
Appendix C:

PHYSIOLOGY INFORMED CONSENT
& MEDICAL QUESTIONNAIRE

Name: ..............................

Age:.........................

Are you in good health?  Yes/No

If no, please explain:

How would you describe your present level of activity? Tick intensity level and indicate approx. duration.

vigorous  moderate  low intensity

Duration (Min).

How Often:  < once per month
once per month
2-3 times per week
4-5 times per week
> 5 times per week

Have you suffered from a serious illness or accident?  Yes/No

If yes, please give particulars:

Do you suffer, or have you ever suffered from:

Asthma  Yes  No
Diabetes  Yes  No
Bronchitis  Yes  No
Epilepsy  Yes  No
High blood pressure  Yes  No

Are you currently taking medication ?  Yes/No

If yes, please give particulars:

Are you currently attending your GP for any condition or have you consulted your doctor in the last three months?  Yes/No

If yes, please give particulars:

Have you, or are you presently taking part in any other laboratory experiment?  Yes/No
PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to do the experimental exercise task if they:

- have a fever, suffer from fainting spells or dizziness;
- have suspended training due to a joint or muscle injury;
- have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
- have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;
- have anaphylactic shock symptoms to needles, probes or other medical-type equipment.
- have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella)
- have a history of infectious diseases (e.g. HIV, Hepatitis B); and if appropriate to the study design, have a known history of rectal bleeding, anal fissures, hemorrhoids, or any other condition of the rectum;

DECLARATION

I agree that I have none of the above conditions and I hereby volunteer to be a participant in experiments/investigations during the period of ...............20___.

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further, my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Signature of Participant: .................................................................

Date: ....................................................

Signature of Experimenter: .............................................................

Date: .....................................................
Name of Participant .................................................................

Age ............................

Are you in good health?  YES  NO

If no, please explain

How would you describe your present level of activity?

Tick intensity level and indicate approximate duration.

<table>
<thead>
<tr>
<th>Vigorous</th>
<th>Moderate</th>
<th>Low intensity</th>
</tr>
</thead>
</table>

Duration (minutes)..............................................................................

How often?

<table>
<thead>
<tr>
<th>&lt; once per month</th>
<th>4-5 times per week</th>
</tr>
</thead>
<tbody>
<tr>
<td>once per month</td>
<td>&gt; 5 times per week</td>
</tr>
<tr>
<td>2-3 times per week</td>
<td></td>
</tr>
</tbody>
</table>

Have you suffered from a serious illness or accident?  YES  NO

If yes, please give particulars:
Do you suffer, or have you ever suffered from:

<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epilepsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High blood pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchitis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Are you currently taking medication?  

If yes, please give particulars:

Are you currently attending your GP for any condition or have you consulted your doctor in the last three months?  

If yes, please give particulars:

Have you, or are you presently taking part in any other laboratory experiment?

---

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to do the experimental exercise task if they:

- have a fever, cough or cold, or suffer from fainting spells or dizziness;
- have suspended training due to a joint or muscle injury;
- have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
- have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;
- have anaphylactic shock symptoms to needles, probes or other medical-type equipment;
- have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella);
• have a history of infectious diseases (e.g. HIV, Hepatitis B); and if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum.

DECLARATION

I agree that I have none of the above conditions and I hereby volunteer to be a participant in experiments/investigations during the period of February – June 2011

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further, my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Signature (participant) .................................................. Date .........................

Print name ..............................................................

Signature (experimenter) .......................................... Date .........................

Print name ..............................................................
Name of Participant .................................................................................................

Researcher ...........................................................................................................

Date .....................................................................................................................

<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>Have you had any kind of illness or infection in the last two weeks?</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Are you taking any form of medication?</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Do you have any form of injury?</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Have you eaten in the last hour?</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Have you consumed any alcohol in the last 24 hours?</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>Have you performed exhaustive exercise in the last 48 hours?</td>
</tr>
</tbody>
</table>

**IF THE ANSWER TO ANY OF THE ABOVE IS 'YES', THEN YOU MUST CONSULT A MEMBER OF STAFF BEFORE UNDERGOING ANY EXERCISE TEST.**

Signature *(participant)* ..................................................... Date ..................

*Print name* .................................................................................................
Appendix D:

McGinnis Thermal Comfort Scale

1. So cold I am helpless
2. Numb with cold
3. Very cold
4. Cold
5. Uncomfortably cold
6. Cool but fairly comfortable
7. Comfortable
8. Warm but fairly comfortable
9. Uncomfortably warm
10. Hot
11. Very hot
12. Almost as hot as I can stand
13. So hot I am sick and nauseated
<table>
<thead>
<tr>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>Absolute maximum</td>
</tr>
<tr>
<td>110</td>
<td>&quot;Maximal&quot;</td>
</tr>
<tr>
<td>100</td>
<td>Extremely strong</td>
</tr>
<tr>
<td>95</td>
<td>Very strong</td>
</tr>
<tr>
<td>90</td>
<td>Strong</td>
</tr>
<tr>
<td>85</td>
<td>Heavy</td>
</tr>
<tr>
<td>80</td>
<td>Somewhat strong</td>
</tr>
<tr>
<td>75</td>
<td>Moderate</td>
</tr>
<tr>
<td>70</td>
<td>Weak</td>
</tr>
<tr>
<td>65</td>
<td>Light</td>
</tr>
<tr>
<td>60</td>
<td>Very weak</td>
</tr>
<tr>
<td>55</td>
<td>Extremely weak</td>
</tr>
<tr>
<td>50</td>
<td>Minimal</td>
</tr>
<tr>
<td>45</td>
<td>Nothing at all</td>
</tr>
</tbody>
</table>

Note: The values are approximate and may vary depending on the specific context or scale used.
Shivering Intensity Scale – Observer rating scale

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No shivering activity</td>
</tr>
<tr>
<td>1</td>
<td>Mild shivering in bursts</td>
</tr>
<tr>
<td>2</td>
<td>Generalised but discontinuous shivering</td>
</tr>
<tr>
<td>3</td>
<td>Very marked and continued shivering movements.</td>
</tr>
</tbody>
</table>
Appendix G:

PAIN SENSATION SCALE

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BARELY COOL</td>
</tr>
<tr>
<td>2</td>
<td>COOL, NO PAIN</td>
</tr>
<tr>
<td>3</td>
<td>COLD, NO PAIN</td>
</tr>
<tr>
<td>4</td>
<td>SLIGHT PAIN</td>
</tr>
<tr>
<td>5</td>
<td>MILD PAIN</td>
</tr>
<tr>
<td>6</td>
<td>MODERATE PAIN</td>
</tr>
<tr>
<td>7</td>
<td>MODERATE – STRONG PAIN</td>
</tr>
<tr>
<td>8</td>
<td>STRONG PAIN</td>
</tr>
<tr>
<td>9</td>
<td>SEVERE PAIN</td>
</tr>
<tr>
<td>10</td>
<td>UNBEARABLE PAIN</td>
</tr>
</tbody>
</table>
Appendix H:

### Wisconsin Upper Respiratory Symptom Survey

**Daily Symptom Report**

<table>
<thead>
<tr>
<th>Date:</th>
<th>Time:</th>
<th>ID:</th>
</tr>
</thead>
</table>

Please fill in one circle for each of the following items.

<table>
<thead>
<tr>
<th>Not sick</th>
<th>Very mildly</th>
<th>Mildly</th>
<th>Moderately</th>
<th>Severely</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

**How sick do you feel today?**

Please rate the average severity of your cold symptoms over the last 24 hours.

<table>
<thead>
<tr>
<th>Do not have this symptom</th>
<th>Very mild</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coughing stuff up</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough interfering with sleep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sore throat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scratchy throat</td>
<td></td>
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Appendix I:

A cross-sectional study examining human thermoregulation during survival bag treatment in the cold

Accidental hypothermia affects approximately 10% of casualties reported in mountainous environments and is a significant contributor to fatalities (Sallis and Chassay, 1999; Sharp, 2007). Rescue times for casualties depend on global location and weather conditions. Yet even where distances are relatively short between emergency services and a casualty (e.g. Scottish Highlands) the reported times for evacuation are 2.25 hours by helicopter and approximately 3.5 hours when individuals are evacuated by others means (e.g. on foot) (Crocket, 1995; Hindsholm et al. 1992).

Simple methods that prevent heat loss and promote re-warming which casualties or first-responders can administer instantly whilst they wait for evacuation will likely reduce hypothermia related fatalities. Polythene survival bags however, have been shown to confer no additional thermal protection for a shivering individual compared to single layer MPS. However it remains unknown whether triple layered MPS reduces heat loss in comparison to single polythene. **PURPOSE:** To determine whether a multi-layered survival bag offers superior thermal protection compared to single-layer polythene in the cold. **METHOD:** 18 individuals (13 m, 5 f) volunteered to participate. Participants completed a 5 day wilderness expedition before being randomly assigned to one of two groups. The groups were 1. Polythene Survival bag (PB) or 2. Blizzard Survival bag (BB). During the trials participants wore shorts only (vests also for females) and completed a 2 h cold air test (CAT, 0°C) in a seated position. Core temperature was assessed throughout. A two-way ANOVA was performed on the core temperature data. **RESULTS:** Core temperature was not different between the groups prior to cold exposure and no further differences occurred at any time point throughout the CAT (**Figure A.1**). **CONCLUSION:** For normothermic, shivering individuals at rest, a multi-layered MPS survival bag conferred no advantage in reducing heat loss compared with a single-layer polythene bag during cold exposure.
Figure A.1. The effect of a 120 minute cold air test on core temperature in two field cold protection interventions (i.e. Polythene survival bag (PB, ■); Blizzard Survival bag (BB, ◆)) Values are Means ± SD.
Appendix J.

The effect of chemical heat pads on skin temperature in 10°C, 20°C and 30°C

Chemical heat pads self activate upon contact with air and can continue to supply heat for up to 24 hours. Given thermal injury may occur when skin is heated to above 44°C (Moritz and Henriques, 1947) and survival devices incorporating chemical heat pads may be employed in ambient conditions that are already warm (e.g. military trauma casualties), it is vital that such heat pads situated inside survival bags do not supply heat great enough for this threshold to be reached.

PURPOSE: The purpose of this investigation was to examine skin temperature during the application of chemical heat pads in an ambient temperature of 10°C, 20°C and 30°C. METHOD: Three volunteers participated in the study (2 f, 1 m). Participants completed three experimental trials in an environmental chamber wearing shorts and vests only. Trials were: 1. Blizzard Heat in 10°C, 2. Blizzard Heat in 20°C and 3. Blizzard Heat in 30°C. During each trial participants remained seated inside the Blizzard Heat survival bag which incorporated four chemical heat pads. Skin temperature was recorded throughout at four sites (calf, forearm, chest and thigh). Data are presented as Means ± SD. RESULTS: The chemical heat pads did not evoke skin temperatures above the threshold deemed to initiate thermal injury in any of the three ambient exposures (Figure A.2). CONCLUSION: The chemical heat pads provided in a Blizzard Heat survival bag do not cause skin temperature to rise above the threshold temperature considered to cause thermal injury even in warm environments. This has significant benefit for trauma casualties who are predisposed to hypothermia even in warm temperatures (e.g. military personnel).
Appendix A.2. The effect of chemical heat pads in three ambient temperatures (10°C, 20°C and 30°C) upon skin temperature. Values are Means ± SD.