STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH
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Esitetään Jyväskylän yliopiston liikuntatieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi Liikunnan salissa L303
kesäkuun 11. päivänä 2014 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Sport and Health Sciences of the University of Jyväskylä,
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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2014

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Publishing Unit, University Library of Jyväskylä

URN:ISBN:978-951-39-5716-2
ISBN 978-951-39-5716-2 (PDF)

ISBN 978-951-39-5715-5 (nid.)
ISSN 0356-1070

Jyväskylä University Printing House, Jyväskylä 2014

“Man should not try to avoid stress any more than he would shun food, love or exercise’’

Hans Selye

ABSTRACT

Gagnon, Dominique D.
The effects of exercising in the cold on energy metabolism, skeletal muscle tissue oxygenation and immuno-endocrine responses.
Jyväskylä: University of Jyväskylä, Finland, 2014, 121 p
(Studies in Sport, Physical Education and Health,
ISSN 0356-1070; 206)
ISBN 978-951-39-5715-5 (nid.)
ISBN 978-951-39-5716-2 (PDF)

Progressively expanding access to the Arctic region has led to an increased number of people who live and work in cold environments, which may interfere with health, safety and performance. The present study examined the effects of cold exposure during exercise on energy metabolism, skeletal muscle tissue oxygenation and immune-endocrine responses. A 56-day, 850 km North Pole expedition study (n = 7) with daily energy intake of 23 MJ · d^{-1}, energy expenditure of 29 MJ · d^{-1} and temperatures of -3˚C to -47˚C induced decreases in serum concentration of testosterone, cortisol and thyroxine post expedition. Endocrine recovery was observed within two weeks but HDL and LDL levels remained elevated at two weeks post. Acute submaximal exercise in the cold (0˚C *vs.* 22˚C) at 50% and 70% of peak O_2 consumption showed no increase in serum non-esterified fatty acids (NEFA), glycerol, or catecholamine concentrations but increased fat oxidation at both exercise intensities. Pre-exercise shivering increased contribution of fat to the energy yield before exercise. Nonetheless, even with an increase in plasma norepinephrine, NEFA, glycerol and beta-hydroxybyturate at 70% VO_{2peak}, fuel selection was not influence by pre-exercise shivering during exercise in the cold. Skeletal muscle tissue oxygenation was decreased by pre-exercise cooling in the early stages of exercise in the vastus lateralis muscle. Endocrine and immunological responses were more elevated in the thermoneutral environment, and in some measures in the cold with shivering. Altogether, these findings demonstrate that: *i)* prolonged and extreme physical stress have minimal influence on hormonal recovery time but blood lipids tended to carry longer-lasting effects, *ii)* when exercising in a cold environment, a dissociation between energy substrates availability and utilization leading to a different energy selection mechanism occurs, *iii)* pre-exercise low-intensity shivering does not modulate fuel selection during submaximal exercise in the cold, *iv)* deep tissue cooling may reduce skeletal muscle oxygenation during the early stages of submaximal dynamic exercise, and *v)* exercising in a cold environment seems to reduce exercise-related inflammatory responses while shivering may evoke immuno-stimulatory effects.

Keywords: Cold exposure, exercise, shivering, fuel selection, muscle, near-infrared spectroscopy, hormones, cytokines

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ACKNOWLEDGEMENTS

The first person I would like to thank is my supervisor, Prof. Heikki Kyröläinen, who believed in me from the start and took me in as his doctoral student. He took time to point me towards the right direction at every corner and provided support whenever needed. My interests in environmental exercise physiology were met with enthusiasm from Heikki as he ensured to build a team of amazing researchers that would meet the demands for our projects. His support is not only seen in the final product of this thesis but in the fact that I was given an opportunity and a positive environment to learn and further advance my research career throughout my years under his tutelage.

When I was introduced to Prof. Hannu Rintamäki, my co-advisor, I could tell from the first handshake that working with him would be a privileged time. He opened the doors of his lab at the Finnish Institute of Occupational Health (TTL) in Oulu for me with little questions or concerns and patiently answered to the many questions I had about cold exposure. He introduced me to his team, Dr. Juha Oksa, Dr. Sirkka Rissanen, and Tero Mäkinen (*post-mortem*) who all kindly showed me all the little things to know at TTL. His kindness, generosity and honesty have helped turned my PhD project into successful research and for that I am forever thankful.

Prof. Karl-Heinz Herzig, who arrived later in the process, became an important mentor for me in his view of what science should be and how should be done. His guidance provided me with a drive in excelling in my work, collecting quality data and always working to become better of a researcher. His medical expertise during data collection proved to be essential and unequaled in quality.

I also want to express my thanks to the co-authors affiliated to the manuscripts comprising this thesis. Dr. Teemu Pullinen from the University of Jyväskylä, Dr. Heikki Karinen from Tampere University, Dr. Stephen Cheung from Brock University, Dr. Timo Törmäkangas from the University of Jyväskylä, Dr. Juha Peltonen from the University of Helsinki University, Dr. Juha Oksa from the Finnish Institute of Occupational Health, Katja Porvari from the University of Oulu, and Katri Puukka from the University of Oulu, thank you all for your wonderful help and support.

The preliminary assessment of this thesis was done by Dr. Benoit Dugué from the University of Poitiers and Dr. John W. Castellani from the U.S. Army Research Institute of Environmental Medicine. I want to thank both reviewers in providing their knowledge and expertise in many facets of cold and exercise physiology for the betterment of the present work with detailed and insightful comments.

The data collection portion of the study was accomplished with the help and support of four other students, including Niina Kantola, Tiina Takalokastari, and Krista Rahunen. I express my utmost gratitude to all of you for spending so much time and effort on a project that was so dear to me. I hope

you have learned as much from me that I have learned from you. I wish you all the best in your future endeavors.

The work presented here was financially supported by multiple institutions and foundations. I want to thank the University of Jyväskylä's Department of Biology of Physical Activity, the Finnish Institute of Occupational Health and the University of Oulu's Department of Physiology for supporting the project. I also would like to thank the Center of Military Medicine and the Jenny ja Antti Wihurin foundation for believing in the contributions of this thesis.

Now that I have been going through the doctoral process, I can truly measure the support my family has given me over the years. To my family spread out from Karkkila, to Winnipeg, to Montréal, to Lancaster, no words can describe the energy you would give me from a simple phone call or an email of encouragement. Although I don't see any of you enough, know that I always think of you. Sheila, I sincerely hope that I can provide you with the same support you have given me in your own work. You have been patient, kind, supportive and even given the extra push when I needed it. The list would be too long to name everything you have helped me with. I love you so much. Eljas, for every word I misspelled, every graph that had the wrong scale, every software that crashed at the worst of time, a simple thought of you fixed everything and made me smile. Don't grow up too fast and remember, *je t'aime mon grand garçon*. To the latest arrival in the family, Sini, *bienvenue au monde ma belle petite fille. On va prendre bien soin de toi.*

Finally, thanks to you Finnish coffee. You have changed my life.

LIST OF ABBREVIATIONS

˚C	Degree celsius
%En	Relative percentage of energy contribution
%Fat	Percentage body fat
ACTH	Adrenocorticotropic hormone
AHS	Adrenomedullary hormonal system
AVA	Arterio-venous anastomoses
BM	Body mass
BMI	Body mass index
BP	Blood pressure
cAMP	Cyclic adenosine monophosphate
CHO	Carbohydrates
COR	Cortisol
diffHb	Difference in oxy-deoxygenated hemoglobin content
ED	Energy deficit
EE	Energy expenditure
EI	Energy intake
EMG	Electromyography
FFA	Free fatty acids
FFM	Fat free mass
FGF2	Fibroblast growth factor
GAS	Gastrocnemius
G-CSF	Granulocyte colony-stimulating factor
GH	Growth hormone
HCT	Hematocrit
HDL	High density lipoprotein
HGB	Hemoglobin
HHb	Deoxygenated hemoglobin
HPA	Hypothalamic-pituitary-adrenocortical
HPG	Hypothalamic-pituitary-gonadal
HPT	Hypothalamic-pituitary-thyroid
HR	Heart rate
IFN- γ	Interferon gamma
IGF-1	Insulin-like growth factor 1
IMTG	Intra-muscular triglycerides
IL	Interleukin
IP-10	Interferon gamma-induced protein 10
KJ	Kilo joules
LDL	Low density lipoprotein
Ln	Natural log
MCP-1	Monocyte chemotactic protein-1
MIP-1 β	Macrophage inflammatory protein-1 β
MJ	Mega joules
MF	Median frequency

MPF	Mean power frequency
NK	Natural killer cells
O_2Hb	Oxygenated hemoglobin
PDGF	Platelet-derived growth factor
PLT	Platelets
RBC	Red blood cells
RER	Respiratory Exchange Ratio
RH	Relative humidity
SHBG	Sex hormone-binding globulin
$Shiv_{peak}$	Peak shivering
SNS	Sympathetic nervous system
tHb	Total hemoglobin
T_3	Triiodothyronine
T_{3free}	Free triiodothyronine
T_{4free}	Free thyroxine
T_c	Core temperature
TC	Total cholesterol
TES_{bio}	Bioavailable testosterone
TES_{tot}	Total testosterone
TES/COR	Testosterone to cortisol ratio
TG	Triglyceride
TSH	Thyroid-stimulating hormone
T_{sk}	Skin temperature
T_{re}	Rectal temperature
UCP1	Uncoupling Protein 1
VEGF	Vascular endothelial growth factor
VL	Vastus lateralis
VLDL	Very low density lipoprotein
VO_2	Oxygen consumption
WBC	White blood cells

LIST OF ORIGINAL PAPERS

The present thesis is based on the following original articles, which are referred to in the text by their Roman numerals.

I. Gagnon D.D., Pullinen T., Karinen H., Rintamäki H., and Kyröläinen H. (2011) Recovery of hormonal, blood lipid, and hematological profiles from a North Pole expedition. Aviation, Space, and Environmental Medicine 82(12):1110-1117.

II. Gagnon D.D., Rintamäki H., Gagnon S.S., Cheung S.S., Herzig K.H., Porvari K., and Kyröläinen H. (2013) Cold exposure enhances fat utilization but not non-esterified fatty acids, glycerol or catecholamines availability during submaximal walking and running. Frontiers in Physiology 4. Article 99, doi: 10.3389/fphys.2013.00099.

III. Gagnon D.D., Rintamäki H., Gagnon S.S., Oksa O., Porvari K., Cheung S.S., Herzig K.H., and Kyröläinen H. (2014) Fuel selection during short-term submaximal treadmill exercise in the cold is not affected by pre-exercise low-intensity shivering. Applied Physiology: Nutrition and Metabolism 39(3): 282-291.

IV. Gagnon D.D., Peltonen J., Rintamäki H., Gagnon S.S., Herzig K.H., Kyröläinen H. (2014) Ambient temperature and body cooling on skeletal muscle oxygenation during exercise. Submitted for publication.

V. Gagnon D.D, Gagnon S.S., Rintamäki H., Törmäkangas T., Puukka K., Herzig K.H., Kyröläinen H. (2014) Immuno-modulation by cold exposure following acute exercise on leukocytes, endocrine and cytokine profiles in humans. Submitted for publication.

CONTENTS

1 INTRODUCTION

Progressively expanding access to the Arctic region has led to an increased number of people who live and work in cold environments. Driving the movement into the Arctic are military interests, as well as the search for, and exploitation of, natural resources. Northern populations are also regularly exposed to cold environments while performing daily tasks and physical activities. Exposure can be limited to a few minutes but, in certain cases, can also be expended to months (e.g. exploratory expeditions, military missions, occupational workers, etc.). Exposure to cold temperatures causes stresses on the body and induces a cascade of physiological changes that may threaten an individual's health and safety and can limit physical performance. Responses to cold expose involve nearly all physiological systems but importantly include changes in energy metabolism, skeletal muscle tissue oxygenation, as well as endocrine and immune responses.

Energy metabolism, more specifically the dependent roles and contribution of carbohydrates (CHO) and fat towards energy production is critical during cold exposure. The capacity to perform work relies on energy supplies and utilization, therefore, a shift in fuel selection from one substrate to another introduces a limiting aspect in physical performance, which in a cold environment, can be hazardous. While exercise on its own requires a greater energetic demand compared to rest, cold exposure and the implication of shivering and non-shivering thermogenesis further increase heat production, therefore requiring more from CHO and fat supplies. Over the years, many studies (Hurley and Haymes 1982; Kruk et al. 1991; Layden et al. 2002, 2004a, 2004b) have examined energy availability and utlization during exercise in various cold settings, but variable experimental designs have made it difficult to determine precise changes in substrates metabolism. Long-term exercise in a cold environment has provided significant evidences pointing towards greater use of fat (Helge et al. 2003; Roschevsky 1993; Stroud et al. 1993, 1997). Acute exercise and cold exposure, however, have demonstrated equivocal results due to methodological inconsistencies across studies including standardization in environmental conditions, exercise intensity and modality, oxygen uptake, and

degree of thermal stress among individuals (Hurley and Haymes 1982; Timmons et al. 1985; Galloway and Maughan 1997; Layden et al. 2002). Exercising in the cold may induce muscle cooling, reduce mechanical efficiency, and therefore increase VO_2 demand for a specific exercise workload (Oksa et al. 2002). The increased VO_2 demand would explain previous studies resulting in an increase of CHO use in the cold. Therefore, precise assessment of fuel selection changes during exercise in the cold remains unknown. Moreover, greater cold stress may induce shivering thermogenesis, known to regulate metabolic fuels differently from exercise (Tipton et al. 1997; Haman et al. 2005, 2007). Although shivering increases VO_2 and therefore fuel selection, previous studies have used pre-determined cooling times or observational shivering onset without standardizing the thermoregulatory effects of cooling across subjects prior to exercise onset (Hurley and Haymes 1982; Kruk et al. 1990, 1991). Metabolic and thermoregulatory state of subjects under cold conditions are known to be affected by body fat content, body surface area, resting metabolic rate, fitness level, muscle mass and fiber composition, and others (Eyolfson et al. 2001; Xu et al. 2005). Shivering and exercise can co-exist but determining the potential effects shivering may have on fuel selection during exercise, thermal stress among subjects needs to be carefully controlled. Energy metabolism during exercise in the cold currently requires a detailed methodological reassessment of factors influencing fuel selection.

Furthermore, cold-induced peripheral vasoconstriction, a protective mechanism to limit heat loss, has recently been linked to an increase in muscle blood volume, thereby providing greater O_2 availability and potentially influencing physical performance (Marsh and Sleivert 1999). Prolonged skin surface cooling, however, is known to induce a reduction in skeletal muscle tissue blood volume and oxygenated hemoglobin content, at rest (Yanagisawa et al. 2007), possibly impeding oxygen transfer and uptake during exercise (Layden et al. 2002). During incremental exercise, progressive skeletal muscle tissue deoxygenation is seen in the working muscles as a function of oxygen uptake (Bhambhani 2004; Rissanen et al. 2012). Muscle oxygenation can be assessed via near-infrared spectroscopy (NIRS) technology, a reliable tool to measure oxygenation levels in various tissues. Peripheral vasoconstriction from cold exposure could influence skeletal muscle blood volume and consequently O_2 availability, subsequently modulating muscle deoxygenationduring exercise. A better understanding of local skeletal tissue oxidative metabolism changes could provide some insights on mechanisms reducing muscle efficiency in the cold.

Severe changes in hormonal concentrations have previously been observed, namely in cortisol, catecholamines, and thyroid and sex hormones during long-term cold exposure (Farrace et al. 1992; Stroud et al. 1997). Certain pathologies involving abnormal hormonal levels are detrimental to health and could present a risk to individuals during and following cold exposure. Acute cold exposure also modulates endocrine axes; to a lesser extent sex and thyroid hormones as opposed to catecholamines and cortisol, which are rapidly

secreted (Leppäluoto et al. 2005; Goldstein and Kopin 2008). Despite the well-known exercise-related endocrine changes (Hansen et al. 2012), the combination of cold and exercise on endocrine modulations have previously been inconsistent due to time differences in hormonal secretions, impact of exercise intensity (i.e. decrease in cortisol below VO_{2max} of 60% and increase above), and the strong response of the sympathetic nervous system (SNS) and catecholamine concentrations from cold exposure. The past decade has also focused on the bi-directional effects of the endocrine and the immune systems (Pedersen and Hoffman-Goetz 2000; Haddad et al. 2002; Fragala et al. 2011a, 2011b). Considerations in endocrine alterations need to also include changes in cytokines and other immune elements modulating hormonal levels.

Both cold exposure and muscular activity stimulate cytokine productions and activate a systemic immune response (Brenner et al. 1999). Alterations in the bi-directional immuno-endocrine system influence our ability to fend off infections and illnesses (Nieman 1994; Gleeson 2007). Since immune responses seem to be modulated by core temperature and its effects on endocrine axes (i.e. mainly increases in catecholamines and cortisol concentration from elevated core temperature) (Cross et al. 1996; Rhind et al. 2001; Peake et al. 2008), changes in thermal balance and heat production during exercise and cold exposure further generate difficulties in assessing accurate changes in the regulation of cytokine secretion. A study by Brenner et al. (1999) observed an immuno-stimulating effect of cold which was enhanced from pre-treatment exercise. Other reports have, however, indicated an immune-depressive effect (Jurankova et al. 1995; Beilin et al. 1998) or no change (Castellani et al. 2002) on immune responses. A review by LaVoy et al. (2011) examined 10 studies investigating the effects of exercising in the cold on immune responses and found no clear tendencies across studies for either an increase or a decrease in immune responses. The use of temperate environmental conditions in other reports (5-10°C) (McFarlin and Mitchell 2003; Peake et al. 2008), as opposed to cold (0°C), prevents sufficient thermal stress to modulate endocrine responses influencing immune functions. Also, the sympathetic nervous system (epinephrine and norepinephrine) and the hypothalamic-putiutary-adrenal axis (cosrtisol, adenocorticotropic hormone) have been the main endocrine axes to explain most changes in immune responses. It is known that other hormones such as testosterone, thyroxine and triiodothyronine further influence immune responses (Olsen and Kovacs 1996; Guevara Patino et al. 2000). To our knowledge, no studies have comprehensively looked at combined acute cold and exercise on the immuno-endocrine system in colder environmental settings and through a larger endocrine panel.

The presence of cold exposure can interfere on energy metabolism, skeletal muscle tissue oxygenation and immuno-endocrine responses during exercise. The extent which this interference may compromises human health, safety and performance remains to be elucidated as guidelines in the cold must balance between minimization of risk and maximization of productivity. Development of guidelines must be based on clearly defined physiological

effects in cold environments. A better understanding on the balance of fat and CHO during exercise, the degree to which tissue oxygenation may be modulated and the thorough examination of relationships between hormonal axes and some immune responses, during cold exposure and exercise is required to advance our understanding of environmental physiology. It is also crucial in determining safety standards for occupational workers and military personnel. Thus, the present study was designed to investigate the effects of cold exposure during exercise on energy metabolism, skeletal muscle tissue oxygenation and immune-endocrine responses.

2 REVIEW OF THE LITERATURE

The following review of literature will present the fundamentals of thermoregulation in the cold prior to reviewing exercise physiology in the cold. More precisely, thermoregulatory, cardiovascular, energetic, oxidative, endocrine, immunological, and lipidic responses to exercise in the cold will be covered.

2.1 Human Thermoregulation

Core temperature balance is constantly maintained through heat production (basal metabolism rate, muscle work, shivering, and brown adipose tissue thermogenesis) and heat loss (radiation, evaporation, convection, and conduction). The balance between heat loss and production resides in behavioral and physiological changes during cold exposure. Humans have always shown ingenuity concerning clothing and sheltering to protect themselves from the environments as behavioral changes can be voluntarily implemented. When observing and quantifying physiological responses to environmental changes, a wide inventory of variables needs to be taken into consideration including environmental and individual factors such as air and radiative temperature, air velocity, contact with cold items, altitude, air/water exposure, length of exposure (prolonged, repeated, short), level of fitness, body surface area, and percentage of body fat, among others. Environmental physiology research involves many physiological systems (endocrine, immunological, neurological, cardiovascular, pulmonary, musculoskeletal, etc.) playing important roles in regulating thermal homeostasis. Prior to examining specific contributions of various physiological systems, we must first explore the basis of what constitutes human thermal balance and its regulatory mechanisms

2.1.1 Thermoregulatory models

The control of thermal homeostasis of the human body is described through three thermoregulatory models: 1) the traditional set point model (Hammel et al. 1963), 2) the reciprocal inhibition model (Bligh 2006), and 3) the heat regulation model (Webb 1995). The first model is defined as a set temperature that implements a counteractive response when a deviation in temperature has occurred. All thermoregulatory signals (cold or warm) from peripheral and central sites are integrated into an overall response to the hypothalamus that either try to increase heat gain or heat loss, thus maintaining the set temperature point. The reciprocal model, although similar in some ways to the set point model, is structurally more complex as it also considers interconnections between warm and cold sensory pathways. A strong cold stimulation and its effector response, for example, would not only induce vasoconsctriction and possibly shivering but would also inhibit warm-related effector response neural pathways as well. Finally, the heat regulation model is setting itself apart from the other models, as it does not use a set temperature point to justify the occurrence of thermal responses. Instead, heat storage or the net balance between heat gain and heat loss is the main variable, which secondarily regulates body temperature.

2.1.2 Thermoregulatory pathways and anotomy

Thermoregulatory mechanisms mediating cold responses include stimulation of thermosensors in various depth of the skin (i.e., 0.15-0.17 mm and 0.6-0.6 mm) (Boulant 2006). Signals are then sent to the hypothalamus, the thermoregulatory center of the body, which in turn sends efferent signals to various organs and systems to adapt to the change in temperature. In the cold, the posterior portion of the hypothalamus controls vasoconstriction and shivering (Edholm and Weiner 1981). While a few effector pathways have been identified in thermoregulation (Edholm and Weiner 1981), the role of the sympathetic-adrenal-medullary axis (SAM), the hypothalamic-pituitary-adrenocortical axis (HPA), and the sympathetic nervous system (SNS) are of particular importance as their roles in vasoconstriction, shivering, cardiac output, heat production, energy mobilization and immune response are crucial in cold exposure (Armstrong 2000). Environmental physiology is a multidisciplinary field that requires a broad perspective of many systems. Figure 1 gives a brief overview of some mechanisms involved during cold exposure.

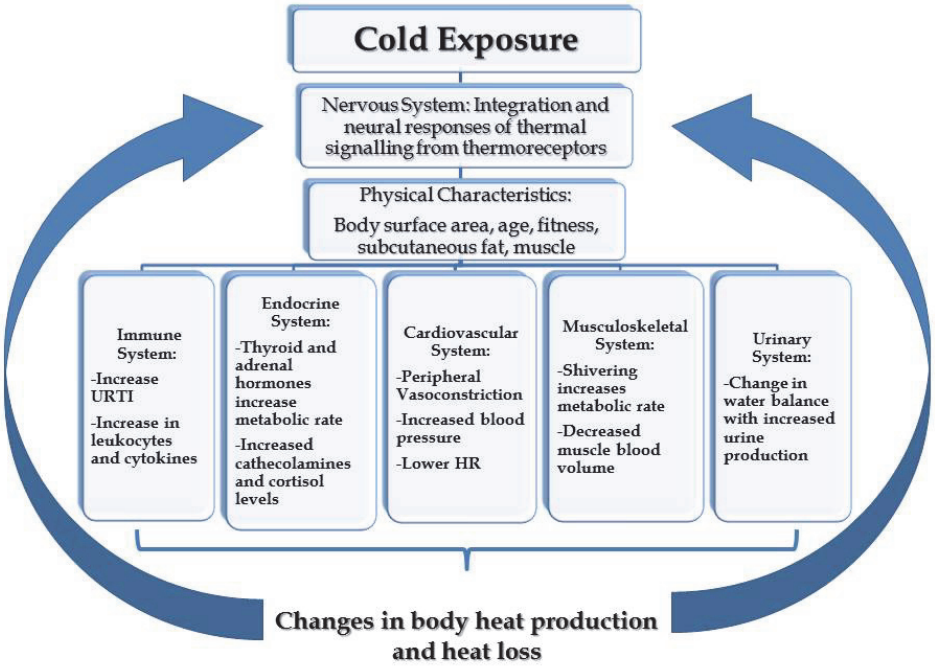

FIGURE 1 Physiological responses to cold exposure.

2.1.3 Thermal balance

Exposure of the human body to stressful environments (hot, cold, altitude, underwater) sets a sequence of responses to maintain homeostasis and body (core) temperature at around 37°C. In the cold, conservation of heat to regulate body temperature at a safe and functional level is paramount. Skin temperature (shell) on the other hand, has much more variability in temperature but generally remains at ~33 - 34°C in thermoneutral conditions. Heat balance equations have been developed to understand the role of the various thermal factors within core temperature regulation. Parsons (2003) expresses the heat equation as M - W = E + R + C + K + S in which metabolic heat production (M) minus mechanical work (W) equals evaporative (E), radiation (R), conductive (C) and convective (K) heat loss plus heat storage (S). The more standard and commonly used equation, in accordance with the Glossary terms of thermophysiology, is expressed as follows: $S = M \pm W_k \pm R \pm C \pm K - E$ ($W \cdot m^{-2}$). Where a net heat balance would be expressed as S = 0, heat gain would positive and heat loss would be negative.

2.1.4 Thermogenesis and shivering

Thermogenesis in humans is achieved via shivering and non-shivering mechanisms to generate heat in order to maintain core temperature. Non-shivering thermogenesis involves stimulation of brown adipose tissue in part mediated by uncoupling of protein 1 (UCP1) in the mitochondria (Ouellet et al. 2012). This mechanism has received little attention until recent evidences demonstrated its clear and significant presence in adults (Cannon and Nedergaard 2004; Nedergaard et al. 2007; van Merken Lichtenbelt 2009; Virtanen et al. 2009). Brown adipose tissue activity tends to be more active during the winter months and less active during the summer months (Au-Yong et al. 2009). Interestingly, under cold stress, brown adipose tissue activity is inversely proportional to shivering activity (Ouellet et al. 2012). As shivering increases, the contribution of brown adipose tissue to total heat production diminishes. In a cold environment, shivering thermogenesis, defined as the repetitive reflex of involuntary muscle contraction, appears from a decrease in skin and/or core temperature. The contribution of core cooling to total shivering activity is approximately 67-80 % respectively while the rest is attributable to skin and peripheral tissue cooling (Castellani et al. 2002; Frank et al. 1999; Toner et al. 1996). Maximum shivering is reached when skin temperature is at 17-20°C and core temperature at ~35°C (Tikuisis and Giesbrecht 1999). The maximum shivering intensity generated by an individual is referred as peak shivering ($Shiv_{peak}$), and corresponds to approximately 5 times resting metabolic rate or 50% of peak oxygen consumption (VO_{2peak}) (Golden 1979; Tikuisis and Giesbrecht 1999). Shivering pattern and intensity are very complex and are presented under different forms. Mainly, an estimated 71% of heat production during shivering is generated by the trunk muscles, 21% by the rectus femoris while the remaining 8% is from smaller muscles in lower leg and upper limbs (Bell et al. 1992). Low-intensity, moderate-intensity and high-intensity shivering recruit skeletal muscle fibers differently and progressively modulate the use of fat towards the use of carbohydrates. Recent work demonstrated that when shivering and exercise intensity are matched in terms of relative percentage of intensity, fuel selection, the contribution of fat and carbohydrate to energy expenditure, is similar (Haman et al. 2005) (Figure 2).

At a given absolute workload or at the same relative VO_2 scale, when we consider that $Shiv_{peak}$ represents approximately 50% of VO_{2max}, the crossover where fat and carbohydrates contribute equally to energy expenditure is considerably lower during shivering. Therefore, shivering and exercise are considered to be metabolically not analogous processes (Tipton et al. 1997; Haman et al. 2005).

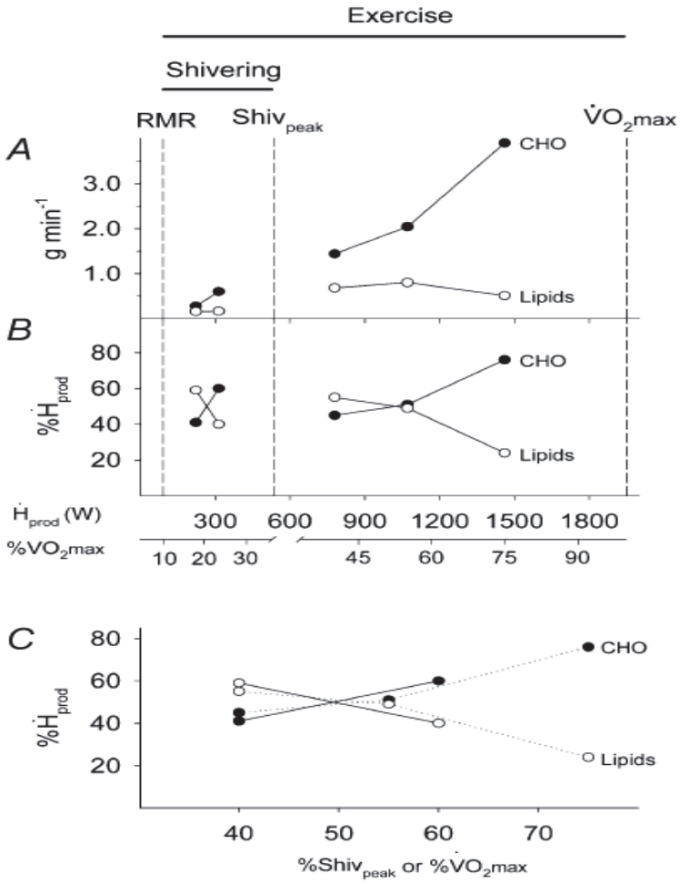

FIGURE 2 Comparison between exercise and shivering for fuel selection (A), heat production (B) and percentage of fat and CHO contribution to energy (C) at a given intensity. (from Haman et al. 2005, reproduced with permission from John Wiley and Sons)

Haman et al. (2002, 2004a, 2004b, 2004c, 2005, 2007) have performed extensive work in the field of shivering and energy metabolism but the simultaneously competing signals from exercise and shivering for muscle activation and its metabolic impacts are still poorly understood. It has been shown that exercise and shivering may co-exist simultaneously (Hong and Nadel 1979) but is generally seen only during low-intensity work (i.e., <50W, Kung et al. 1980) and during low force output (i.e., 10% maximal voluntary contraction) (Meigal et al. 1998). Nadel et al. (1973) proposed that the combined metabolic effects of shivering and exercise are additive as measured by an increase in oxygen consumption. The increase in thermoregulatory VO_2 over exercise-related VO_2 does not necessarily indicate the presence of shivering as muscle cooling increases metabolic demand at a given workload (Oksa et al. 2002, 2004). Reduced muscle temperature requires an increase in muscle fiber recruitments for a given work intensity, and therefore an increase in oxygen consumption

(Oksa et al. 2002). Muscle activation via electromyography (EMG) activity can determine the presence of shivering at rest, but this proves to be difficult during exercise as movements and voluntary muscle activation will blunt shivering-related EMG readings (Linnamo et al. 2003).

2.1.5 Individual characteristics and thermal responses

Morphological characteristics of individuals will, in part, determine cold tolerance, and, according to some thermoregulatory models, predict cold related responses (i.e., shivering, core cooling) (Eyolfson et al. 2001; Xu et al. 2005). Height and body mass are used to determine body surface area (BSA) (Dubois and Dubois 1916), which determined how much surface can be exposed to the cold. The BSA to mass ratio further provides support in determining thermal response as greater the ratio, greater the heat loss will be for a determined temperature, subsequently followed by a greater core cooling rate. This is of particular importance for children and adolescents who tend to have a bigger ratio compared to adults (Smolander et al. 1992). Body composition also plays an important role in thermal responses. The thermal resistance of adipose tissue is twice as much as that of muscle (Hatfield and Pugh 1951), which gives a cold-tolerance advantage to individuals with greater subcutaneous fat mass. Although subjects that are considered more fit, and consequently with lower fat mass, have been known to have more efficient thermoregulatory systems and even a greater control over core temperature via thermogenesis (Jett et al. 2006). This may be due to reduced sympathetic sensitivity in subjects with greater fat mass, therefore not responding as much to cold stimuli (Shepard 1992; Jett et al. 2006).

2.2 Exercise Physiology in the Cold

2.2.1 Cardiovascular response

Exercising in a cold environment induces a series of cardiovascular changes compared to a thermoneutral or hot environment including shifts in heart rate, blood pressure, cardiac output, and stroke volume (Gonzalez-Alonso 2000, 2012; Stocks et al. 2004a). More precisely, vasoconstriction appears very quickly upon cold exposure and is the primary factor in the subsequent cascade of cardiovascular changes. The sudden decrease in skin temperature (T_{sk}) provides a strong sympatho-adrenal response that rapidly signals the secretion of catecholamines (epinephrine, norepinephrine) and cortisol, acting as constriction agents (Wilkerson et al. 1974; Johnson et al. 1977). This occurs primarily in subcutaneous areas containing high amounts of arterio-venous anastomoses (AVA's) (connection between arteries and veins), namely the hands, feet, ears, nose, lips, elbows and cheeks (Daanen 2003). Thereafter, vasoconstriction occurs as a whole in the periphery such as arms and legs. This

response aims primarily at redirecting blood flow to the core organs to maintain functionality but also to minimize heat loss by preventing warm blood to circulate in cooled tissue (i.e., skin, subcutaneous fat and after prolonged cooling muscle as well). Previous work has demonstrated an important temporary increase in core cooling following the initial moments of rewarming after whole-body cooling (Giesbrecht et al. 1994: Giesbrecht at al. 1997). This decrease in core temperature (T_c), called 'afterdrop', lasts only a few minutes (i.e. 5-10 min) and is due to the return of cold blood from its redistribution to cooled peripheral tissue during rewarming.

In the presence of skin cooling, SNS activity promotes greater peripheral vasoconstriction (Castellani et al. 1998), thereby redirecting peripheral blood flow to the core as a thermal protective mechanism. The combination of increased central blood volume and the activation of the baroreceptor reflex from vasoconstriction and stimulation of the trigeminal nerve from cold air in the face are all phenomena associated with a reduced heart rate in the cold (Williams and Kilgour 1993). Therefore the mechanisms responsible for decreased heart rate in the cold are both neural (facial cooling) and metabolic (blood flow redistribution) indicating its strong physiologic significance. Finally, vasoconstriction also induces an increase in total peripheral vascular resistance and blood pressure (Jansky et al. 1996; Gagnon et al. 2013). This change ensures that despite the decreased blood flow to peripheral tissue, adequate oxygenation is maintained.

2.2.2 Energy metabolism

2.2.2.1 Fuel selection and availability

The proper mixture of carbohydrates (CHO; circulating glucose and intra-muscular glycogen), fat (circulating free fatty acids (FFA) and intra-muscular triglycerides (IMTG)) and proteins (amino acids), to fuel the energy systems, depends on various factors including exercise type and intensity, environmental conditions (i.e. temperature, wind, humidity, etc.), training status of the subjects, diet, and many others (Venables et al. 2005). For the purpose of this review, amino acids availability and utilization during cold exposure and exercise will not be discussed as protein contribution to energy during exercise is only ~5% and negligible (Carraro et al. 1990; Rennie et al. 1982) and remain unchanged during resting cold exposure (Haman et al. 2007). The selection of a substrate to perform work is also greatly dependent on its availability. Glycogen loaded or depleted protocols prior to either cold exposure alone or exercise have demonstrated a clear shift towards alternative fuel sources (Haman et al. 2004; Wagenmakers et al. 1991). The same has been observed with altered fatty acids availability (see review by Hawley 2002). In general, an increase in substrate availability will lead to an increase in its oxidation, whereas a reduction in availability will lead to the use of alternate fuel sources.

2.2.2.2 Exercise Intensity

The crossover concept is the point at which the contribution of fat and CHO to the energy yield is equal (Brooks and Mercier 1994) (see Figure 3). During exercise in a thermoneutral environment, that point will be ~50% of VO_{2max}. As exercise intensity increases, a greater reliance on CHO will be observed, whereas fat sources will dominate at intensities below that point. Factors including diet, substrates availability, physical activity and fitness level, and gender can modulate the contribution of fat and CHO at a given workload, (Venables et al. 2005), although exercise intensity itself is known to be the strongest modulator.

Romijn et al. (1993) reported a similar contribution between fat and CHO at 65% VO_{2max} compared to 25% and 85%. In addition, they determined rates of appearance for FFA and glycerol which relatively paralleled their venous concentrations. They also measured net glycogen utilization as an important energetic factor of exercise, which became the dominant source of energy as exercise intensity increased. A review by Brooks (1998) clearly summarizes alterations in energy sources as exercise intensity increase (see Figure 4). A comparable report from van Loon et al. (2001) observed similar contributions at 55% between CHO and fat compared to 40% and 70% $VO_{2max.}$ They also observed reduced oxidation rates of fat sources in conjunction to a greater glycogen utilization as exercise intensity increased. While glucose and glycogen utilization increased, the increase to the energy yield from glycogen, from 40% to 70% VO_{2max} in their study, was much greater compared to glucose (23 *vs*. 8% increases). Venables et al. (2005) further examined the crossover concept but more precisely with exercise intensities of 41 %, 43%, 48%, 58%, and 61% of VO_{2max} in a group of men and a group of women. While the combined results for men and women indicated that the equal contribution between CHO and fat was at 48%, men tended to reach the crossover much earlier (43% VO_{2max}) compared to women (58% VO_{2max}). They also confirmed previous observations that with increased exercise intensity, CHO oxidation increases linearly or slightly exponentially whereas fat follows a hyperbolic curve.

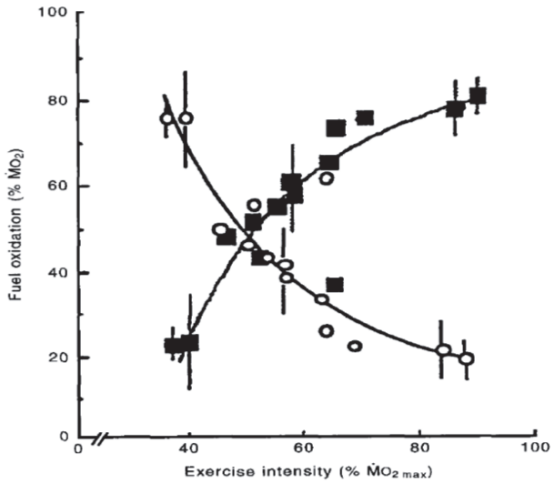

FIGURE 3 Relative increase in CHO and decrease in fat to the energy yield as a function of exercise intensity in various mammals. (from Brooks 1997, reproduced with the permission from Elsevier)

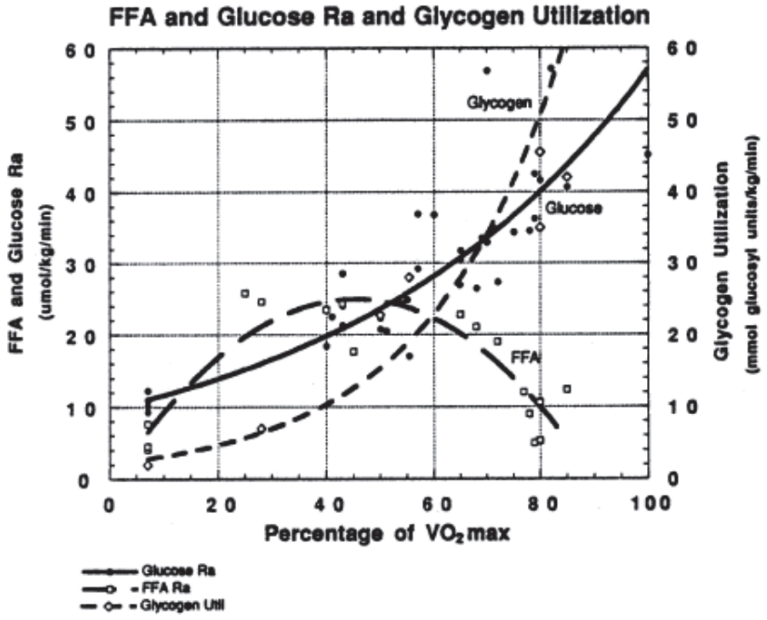

FIGURE 4 Rates of appearance of FFA and glucose and glycogen utilization as a function of exercise intensity. (from Brooks 1998, reproduced with the permission from Elsevier)

2.2.2.3 Thermoneutral *vs*. cold environment

Galloway and Maughan (1997) observed an increase in oxygen consumption when cycling in 4˚C compared to 11˚C, 21˚C and 31˚C. This was accompanied by a lower RER in 11˚C compared to 4˚C and 21˚C and greater total fat and CHO oxidation in the 11˚C compared to 21˚C. The exercise intensity was set at 70% VO_{2max} which established in a thermoneutral environment prior to the experimental trials. Exercising in the cold condition increased muscular metabolic demand and therefore increased oxygen consumption by ~1 L·min^{-1}, complicating the interpretation of energetic data. Layden et al. (2002) examined the effects of cycling at 65% VO_{2max} in -10˚C, 0˚C, 10˚C and 20˚C. They observed no statistical difference in VO_2 but an increase in RER in the -10˚C and 0˚C compared to the other conditions. Their results also indicated a decrease in glycerol in the -10˚C and 0˚C conditions and lower glucose concentrations in -10˚C only. They explained their results by suggesting a decreased lipolysis and a decrease in peripheral blood flow from adipose tissue sites that would impede FFA mobilization. A 30˚C difference between the warmest and coldest conditions should technically bring a difference in oxygen consumption from an increase in muscular metabolic demand (i.e. cooled muscles increases oxygen consumption) (Oksa et al. 2002, 2004). Also, no differences in HR were observed between 10˚C and -10˚C.

Conversely, Hurley and Haymes (1982) demonstrated a decrease RER and increased FFA availability combined with no changes in glucose or lactate concentrations at the end of a 90-min cycling session when both skin and core temperatures were lowered. This was performed at very low cycling workloads of 50-60W. Moreover, Weller et al. (1997a, 1997b) examined the physiological responses to prolonged (i.e., 6 hours) intermittent low- (5 km · h^{-1}, 0% incline) and high-intensity (6 km · h^{-1}, 10% incline) walking in cold (5^{o}C) and neutral (15^{o}C) environmental conditions demonstrating that low-intensity walking in the cold increased CHO oxidation, venous glucose and lactate concentrations, and oxygen consumption (VO_2) compared to thermoneutral conditions. The authors concluded that the difference in VO_2 was due to the added shivering thermogenesis that was gained during the long-term exercise, attempting to compensate for the increased heat loss in the cold as heat production from exercise may have been insufficient. The likely presence of muscle cooling may also have led to an increase in VO_2, as reduced mechanical efficiency requires more energy to perform the same workload (Oksa et al. 2002). Although, similar to Galloway and Maughan (1997), oxygen consumption was greater in the cold which makes data interpretation difficult as the additional thermoregulatory metabolic demand was not present in the 15˚C condition. The lack of standardization between environmental conditions, exercise intensities and oxygen consumption minimizes our ability to cross-validate studies examining the effects of cold on energy metabolism during exercise.

Few studies also examined the effects of a pre-cooling protocol, inductive of shivering, prior to exercise on exercise metabolism. Kruk et al. (1991) had subjects sitting in 5˚C until shivering onset would occur before sessions of

cycling at 40%, 60% and 80% of VO_{2max}. For the three exercise sessions, respiratory quotient (RQ) values were lower in 5˚C compared to the 24˚C condition despite greater oxygen uptake during the rest periods, likely from shivering. Interestingly, RQ had a tendency to be lower during rest periods as well despite the increase in VO_2. Hurley and Haymes (1982) also observed a decrease in RQ in addition to an increase in FFA in the cold during exercise when both skin and core temperature were lower compared to baseline. Their subjects cycled at very low intensity (50W) for 90 minutes, providing an adequate thermal environment to maintain lower skin and core temperatures. Tipton et al. (1997) compared the metabolic changes of exercise and shivering, individually, at matching incremental VO_2 intensity. They observed a shift from CHO towards fat metabolism from increase FFA and beta-hydroxybutyrate (BHB) during shivering compared to exercise at matching VO_2. The metabolic shift towards fat as an energy source from shivering could explain some previous results indicating a lower RQ in the cold following a pre-cooling protocol.

Some studies examining changes in fuel selection during long-term strenuous exercise and cold exposure has focused on blood cholesterol alterations post event as markers. The 1989 'Bering Bridge' expedition (33 days) (Roschevsky et al. 1993) demonstrated important decreases in very-low (VLDL) and low-density lipoprotein (LDL) early in the expedition, indicating greater use of fat and healthier blood cholesterol profile. Another expedition lasting 42 day and crossing the Greenland icecap (Helge et al. 2003) also showed decreases in LDL and total cholesterol (TC) but no change in high-density lipoprotein (HDL). Interestingly, however, a 95-day expedition in Antarctica showed no change (Stroud et al. 1997). Important energy deficits, changes in diet, and muscle/cellular adaptation may explain these results as a metabolic shift towards endurance capability.

2.2.2.4 Exercise modality

Walking and running are two of the most commonly used modalities of exercise. Interestingly, most cold exercise studies have been performed during cycling despite the fact that treadmill type exercise will demonstrate greater fat utilization compared to cycling (Snyder et al. 1993; Achten et al. 2003; Basset et al. 2003). Previous reports explaining this important difference pointed out towards a synergy between muscle mass, blood flow and catecholamine release. Firstly, treadmill exercise relies on a larger muscle mass, compared to cycling, performing at a lower intensity, which has shown to increase catecholamine release (Hermansen and Saltin 1969; Kjaer et al. 1991; Savard 1989). Secondly, the use of a greater muscle mass has also been associated with greater local blood flow providing a more suitable environment for catecholamine release and transport. Cold exposure is generally associated with a strong sympatho-adrenal stimulation influencing lipolytic activity in adipose tissue beds through ß-adrenergic receptors (Castellani 1998, 1999; Frank et al. 1997).

2.2.3 Skeletal muscle tissue oxygenation

2.2.3.1 Theory and limitations of near-infrared spectroscopy

Near infrared spectroscopy (NIRS) technology in biological sciences has recently been determined as a reliable tool to measure oxygenation levels in various tissues, mostly in skeletal muscles and the brain. This non-invasive technology provides real-time information and responds to rapid changes in oxygen concentration of hemoglobin and myoglobin as well as tissue blood flow. An optical fiber bundle delivers a light at different wavelengths (within 760 – 905 nm) that travels within the tissue in a banana shaped curve that is reabsorbed by another bundle. The differential absorption properties of oxygenated (O_2Hb) and deoxygenated (HHb) hemo/myoglobin wavelengths provide valuable information on tissue deoxygenation. The distance between both light emitting and receiving fiber bundle, called optode, determines the depth the light travels into and is essential in tissue selection as the wider the distance the deeper the light will travel through (the depth of signal to distance of optode ratio being ~0.5:1) (Hiroyuki et al. 2002) (see Figure 5). Other than O_2Hb and HHb, NIRS measurements also include total hemo/myoglobin content (tHb) (O_2Hb + HHb = tHb).

Some limitations of this technology include the lack of differentiation between hemoglobin and myoglobin molecules in the muscle under the light spectrum (Mancini et al. 1994). It is difficult to completely justify oxygen saturation changes from muscle activation since myoglobin absorbency overlaps the infrared region of that of hemoglobin. This validity issue remains relatively small and is generally neglected by researchers. Adipose tissue thickness has also been found to have a confounding effect on NIRS measurement (Beekvelt et al. 2001; Costes et al. 1996; Homma et al. 1996) and need to be considered with larger groups that include obese and non-obese subjects where significant inter-variability in adipose tissue measurements is present. The placing of the optodes for data collection has also received some attention, as site selection will highly affect the NIRS signals. While much research has been performed using NIRS, lack of standardization in optodes placement has made it difficult to compare results between studies (see review by Bhambhani 2004). Finally, conflicting results on the effects on skin blood flow on the NIRS signal remain important in determining the validity of previous results. Previous work (Buono et al. 2005; Davis et al. 2006) has demonstrated that either an increase or a decrease in skin blood flow will modulate light absorption and consequently the muscle-derived NIRS signal. NIRS data provides relative changes in tissue oxygenation from a pre-determined baseline period. It is therefore essential to establish, prior to data collection, a time point which the changes in O_2Hb, HHb, and tHb will have some physiological significance.

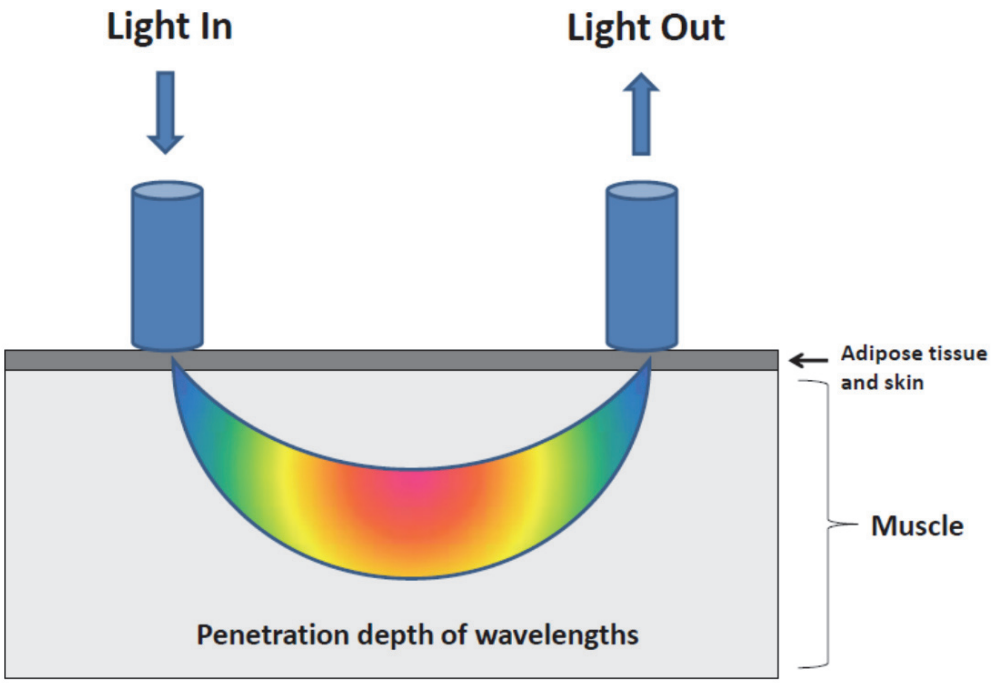

FIGURE 5 Near-infrared spectroscopy optode and wavelengths schematic (modified from Yanagisawa et al. 2007with permission from Springer).

2.2.3.2 NIRS applications during exercise

The application of NIRS has been seen during isometric (Beekvelt et al. 2002; Boushel et al. 1998; Hicks et al. 1999) and dynamic (Bhambhani et al. 1998; Martin et al. 2009; Neary et al. 2002; Rissanen et al. 2012) exercise in both maximal and submaximal settings. During treadmill incremental exercise, a four-phase response defines the muscle oxygenation pattern. As exercise commences, an increase above baseline in muscle oxygenation is seen from increased blood flow and redistribution to the working muscles at a speed between 4 – 6 km · h^{-1} (Hiroyuki et al. 2002; Rissanen et al. 2012). Subsequently a decrease below resting value is observed followed by a leveling off when maximal intensity is attained. Finally, a very rapid increase is seen in muscle oxygenation during the first 1 - 2 min of recovery above resting values (see review by Bhambhani 2004). While there are similarities in muscle oxygenation patterns between cycling and treadmill exercise (Hiroyuki et al. 2002; Rissasen et al. 2012; Peltonen et al. 2013), walking and running bring more complexities as these exercise modalities rely on muscular activation of the vastus lateralis, gastrocnemius, and some smaller muscles. There are significant differences in muscle oxygenation pattern originating from inter-individual variation as well as between the vastus lateralis and gastrocnemius muscles (Hiroyuki et al. 2002; Quaresima et al. 1996).

2.2.3.3 NIRS applications during cold exposure

Yanagisawa et al. (2007) examined oxygenation levels of the ankle dorsiflexors following 30 min of cooling in 0˚C, 10˚C and 20˚C. Their cooling protocol induced decreases in skin and muscle temperatures in addition to tissue oxygenation in all conditions. A marked decrease in blood volume was also seen in all conditions likely due to cold-induced vasoconstriction. Despite these relative changes, the range of decreases in tissue oxygenation and blood volume were non-dependent on temperature as no significant differences were observed between 0˚C, 10˚C or 20˚C. We are currently unaware if the effects of cooling during exercise would be additive and consequently increase deoxygenation rate.

2.2.4 Immuno-endocrine response

2.2.4.1 Immune system

The immune system is comprised of an innate (non-specific and short-term response) and an adaptive (specific and long-term response) component (Gleeson 2006). As an acute first line of defense against infections and/or invading micro-organisms, the innate part of the immune system is activated and increases leukocytes in circulation, a phenomenon called leukocytosis. Leukocytes sub-populations include neutrophils and monocytes, which are phagocytic cells that can ingest and eliminate foreign agents in the tissues (Walsh et al. 2011). The adaptive part of the immune system, with more specificity in combating pathogens and an ability to adapt over time, also increases leukocytes when activated via lymphocytes proliferation, which are known to be an important site of cytokine production (Walsh et al. 2011). Cytokines are another important element of adaptive immune function which will be discussed further below. Cell differentiation of lymphocytes in the thymus or the bone marrow produces T-lymphocytes, B-lymphocytes and natural killer (NK) cells, each responsible for various immune functions.

The relationship between exercise intensity/volume and immune responses has received much attention since the concretization of the conceptual J-shape curve developed by Nieman (1994) describing the risk of upper respiratory tract infection (URTI) in relation to exercise intensity / training volume (see Figure 6). More specifically, previous studies (Nieman et al. 1990, 1993) have indicated that moderate intensity exercise seems to increase immunity, which consequently reduces the likelihood of infections, described here as URTI. Work by Nieman et al. (1990) has demonstrated that a walking exercise regimen of 15 weeks in obese women did not reduce the number of URTI episodes but did decrease the length of each infections from 10.8 to 5.1 days compared to control. Although original immunological work used URTI as a measurable outcome, the acute effects of exercise on immune responses are now a growing interest with more accurate markers of immune functions. Exhaustive and supra-maximal intensities provide clear changes in cytokine production (see Figure 7) and leukocytes count and sub-populations (see Figure 8). Importantly, the immune responses will be greatly modulated by stress

hormones as Castellani et al. (2002) demonstrated that cortisol, epinephrine and norepinephrine, in addition to heart rate and core temperature, account for 2-42% of the variation in leukocytes, granulocytes, monocytes and lymphocytes subsets. Therefore, the exercise intensity and duration influence stress hormones secretion, which thereafter modulates immune responses. While catecholamines are secreted very rapidly upon initiation of exercise, cortisol secretion is delayed and only increases during exercise intensity of ~>60 VO_{2max}, whereas at lower intensities, a decrease in cortisol can be seen (Hill et al. 2008). This temporal differentiation between stress hormones, each affecting specific cytokines and leukocytes sub-populations, make the immune response difficult to interpret.

Few studies have adequately assessed laboratory-based cold exposure on the immune system. Brenner et al. (1999) examined leukocyte and cytokine responses after a 2 h cold exposure in 5˚C following resting and exercise conditions in warm and cool water. Cold exposure preceded by the resting thermoneutral condition induced an increase in leukocytes, granulocytes and lymphocytes as well as plasma interleukin-6 (IL-6). They further demonstrated when preceded by exercise, immune responses in the cold can be further enhanced leading to the argument that immune impairment in moderate cold, if present, is very limited.

Rhind et al. (2001) provided further indication that cold does modulate the immune response by observing an increase in IL-6 and IL-1 antagonist receptor (IL-1ra) as well as a decrease in IL-1β and TNF-α following wet cold exposure. Additionally, stress hormones were also correlated with cytokine expression, further indicating the importance in hormonal modulation on immune responses during thermal stress. Finally, thermal clamping has been used in cold studies as Cross et al. (1996) and other recent work determined that changes in core temperature (Peake et al. 2008) or thermal habituation (Dugué et al. 2000) will impact immunity.

Rhind et al. (1999, 2001, and 2004) have hypothesized that it is not core temperature per se that modulates immune responses but rather the change in hormonal concentrations, brought upon from core temperature alteration. The increases in heat production from exercise and heat loss from cold exposure both affect core temperature, an important driver on endocrine fluctuations. Some studies have evaluated immune differences between a cool and a hot environment, neglecting the comparison to a thermoneutral setting where immunity should be unchallenged and represent a control group comparison (McFarlin and Mitchell 2003; Peake et al. 2008). Therefore, differences in immune responses were determined between conditions inducing various levels of core temperature increase. The effects of cold exposure sufficient to provide a thermal challenge (i.e. decreased skin and core temperature) combined to exercise on immuno-endocrine responses has not been investigated. Additionally, acute cold exposure and exercises are known to have little influence on some endocrine axes, namely the hypothalamic-pituitary-gonadal and the hypothalamic-pituitary-thyroid axes (Leppäluoto et

al. 1988, 2005; Kraemer et al. 1991). A stronger cold stress may modulate testosterone and thyroid hormones, involved in immune responses (Araneo et al. 1991; Klecha et al. 2000, and 2006). Whether exercise further modulates the potential changes in these hormones from significant cold exposure remains unclear. The effects of the complex balance of heat production and heat loss on core temperature, endocrine changes and subsequent immune responses during exercise in the cold as well as the interactions between exercise and changes in testosterone and thyroid hormones from a strong cold stimulus is unknown.

2.2.4.2 Cytokines

Cytokines are messenger molecules from the immune system that act in an autocrine, juxtracrine, paracrine and endocrine fashion across tissues. One of the first cytokine examined in exercise studies, IL-6, is mainly produced in the muscle and is now known to increase greatly during exercise promoting glycogenolysis in the liver and lipolysis in adipose tissue (Steenberg et al. 2000). Many pro-inflammatory anti-inflammatory cytokines are produced in monocytes and lymphocyte subsets during exercise (i.e. IL-6, IL-1ra, IL-8, IL-10, MIP-1β, TNF-α, IL-1, etc.) (Pedersen 2000a, 2000b), and some are also secreted from the contracting muscle during exercise as myokines (i.e. IL-4, IL-6, IL-7, IL-15, etc.) (Pedersen and Febbraio 2012). Mechanisms of secretion and action on tissues are still debated for many cytokines. The interaction between cytokines on each other's responses is also a field of interest requiring further investigation. An importance consideration in cytokine studies is the transient appearance in plasma concentration that can last from 15-min to a few days (see Figure 8) (Kock 2010).

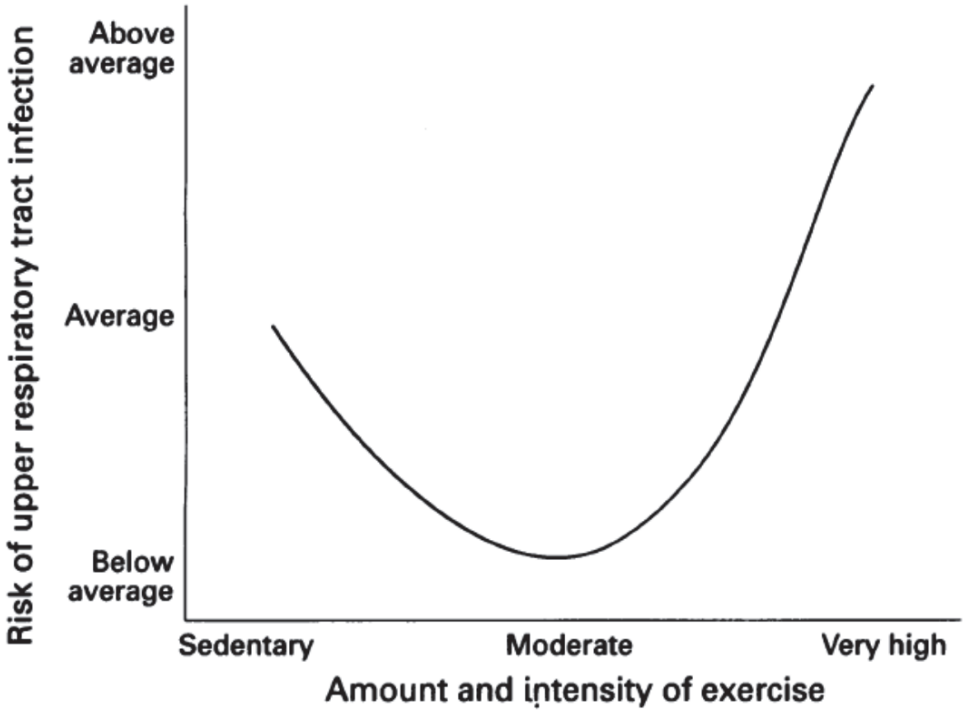

FIGURE 6 J-shape model of relationship between varying amounts of exercise and risk of URTI. (from Nieman 1994, with permission from Wolters Kluwer Health)

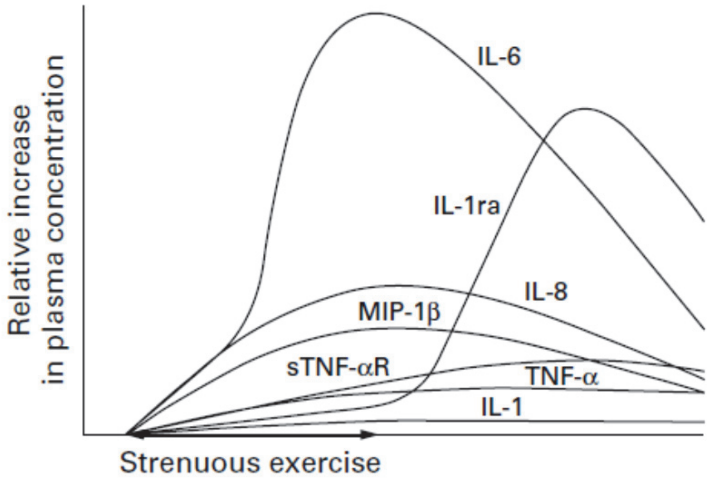

FIGURE 7 Rate of changes in various cytokines in relation to exercise. (from Pedersen and Toft 2000, with permission from BMJ Publishing Group Ltd)

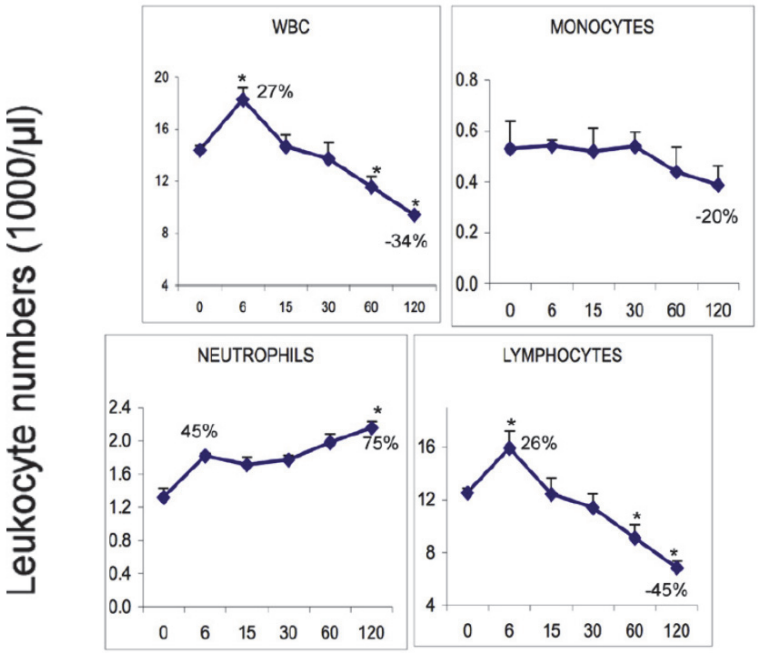

FIGURE 8 Changes in leukocytes, monocytes, neutrophils and lymphocytes during stress in rats. (from Dhabhar et al. 2012, with permission from Elsevier)

2.2.4.3 Catecholamines

Catecholamines are a grouping of three hormones, namely epinephrine (Epi), norepinephrine (NE) and dopamine. NE provides important information concerning the SNS while Epi signals changes in the adrenomedullary hormonal system (AHS) axis during stressful events. Both NE and Epi are secreted from the adrenal medulla prior to being transported in the blood stream acting as hormones, although NE is mainly produce in nerve endings and can also act as a neurotransmitter. Acute cold exposure or exercise increases plasma concentrations of Epi and NE. Some reports on Epi increases during acute cold exposure, however, are less consistent and have shown no change, indicating that NE, therefore the SNS as a whole, responds more strongly and rapidly to physical stress (Leppäluoto et al. 1988; Sramek et al. 2000). Following repeated bouts of exercise at the same workload or following long-term cold exposure, the Epi and NE responses tend to decrease, suggesting that the stress from exercise or cold is not as physiological significant on the body.

There is a well-known relationship between catecholamines and the immune system. Systemic release of catecholamines and norepinephrine from nerve terminals bind to target tissue to β-adrenergic receptors and interact with intracellular cyclic adenosine monophosphate (cAMP), increasing cellular activity (Carlson et al. 1989). This impacts various tissue including circulating leukocytes and its sub-populations. The density of adrenergic receptors on

leukocytes cell surface is a determinant factor its mobilization and action in response to changes in catecholamines concentrations. Natural killer cells have the most adrenergic receptors compared to other lymphocyte subsets and consequently are considered the first line of defense during infections, increasing rapidly, similarly to catecholamines (Maisel 1990; Pedersen 2000a, 2000b).

2.2.4.4 ACTH and Cortisol

Adrenocorticotropic hormone (ACTH) secretion from the pituitary gland is the precursor hormone of cortisol (COR), secreted from the adrenal cortex, both dynamically responding to each other in a feedback loop and are part of the hypothalamic-pituitary-adrenal hormonal axis (HPA). Cortisol principally acts as a stress marker (i.e. from exercise, environmental challenges, infections, etc.) which mobilizes fuels (glucose and fat), reduce immune and inflammatory responses, alter behavior, and inhibit muscle protein synthesis in the muscle through inhibiting actions of actin and myosin (Mastorakos et al. 2005). Exercise modulates ACTH and COR similarly as responses with each increases linearly with increased exercise intensity and duration (Friedmann and Kindermann 1989: Goldstein and Kopin 2008: VanBruggen et al. 2011), although this increase debuts at approximately at 60% VO_{2max}. Other studies have also recorded a decrease in COR at 40% with no change at 60% VO_{2max} indicating that moderate to high intensity exercise induced an increase in COR while low to moderate intensity, instead, induces a decrease (Farrell et al. 1983; Hill et al. 2008). Cold exposure is well known to induce an increase in both ACTH (Goldstein and Kopin 2008) and COR (Wilkerson et al. 1974). Overtime, nonetheless, physical stress from cold stress is reduced and a habituation to cold nullifies adrenocortical responses (Leppäluoto et al. 2008).

Unlike responses observed with an increase in catecholamines, changes in immune responses from alterations in corticosteroids are slightly delayed and have been associated to post-exercise drop in leukocytes subsets, including lymphocytes, monocytes and neutrophils (Rabin et al. 1996). Circulating COR interact with intracellular steroid hormone receptors (GRs), located in the cytoplasm, which subsequently enters the nucleus and modulate gene expression by DNA binding. Increases in circulating corticosteroid levels have previously demonstrated immune cells death (Cohen and Duke 1984; Hoffman-Goetz 1998) and inhibit NK cell response (Pedersen et al. 1984) and as a whole seems to regulate immune processes by inducing a down-regulation of inflammatory functions.

2.2.4.5 Thyroid hormones

Thyroid-stimulating hormone (TSH) is secreted by the anterior pituitary gland and exacerbates its effects on the thyroid gland that in turn secrete thyroxine (T_4), and to a lesser extent triiodothyronine (T_3). The 20:1 production ratio of T_4 over T_3 is not of great importance as T_4 is subsequently deiodinated to T_3, the more active thryroid hormone on metabolism (Leppäluoto et al. 2005). Short-term exercise or cold exposure does not have any significant effects of thyroid

functions despite its roles on whole-body energy metabolism (Leppäluoto et al. 2005). Although some work have indicated increases in TSH during short but severely cold exposures (Deligiannis et al. 1993). Instead, prolonged cold exposure will lead to increased production of TSH and lower levels of T_3 as its clearance is upregulated as it stimulates BAT activity and non-shivering thermogenesis. The disposal of thyroid hormones is accelerated during cold exposure to activate UCP-1 found in mitochondria of BAT (Ribeiro et al. 2001).

Klecha et al. (2000, 2006) have demonstrated clear bi-directional relationships between the immune and the hypothalamic-pitiutary-thryroid systems and thyroid hormones binding receptors on monocytes and lymphocytes. Changes in serum levels of T_4 and T_3 have led to a decrease in cytokine production in the lymphocytes as well as modulation of IL-2, IL-6, interferon-gamma and proliferation of T and B cells. Much of the studies involving the immune system and thyroid hormones have been done with ablation of the thyroid gland. Additionally, TSH have also been shown to interact with immune responses and seems to be involved as a buffer for T_4 and T_3 production through a negative feedback loop mechanism involving immune responses (Klecha et al. 2006).

2.2.4.6 Sex hormone-binding globulin

Our knowledge of sex hormone-binding globulin (SHBG) and its functions debuted only 40 years ago with some basic consensus of its role on binding steroids in plasma. Since then, various studies has been performed on modulating factors, leading to either an increase (i.e. aging, stress, increased thyroid hormones and estrogens) or a decrease (obesity, exogenous testosterone does, menopause) (Rosner 1990). The main function of SHBG, a glycoprotein produced in the liver and testes, is to regulate androgen-estrogen balance in plasma, mainly that of testosterone (TES) as it affinity to bind to SHBG is greater than other steroids, and inhibits its effect on tissues. Therefore, much research on the impact of fluctuating levels of SHBG and TES have been done in the field of exercise since TES strongly influence acute and chronic exercise outcomes. Although considered mainly as a binding globulin, recent findings seem to indicate that SHBG plays more pivotal in physiological functions than previously believed (Gates et al. 2013). Additionally, a steroid-SHBG receptor complex can initiate a cascade of events leading to steroid effects in the cell without actually penetrating the cell as it is bound to SHBG (Hryb et al. 1989). The role and functions of SHBG in modulating the availability and effects of circulating steroids require further examination. Finally, in the healthy population of men between 19 – 40 years old, resting SHBG levels range at about 276 $ng \cdot dl^{-1}$ (Bhasin et al. 1996). A dearth of data exist concerning the effects of cold on SHBG and moreover, the little information concerning the effects of exercise seem to indicate, that low SHBG levels is a predictor of type 2 diabetes and other pathologies related to the metabolic syndrome (Ding et al. 2009).

Modulating immune responses through SHBG changes remain partly unclear. Current work examining interactions between SHBG and the immune

system relies on the binding and cross-talk effects of SHBG on TES and thyroid hormone levels, which subsequently affect immunity. Sex hormones, including testosterone, directly impact many immune responses and will be discussed below.

2.2.4.7 Testosterone

Testosterone is an anabolic hormone derived from cholesterol and is a part of the HPG (hypothalamic-pituitary-gonadal) axis. It travels in circulation bound to SHBG, albumin, or unbound (free). The affinity of TES to bind to SHBG is much greater than albumin but since albumin is in greater concentration in the blood stream, approximately the same amount of TES is bound on SHBG and albumin. Both continuous physical stress and cold exposure have demonstrated important decreases in testosterone (TES) concentrations that, depending of the physical load, can reach hypogonadal levels (Stroud et al. 1997; Friedl et al. 2000; Nindl et al. 2007). Short-term resistance exercise may induce an increase in TES levels, mostly in men (Kraemer et al. 1991), but endurance type activities generally lead to no changes. A two-hour stay at 10˚C did not seem sufficient in eliciting a drop in TES (Leppäluoto et al. 1988), demonstrating that greater thermoregulatory stress is required for changes in TES levels. Both total (TES_{tot}) and bioavailable (TES_{bio}) testosterone provide essential information on the physical dynamics on its physiological effects on body tissue, even if TES_{bio} has a more active function. The roles of TES includes the anabolic effects of protein synthesis leading to muscle growth, increase bone density and maturation and androgen effects including, stimulation of spermatogenesis and gender characteristics and differentiation (Griffin and Ojeda 2004).

Physiological concentrations of TES with the presence of cortisol inhibited IL2-, IL4, and IL-10 and tended to inhibit interferon-gamma as well in 9 healthy males (Janele et al. 2006). This immunosuppressive effect of testosterone on immunity if further seen in vitro with a reduction in lymphocytes secreted IL-5 (Araneo et al. 1991). Mechanisms of actions of testosterone on leukocytes and cytokines are also regulated by GRs, similarly to cortisol. Importantly, as previously described, the density of GRs on target cells will modulate the ability of sex hormones to influence immunity, and finally, the magnitude of change in sex hormones is not as severe as catecholamines and cortisol, therefore not a strong contributor to immune changes.

2.2.4.8 IGF-1

Growth hormone (GH) stimulates the production of insulin-like growth factor 1 (IGF-1) in the liver which subsequently acts on muscle mass, increasing muscle protein synthesis and hyperthrophy (Kahn et al. 2002). Acute endurance exercise does not seem to modulate the IGF-1 response but some studies using resistance exercise have observed an increase (Kraemer et al. 1991). Current information on this hormone seems to demonstrate that the rise in IGF-1 associated with exercise is delayed due to slow GH production, the main mediator of IGF-1 (Kraemer and Ratamess 2005). The presence or role of IGF-1 during cold exposure is currently unknown. Cold water immersion following

intense cycling did not modulate IGF-1 levels despite its effect on heart rate and skin temperature (Halson et al. 2008).

The influence of IGF-1 on innate immunity includes an enhanced NK cell activity and increased TNF-α (Heemskerk et al. 1999). Moreover, IGF-1 receptors, similar in molecular structure to insulin receptors, are expressed in almost all immune cells including T and B cells and blood peripheral mononuclear cells (Heemskerk et al. 1999). The extent to which IGF-1 receptors are distributed provides a large window of effects on immunity as IGF-1 is known to be strongly modulated by exercise.

3 AIMS OF THE STUDY

This research project aimed at investigating the physiological effects of cold exposure during exercise on energy metabolism, skeletal muscle tissue oxygenation, and immuno-endocrine responses. One field study, a North Pole expedition, aimed at examining endocrine and energy metabolism changes following prolonged strenuous exercise and cold exposure whereas a laboratory study was designed to assessed, in a controlled environment, acute changes in energy metabolism, skeletal muscle tissue oxygenation and immune-endocrine responses. The specific aims were:

1. To investigate the effects of prolonged cold exposure and exercise on metabolic and endocrine responses.
2. To investigate acute changes in energy metabolism, more precisely substrates availability and utilization from exercising in a cold environment compared to a thermoneutral environment with oxygen consumption and core temperature maintained.
3. To investigate changes in substrates availability and utilization from exercising in the cold with and without a pre-exercise low-intensity shivering protocol with oxygen and environmental temperature maintained.
4. To determine the changes in dynamics of oxygenated (O_2Hb), deoxygenated (HHb), total (tHb) and difference between oxy/deoxygenated (diffHb) hemoglobin content in the vastus lateralis and the gastrocnemius muscles from exercising at different intensities in a cold compared to a thermoneutral environment and following a pre-exercise whole-body cooling period inducing deep tissue cooling.
5. To examine the effects of combined exercise and cold exposure on endocrine changes, leukocyte count and subsets as well as cytokine responses in addition to investigate relationships between immunological (leukocytes and cytokines) and endocrine measures.

The hypotheses were:

1. It was hypothesized that severe changes in hormonal concentrations and blood lipids will occur following the expedition but full recovery will be seen within 2 weeks of return.
2. It was hypothesized that the fuel selection response during treadmill exercise at 50% and 70% of peak VO_2 with maintained core temperature and relative oxygen uptake will be more dependent on fat as an energy source in a cold compared to a thermoneutral environment.
3. It was hypothesized that a pre-exercise low-intensity shivering period would increase fat as an energy source during subsequent submaximal treadmill exercise in the cold. We hypothesized that this energetic shift would be stronger at 50% compared to 70% of peak VO_2.
4. It was hypothesized that exercising in a cold environment would reduce skeletal muscle deoxygenation while exercising following the pre-exercise low-intensity shivering period, inducing skin and core cooling, would increase tissue deoxygenation similarly in both the vastus lateralis and the gastrocnemius muscles.
5. It was hypothesized that environment-modulated endocrine changes will influence leukocyte count and subsets as well as cytokine responses. We also hypothesize that all endocrine axes will have some relationships with immune responses

4 METHODS

4.1 Participants

4.1.1 Field Study (Paper I)

Seven male subjects completed an 850 km unsupported North Pole cross-country skiing expedition. The mean (±SD) age of the subjects was 33 ± 4 yrs, height 178 ± 7 cm, body mass 84 ± 7 kg, and peak oxygen consumption 52 ± 3 $ml \cdot kg^{-1} \cdot min^{-1}$. Nine male subjects were subsequently selected as a control group. The mean (±SD) age of the subjects was 30 ± 5 years, height 176 ± 7 cm, body mass 75 ± 8 kg, and maximal aerobic capacity 48 ± 4 $ml \cdot kg^{-1} \cdot min^{-1}$. Subjects were fully aware of the risks related to the experiment and provided informed, written consent. The Ethical Committee of the University of Jyväskylä approved this study.

4.1.2 Laboratory Study (Paper II-V)

Eleven male participants volunteered for the study. The participants were moderately active and not cold acclimatized. They each provided written informed consent and were screened with a PAR-Q questionnaire and for cardiovascular and respiratory conditions that could be aggravated by cold air exposure or exercise. Mean (±SD) characteristics of the participants were determined during a familiarization trial and were: age 24 ± 3 yrs; height 180.0 ± 9.8 cm; body mass 80.0 ± 11.0 kg; body fat 19.0 ± 4.5 %; body surface area 1.99 ± 0.19 m^2; peak oxygen consumption 52.9 ± 1.7 $ml \cdot kg^{-1} \cdot min^{-1}$; and shivering peak intensity ($Shiv_{peak}$) 20.9 ± 2.3 $mlO_2 \cdot kg^{-1} \cdot min^{-1}$. Body fat percentage was estimated by hydrostatic underwater weighing (Brosek et al. 1963) with the water temperature maintained at 32°C. Total body surface area (A_D) (m^2) was calculated from height (H) and body mass (BM) measurements as follows: $A_D = 0.202 \times H^{0.425} \times BM^{0.725}$ (Dubois and Dubois 1916). The study was performed according to the declaration of Helsinki and was approved by the Ethical Committee of the Central Finland Health Care District.

4.2 Experimental Designs

4.2.1 Field Study (Paper I)

Measures for body mass (BM), body fat percentage (%fat), fat free mass (FFM), and body mass index (BMI) and blood samples were all collected two weeks before (Pre) the start of the expedition. Skinfold thickness at four sites (biceps, triceps, subscapular and iliac crest) was measured and % fat was calculated based on body density, estimated from the sum of four skinfolds (Durnin and Rahaman 1967). The same series of tests were also performed two weeks after (Post 1) and two months after (Post 2) the expedition to measure recovery for the expedition group. Additional blood samples were collected on the following days after reaching the North Pole: the first day of return (R1), third day of return (R3), and fifth day of return (R5), to measure short-term hormonal responses. Blood samples were drawn from the antecubital vein after a night of fasting. Recovery measurements for the control group were collected at Pre and Post 2 as indicative of the beginning and the end of the study. During the expedition, subjects from the control group were required to maintain their personal lifestyle and level of daily activity.

Members of the expedition spent two weeks in Resolute Bay, Canada, to acclimatize to the cold, maintain training regime and pack equipment. The expedition started on March 5th from Ward Hunt, in northern Canada, and ended when subjects reached the geographic North Pole on April 29th after 55 days. It consisted of cross-country skiing combined with occasional walking, climbing and short periods of swimming. The distance covered was approximately 850 km. The whole expedition was unsupported, meaning that the participants had to carry all their food, fuel and equipment themselves without any resupplying. Each participant pulled a sledge which weighed about 140 kg at the start of the expedition. They slept approximately 8-hours between 22:00 and 6:00 daily. Towards the last few days of the expedition, however, sleeping patterns were shortened in order to cover more distances (i.e., two to four hours of sleep only). Distances covered daily by the group ranged from 0 to 34.9 km. The mean distance traveled daily increased throughout the expedition from the first two weeks (4.3 km only), to the third and fourth weeks (10.6 km) and to the remaining four weeks (22.4km). The temperature during the trip ranged from -47°C during the earlier weeks of the northern winter to -3°C measured in latter part of the expedition.

4.2.2 Laboratory Study (Paper II-V)

Each participant took part in 6 experimental sessions, wearing shorts and t-shirt (clothing equivalent of ~0.2 to 0.3 clo), separated by at least 72 h and at the same time of day to control for circadian rhythms. They arrived at the laboratory between 0700 and 0800 h, preceded by a 24-h period without alcohol, caffeine, tobacco and vigorous exercise. The participants were requested to arrive at the

laboratory in a fasted state to avoid loading of their glycogen stores, a determinant factor in fuel selection during exercise and shivering (Haman et al. 2004b). They were also instructed to record their dietary and fluid intake 24 hours before the first session and to keep the same nutritional guidelines for each day preceding a session. Water was available *ad libitum* before all sessions.

The participants were instrumented for a duration of approximately 45 min (see appendix 1) while standing in the instrumentation chamber, a temperature and humidity-controlled chamber set at 25.0 ± 0.2°C, 40% relative humidity (RH) and 0.2 $m \cdot s^{-1}$ wind speed. This was to ensure that, together with the 15 min stabilization period, the subjects were in a thermoneutral condition prior to testing. After instrumentation, the participants sat for a baseline period of 15 min in a climatic chamber set at 25.0 ± 0.2°C, 40% relative humidity (RH) and 0.2 $m \cdot s^{-1}$ wind speed. Thereafter, the participants moved to the adjacent experimental chamber set at 0.0 ± 0.2°C or 22.0 ± 0.2°C, 40% RH and 0.2 $m \cdot s^{-1}$ wind speed. Then, they either immediately started exercising or remained seated until oxygen consumption from shivering activity averaged 40% of peak shivering intensity ($Shiv_{peak}$), over a period of 5 min, and then started to exercise (see appendix 2). The low-intensity shivering was chosen as moderate and high-intensity shivering may impede neuromuscular function and prevent task completion such that combined exercise and shivering activity would typically only occur during low-intensity shivering only. Low-intensity shivering at 40% $Shiv_{peak}$ is known to rely mainly on fat as a fuel source (Haman et al. 2005, 2007). This pre-exercise protocol was deemed adequate to also decrease skin and peripheral tissue temperature and have minimal impact of core temperature. Exercise was comprised of 60 min of treadmill exercise at a grade of 1.0 % (Tunturi T40, Accell Group, Heerenveen, The Netherlands). After the exercise session, the subject was moved back to the instrumentation chamber for a recovery period of 30 min in a sitting position. $Shiv_{peak,}$ was determined from the formula by Eyolfson et al. (2001), which has previously been used (Haman et al. 2004b; Xu et al. 2005):

$Shiv_{peak}$ (ml $O_2 \cdot kg^{-1} \cdot min^{-1}$) = 30.5 + 0.348 $\cdot VO_{2max}$ (ml $O_2 \cdot kg^{-1} \cdot min^{-1}$) – 0.909 $\cdot$ body mass index ($kg \cdot m^{-2}$) – 0.233 age (years).

Experimental sessions (see Figure 9) followed a balanced order design using low- (LOW) and moderate-intensity (MOD) exercise and involved the following conditions:

1) Exercise at 50% VO_{2peak} in the cold at 0˚C (LOW COLD);
2) Exercise at 50% $VO_{2\,peak}$ following pre-exercise shivering protocol at 0˚C (LOW SHIV);
3) Exercise at 50% $VO_{2\,peak}$ in a thermoneutral environment at 22˚C (LOW NT);
4) Exercise at 70% $VO_{2\,peak}$ in the cold at 0˚C (MOD COLD);

5) Exercise at 70% VO_{2peak} following pre-exercise shivering protocol at 0˚C (MOD SHIV); and
6) Exercise at 70% VO_{2peak} in a thermoneutral environment at 22˚C (MOD NT).

Low-intensity exercise was performed via walking while moderate-intensity exercise was done via running. Exercise intensities were determined based on the fact that 50% $VO_{2\ peak}$ is the crossover point in dominance of energetic contribution between fat and CHO, thereby not privileging either energy sources during exercise. The use of another exercise intensity provided a thermoregulatory shift of greater heat production as opposed to 50%. Greater heat storage over the course of 60 min of exercise may influence the rewarming rate of cooled limbs following the pre-exercise low-intensity shivering protocol or from the initial moments of cooling when commencing exercise at 0˚C. This change in heat production capacity between both exercise intensities could therefore affect fuel selection at different time points. Treadmill speed for each subject corresponding to the protocol intensities was determined individually during the familiarization trial and manually adjusted if needed during experimental trials to ensure that relative $\%VO_{2peak}$ was consistently at their respective target level throughout the full 60 min of exercise.

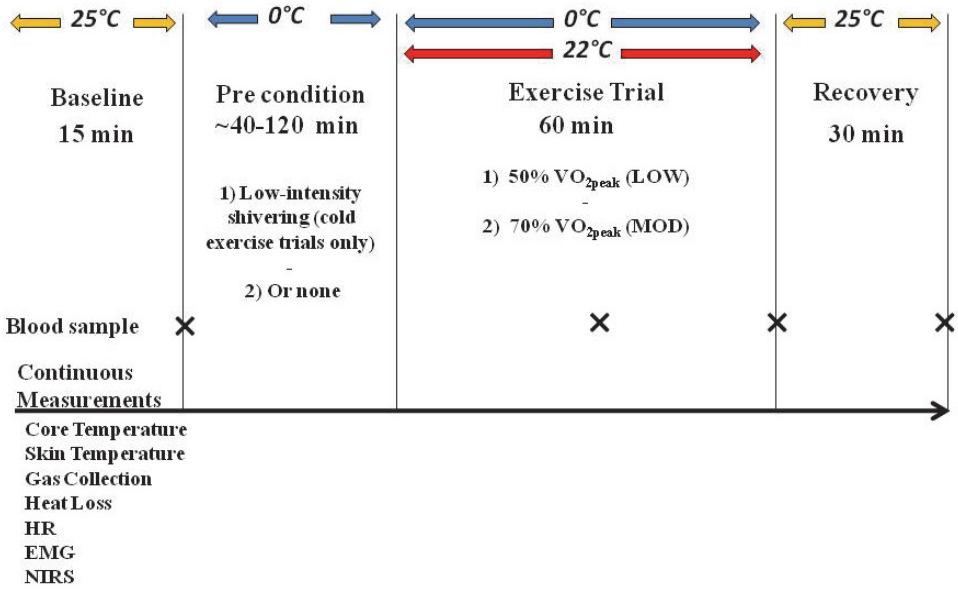

FIGURE 9 Laboratory study exercise protocol

4.3 Instrumentation and Measurements

4.3.1 Energy intake and expenditure (Paper I)

Daily energy intake (EI) during the expedition was estimated from prepackaged daily rations that were the same for all subjects and prepared so that every two weeks, EI would increase to meet the nutritional requirements of the expedition. Energy intake for the first two weeks of the expedition was 19.5 MJ · day^{-1} (51.0% fat, 39.4% carbohydrates and 9.6% proteins), the third and fourth weeks were 23.4 MJ · day^{-1} (56.6% fat, 34.4% carbohydrates and 9.0% proteins) and the remaining weeks were 26.2 MJ · day^{-1} (58.1% fat, 32.9% carbohydrates and 9% proteins). The diet mostly consisted of butter, oil, rye bread, chocolate, biscuits, fruit soups, and nuts. Nutrients and fluid intake were scheduled to occur upon waking up in the morning, mid-day and when subjects decided to stop for the day. Moreover, a 5-min break was taken every hour for additional nutrient and fluid intake when necessary. During these replenishment periods, water was taken ad libitum. Energy expenditure (EE) was estimated from the difference in body mass knowing that 1g of fat is equal to ~37 kJ (Jeukendrup and Wallis 2005), assuming that most of the weight loss from the expedition was from adipose tissue.

4.3.2 Core and skin temperature (Papers II-V)

Rectal temperature (T_{re}) was measured using a rectal thermistor (YSI 401, Yellow Springs Instruments, USA) inserted 10 cm beyond the anal sphincter and is representative of core temperature. Skin temperature was measured from 6 sites (face, chest, forearm, hand, thigh, and back) using thermistors (NTC DC95, Digi-Key, USA). Both core and skin temperature data were recorded by a portable data logger (SmartReader Plus 8, ACR Systems Inc., Surrey, Canada). Weighted mean skin temperature ($\bar{T}_{sk}$) was subsequently calculated using the weighted average of the 6 sites (Palmes and Park 1947):

$$\bar{T}_{sk} = 0.14\,(T_{face}) + 0.19\,(T_{chest}) + 0.11\,(T_{forearm}) + 0.05\,(T_{hand}) + 0.32\,(T_{thigh}) + 0.19\,(T_{back})$$

4.3.3 Whole-body heat loss (Paper III)

Heat flux transducers (Transducers Model Ha13-18-10-P, Thermonetics, Co, USA) connected to portable data loggers (SmartReader Plus 8, ACR Systems Inc., Surrey, Canada) measured dry heat loss from the skin and were positioned on ten sites: head, chest, abdomen, lower back, arm, forearm, hand, thigh, calf and foot and were fixed by adhesive tape which covered the rim of the transducer. Heat flux (W · m^{-2}) values indicated the rate of heat exchange from

the skin towards the environment and, therefore, were considered as dry heat loss. Whole-body dry heat loss was subsequently calculated from heat flux values, the regional contribution of each site measured (face 7%; chest, abdomen and back 35%; arms and forearms 14%; hands 5%; legs 19%; calves 13%; feet 7%) (Hardy and Dubois 1938) and body surface area (BSA) as follows:

Whole-body dry heat loss (W) = site ($W \cdot m^{-2}$) · regional % contribution of site · BSA

4.3.4 Shivering (Paper III)

Shivering was assessed by measuring surface EMG activity (model ME6000, Mega Electronics, Kuopio, Finland). Electrodes were placed longitudinally over the muscle belly between the centre of the innervations zone and the distal tendon (Hermens et al. 2000) of each of the following four muscles: *pectoralis major, latissimus dorsi, rectus femoris* and *sternocleidomastoid*. Distance between recording contacts was 2 cm with the ground electrodes were placed on inactive tissues. A sampling rate of 1000 Hz was used for the EMG signals, which were amplified 2000 times with a preamplifier positioned 6 cm after the electrodes and were recorded continuously. The signal band between 20-500 Hz was full-wave rectified and averaged with a 0.1 s time constant. A power spectrum analysis was subsequently conducted to assess median frequency (MF), and mean power frequency (MPF) (Megawin PC-Software 3.1, Mega Electronics, Kuopio, Finland). The combined data of the four established sites during the last minute of baseline and the first minute of recovery were used to determine both averaged EMG (aEMG) and frequency components.

4.3.5 Heart rate (Papers II-III-V)

Heart rate (HR) was continuously monitored and recorded with a heart rate monitor (T6, Suunto, Vantaa, Finland).

4.3.6 Oxygen consumption (Papers II-V)

Oxygen consumption (VO_2) and respiratory exchange ratio (RER) were measured using an open circuit ergospirometer with a gas-mixing chamber (Medikro 919, Kuopio, Finland). A one-way Hans-Rudolph valve, connected to a breathing tube, was used in all trials to collect expired gases. Gas collection and mixing was done outside of the experimental climatic chamber set at temperature (25°C).

4.3.7 Indirect calorimetry (Papers II-III)

Oxidation of CHO and fat was calculated using stoichiometric equations (Jeukendrup and Wallis 2005) from VO_2 and VCO_2 values as follows:

$CHO\ (g \cdot min^{-1}) = 4.21 \cdot VCO2 - 2.962 \cdot VO_2 - 0.4 \cdot n$

$Fat\ (g \cdot min^{-1}) = 1.695 \cdot VO_2 - 1.701 \cdot VCO2 - 1.77 \cdot n$

where n represents nitrogen excretion from protein oxidation (estimated at 135 $\mu g \cdot kg^{-1} \cdot min^{-1}$) (Carraro et al. 1990). Protein oxidation was not directly calculated as short term cold exposure does not tend to alter its contribution to energy expenditure (Vallerand and Jacobs 1989). Metabolic rate, energy expenditure and relative contribution of CHO, fat and protein was subsequently calculated for the entire 60-min of exercise using the energy potentials for CHO (mixture of 20% glucose and 80% glycogen; 4.07 $kcal \cdot g \cdot min^{-1}$), fat (9.75 $kcal \cdot g \cdot min^{-1}$), and protein (4.09 $kcal \cdot g \cdot min^{-1}$) (Jeukendrup and Wallis 2005). Protein energy contribution was estimated from the assumption that 5.57 g of protein is oxidized for each g of nitrogen excreted (Jeukendrup and Wallis 2005).

4.3.8 Near-infrared spectroscopy (Paper IV)

Local oxygenation profiles of the vastus lateralis (VL) and gastrocnemius (GAS) muscles were assessed simultaneously using a continuous wave NIRS system (Oxymon Mk III Near-Infrared Spectrophotometer, Artinis Medical Systems, Zetten, The Netherlands). The validity and the technical aspects of NIRS have previously been detailed (Mancini et al. 1994; Scheeren et al. 2012). Light transmitting and receiving optodes were positioned in optdode holders longitudinally located on the medial axis of VL, ~10 cm above the knee joint and on the medial head of the GAS ~15cm below the knee joint. The holders were safely affixed to the skin using microporous hypoallergenic tape (Micropore ™, 3M Finland, Espoo, Finland). To ensure that no outer sources of light or movements would interfere with the signal from the investigated areas, a 3 inches tensor elastic bandage (TENSOR ™, 3M Finland, Espoo, Finland) was lightly wrapped around each sites further stabilizing optodes. Distance between the light emitting and receiving optodes were 40 mm for VL and 35 ml for GAS. Four optical wavelengths were used (766, 784, 859, 905 nm) as light sources. The intensity of incident and transmitted light was recorded continuously at a sampling rate of 1Hz and provided concentration changes (ΔμM) from resting baseline of oxygenated (O_2Hb), deoxygenated (HHb), total (tHb) hemoglobin and the difference between oxy – deoxy hemoglobin (diffHb) content in the investigated sites. The myoglobin chromophore has a similar optical wavelength as hemoglobin and is assessed within the NIRS signal. Its contribution is, however, limited (5%) and negligible.

Deoxygenated Hb has been used as reliable indicators of muscle tissue deoxygenation during exercise while tHb provides an estimate of tissue blood volume (Boushel et al. 2001; Delorey et al. 2003). When tHb is not constant, diffHb has also been used as an indicator of tissue deoxygenation as it takes into consideration the influence of tHb on O_2Hb while HHb on its own is

insensitive to tHb fluctuations during exercise (van Beekvelt et al. 2002; Kime et al. 2013). Furthermore, O_2Hb has also been strongly correlated to local blood flow (Fadel et al. 2004).

4.3.9 Blood sampling and analyses (Paper I-V)

Upon returning from the North Pole expedition, cortisol (COR), total testosterone (TES_{tot}), sex-hormone-binding globulin (SHBG) and free thyroxine (FT_4) were analyzed by Immulite 1000 (DPC Diagnostics Corporation, Los Angeles, USA) using respective commercial luminoimmunoassay kits (Ortho Clinical Diagnostics, Amersham, UK). The sensitivity and intra-assay coefficients of variance for these assays were 5.5 nmol · L^{-1} and < 4.6%, 0.5 nmol · L^{-1} and < 5.7%, 3.8 pmol · L^{-1} and < 5.8%, and 0.2 nmol · L^{-1} and < 2.4%, for COR, TES_{tot}, F_{4free} and SHBG, respectively. Bioavailable testosterone (TES_{bio}) was estimated using the equation of Morris et al. (2004): $lnTES_{bio}$ = -0.266 + (0.955*lnTES) – (0.228*lnSHBG) (ln = natural log, units are in nmol/L). Calculated TES_{bio} and measured TES_{bio} have previously demonstrated to be highly correlated (r=0.90) with each other (Morris et al. 2004). The TES/COR ratio was subsequently calculated to indicate whether subjects were in an anabolic (high ratio) or catabolic (low ratio) state at any point during recovery (Häkkinen et al. 1985). This ratio has previously been used in exercise studies to determine the resulting physiological state (catabolic *vs.* anabolic) following specific training programs (Santtila et al. 2009; Tyyskä et al. 2010).

Total cholesterol (TC), high-density lipoprotein (HDL), triglycerides (TG), glucose, protein, and urea were analyzed by VITROS DT60 Chemistry System (Ortho-Clinical Diagnostics, Inc., Rochester, NY, USA). Low-density lipoprotein (LDL) was derived from TC and HDL values using the Friedewald equation (Friedwald et al. 1972). Measured hematological parameters included white blood cells [WBC], red blood cells [RBC], hemoglobin [HBG], hematocrit [HCT], platelets [PLT], lymphocytes [LYM], and neutrophils [NEUT], and were all analyzed by Sysmex KX 21N-analyzer (Sysmex Co., Kobe, Japan).

In the laboratory study, an OCRILON®polyurethane catheter (Optivia I.V 18G, Jelco, Smith's Medical, Ashford, UK), positioned in the antecubital vein before the start of the experiment and maintained throughout exercise, was used to collect blood samples in 3.5-ml vacuum-sealed serum tubes with silicon coating (BD Vacutainer® SST™ tubes, BD, New Jersey, USA) and in 3-ml K_2EDTA whole blood tubes (BD Vacutainer® Plus Plastic K_2EDTA tubes, BD, New Jersey, USA). Catheters were maintained using adhesive hypoallergenic, water-resistant tape (3M™Transpore Surgical Tape, 3M Health Care, London, Canada). The blood samples in serum tubes were given 30 min to coagulate as recommended by the manufacturer. Centrifugation was then performed at 3500 rpm for 10 min followed by isolation of plasma and serum samples in Eppendorf tubes and frozen at -80°C for future analysis.

Plasma catecholamine concentrations (epinephrine [Epi] and norepinephrine [NE]) were analyzed via a commercial ELISA kit (DRG Instruments GmbH, Germany). Coefficients of variance for intra-assay precision

were 15.0 % for Epi and 16.1% for NE at 2.5 ng·ml^{-1}and 24.4 ng·ml^{-1} levels, respectively. Serum energy substrates (non-esterified fatty acids [NEFA], glycerol, glucose, beta-hydroxybutyrate [BHB]) and lactate were analyzed by Konelab 20XTi (MedWOW, Nicosia, Cyprus). Their sensitivities of intra-assay coefficient of variances were 7.4%, 4.6%, 2.4%, 0.8% and 1.7%, respectively.

Serum cortisol (COR), adrenocorticotropic hormone (ACTH), total testosterone (TES_{tot}), sex-hormone-binding globulin (SHBG), thryroid-stimulating hormone (TSH) free thyroxine (FT_4), triiodothyronine (F_3) and free triiodothyronine (FT_3) were analyzed by Immulite 1000 (DPC Diagnostics Corporation, Los Angeles, USA) using respective commercial luminoimmunoassay kits (Ortho Clinical Diagnostics, Amersham, UK). The sensitivity and intra-assay coefficients of variance for these assays were 5.5 nmol · L^{-1} and < 7.4%, 5 ng · L^{-1} and < 3.8%, 0.5 nmol · L^{-1} and < 8.4%, 0.2 nmol · L^{-1} and < 7.3%, 0.004 mU · L^{-1} and < 7.9%, 1.67 pmol · L^{-1} and < 5.5%, 0.54 nmol · L^{-1} and < 7.5%, and finally, 1.0 pg · ml^{-1} and < 5.7%, for COR, ACTH, TES_{tot}, SHBG, TSH, FT4, F3 and FT3. Bioavailable testosterone (TES_{bio}) was estimated using the equation of Morris et al. (2004): $lnTES_{bio}$ = -0.266 + (0.955*lnTES) – (0.228*lnSHBG) (ln = natural log, units are in nmol/L). Calculated TES_{bio} and measured TES_{bio} have previously demonstrated to be highly correlated (r=0.90) with each other (Morris et al. 2004).

Plasma cytokines were measured with the Bio-Plex 200 system based on Luminex xMAP technology (BioRad Laboratories Inc., CA, USA) and Bio-Plex Pro Human Cytokine 27-plex Assay (CAT# M50-0KAF0Y, BioRad Laboratories Inc., CA, USA). The assay was performed according to manufacturer´s instructions as previously described (Lehto et al. 2010, Myhrstad et al. 2011). The plasma samples were thaw on ice and centrifuged in 13 000 G for 10 min in 4°C before the assay. For the assay the samples were diluted 1:2 in assay buffer. The washing of the plate was done with BioTek ELx405 plate washer (BioTek Instruments, Inc., VT, USA) to minimize variation. The results were calculated with Bio-PlexManager Software 6.0 with fiveparameter logistical equation.

Leukocytes and other hematological parameters were analyzed by Coulter® Ac · T diff™ (Beckman Coulter Inc, Vaanta, Finland). Whole-blood samples were mixed according to manufacturor's instruction for 1-2 min and analyzed in triplicate for WBC, lymphocytes, monocytes, granulocytes.

4.4 Statistical Analyses

Concerning the North Pole expedition study, a one-way analysis of variance (ANOVA) for repeated measures or Kruskal-Wallis test was used for statistical analyses depending on whether assumptions were met, to determine significant differences between pre-expedition values and recovery time periods for the expedition group. Post hoc analysis was performed using Bonferroni multiple comparisons or Wilcoxon sum of ranks. Statistical significance was set at $P<0.05$. Student's t-tests were used to determine differences, if any, between Pre and

Post 2 within the control group. No differences were found in any parameters. This served as a reference to validate any changes seen in the expedition group over the entire timeline of the study.

To determine the effects of the cold compared to a thermoneutral environment on bioenergetics in a laboratory setting, a three-way repeated measures (RM) analysis of variance (ANOVA) was used with the factors of time, ambient temperature (levels: COLD and NT), and exercise intensity (levels: LOW and MOD). A two-way repeated measure ANOVA with the factors of ambient temperature (levels: COLD and NT), and exercise intensity (levels: LOW and MOD) was used to determine statistical differences for total energy expenditure during the entire exercise session (60 min) as well as CHO and fat contribution. Post-hoc analyses were conducted using independent Tukey's HSD test when appropriate.

A three-way ANOVA was also performed to determine the effects of a pre-exercise shivering protocol during exercise in the cold with the addition of adding a recovery as a level of time. A two-way RM ANOVA was used to determine statistical differences in relative energy contribution from CHO and fat (%En) for the entire 60 min of exercise with the factors of shivering (levels: COLD and SHIV), and exercise intensity (levels: LOW and MOD). To isolate the electromyographic and metabolic effects of pre-exercise low-intensity shivering protocol, a paired t-test was performed for aEMG, %En, %$VO_2$and RERwith combined data of both SHIV conditions between baseline and the last 5 min of the shivering protocol.

Near-infrared spectroscopy data (O_2Hb, HHb, tHb, and diffHB) was analyzed by a two-way RM ANOVA with the factors of temperature (levels: NT, COLD, and SHIV) and time (levels: Δ REST – Exercise at 10 min, Δ Rest – Exercise at 60 min, and Δ 60 min – recovery at 10 min) where the changes in amplitude (Δ) between Rest, 10 min (Ex10), 60 min (Ex60) and 10 min in recovery (Rec10) were used. This was done for both the GAS and VL for walking and running (LOW and MOD). The effects of exercise intensity (LOW vs. MOD) were not included in the analysis as we already known that increased exercise intensity induces a greater deoxygenation response. Also, exercise intensities were determined based on whole-body oxygen consumption measurements and not only from oxidative local muscle changes. Finally, two exercise modalities were used (walking and running) which would render statistical comparisons inadequate. The use of 10 min during exercise and during recovery for statistical purposes was used as these times provided that greatest change among conditions from the preceding period.

Immuno-endocrine response were assessed via three-way repeated measures (RM) ANOVA with the factors of environment (levels: NT, COLD, and SHIV), exercise intensity (levels: LOW and MOD), and time (levels: baseline (BL) and 60 min) to determine changes in HR, T_{re}, $\bar{T}_{sk}$, leukocyte count and subsets, hormones and cytokines. Post-hoc analyses were conducted using independent Tukey's HSD test when appropriate. Heart rate, core and skin temperature at 60 min were calculated from the average of the last 15 min of

exercise. Pearson's product-moment correlation coefficients were calculated to assess potential relationships between cardiovascular, thermoregulatory and endocrine responses with cytokine responses, separately in NT, COLD, and SHIV. To examine the effects of exercise growth factors, anti-inflammatory and pro-inflammatory cytokines in NT, COLD and NT, values were divided, converted into a z-score and then computed into a weighted index to be subsequently analyzed via a one-way (RM) ANOVA with the factor of environment (levels: NT, COLD, and SHIV).

All data is presented as mean ± SD. All analyses were performed using the statistical software package SigmaPlot 12 for Windows (Systat Software Inc. SanJose, CA, USA), SPSS 20 for windows (IBM, Armonk, NY, USA), and Statistica 7 for windows (StatSoft Inc, Tulsa, OK, USA).

5 RESULTS

5.1 Prolonged Cold Exposure and Exercise

5.1.1 Anthropometry and energy expenditure (I)

Estimation of EE for the whole expedition averaged 29.6 MJ · day^{-1}. Participants demonstrated a decrease of 10 kg in body mass immediately upon return that was fully regained at Post 1 (Table 1). Fat free mass was also lower at Post 2 ($P <$ 0.005). No changes were observed between pre and post expedition in body fat percentage (23 ± 2 vs. 21 ± 3) or BMI (26 ± 2 vs. 27 ± 2).

5.1.2 Serum hormones (I)

Cortisol concentration was lower from R1 to R5, the lowest point (215.71 ± 76.03 nmol/L) which was lower than Pre, Post 1 and Post 2 ($P < 0.001$), before returning to baseline value (Figure 10). TES_{tot} declined to 11.57 ± 3.50 nmol/L at R1 which was significantly different from R5, Post 1 and Post 2 ($P < 0.001$) but not pre-expedition values (16.65 ± 0.79 nmol/L). TES_{bio} demonstrated a similar response with a decline at R1 (0.13 ± 0.23 nmol/L) that was significantly different compared to R5, Post 1, Post 2 and Pre (0.49 ± 0.06 nmol/L) ($P < 0.001$). Thyroxine showed a decline until R3 (12.45 ± 1.24 pmol/L), which was different compared to Pre, Post 1, and Post 2 ($P < 0.001$). Sex hormone-binding globulin levels were higher immediately upon return from the expedition (48.33 ± 11.18 nmol/L) compared to Pre (30.83 ± 9.93 nmol/L). It was also subsequently higher compared to the recovery values at R3, Post 1 and Post 2 ($P < 0.001$). The TES/COR ratio peaked at R5 (0.097 ± 0.05), and it was greater than Pre, R1, and R3 ($P < 0.001$).

5.1.3 Serum lipids (I)

Venous serum concentrations at Post 1 for TC (6.4 ± 1.36 mmol/L), HDL (1.86 ± 0.39 mmol/L) and LDL (4.23 ± 1.27 mmol/L) were greater compared to Pre, and Post 2 (Figure 11). No significant changes were observed in triglyceride levels despite a small decrease (0.69 ± 0.27 mmol/L) at Post 1.

5.1.4 Hematological changes (I)

Red blood cell count, HGB and HCT were all decreased at Post 1 compared to Pre (Table 1). White blood cell count increased at Post 1 compared to Pre. Platelet count and NEUT were greater at Post 1 compared to Pre and Post 2.

5.2 Acute Cold Exposure and Exercise: Energy Metabolism

5.2.1 Thermoregulatory measures (Papers II-V)

When comparing COLD *vs.* NT conditions (Figure 12), the increase in core temperature was greater during MOD compared to LOW ($P < 0.001$). The only interaction observed was at 60 min with T_{re} being lower in COLD *vs.* NT ($P < 0.001$). Skin temperature was constant in NT but decreased sharply in COLD. A three-way significant interaction indicated that from 15 min to the end of exercise $\bar{T}_{sk}$ values were lower in COLD compared to NT within each exercise intensity ($P < 0.05$). The LOW condition also demonstrated lower $\bar{T}_{sk}$ compared to running at 45 min and 60 min ($P < 0.05$).

Concerning SHIV *vs.* COLD conditions (Figure 12), core temperature values were lower in SHIV *vs.* COLD within both exercise intensities in the first 45 min of exercise ($P < 0.001$). At 60 min, T_{re} in the SHIV condition was also lower than in COLD but in the LOW condition only. During recovery, T_{re} in SHIV was higher than COLD in MOD only. A rapid decrease was observed in $\bar{T}_{sk}$ during exercise, followed by a sharp increase during recovery. Lower values were also seen in the SHIV condition within both LOW and MOD in the first 45 min of exercise ($P < 0.001$). From 60 min to the end of recovery, $\bar{T}_{sk}$ was lower only during SHIV in LOW ($P < 0.001$).

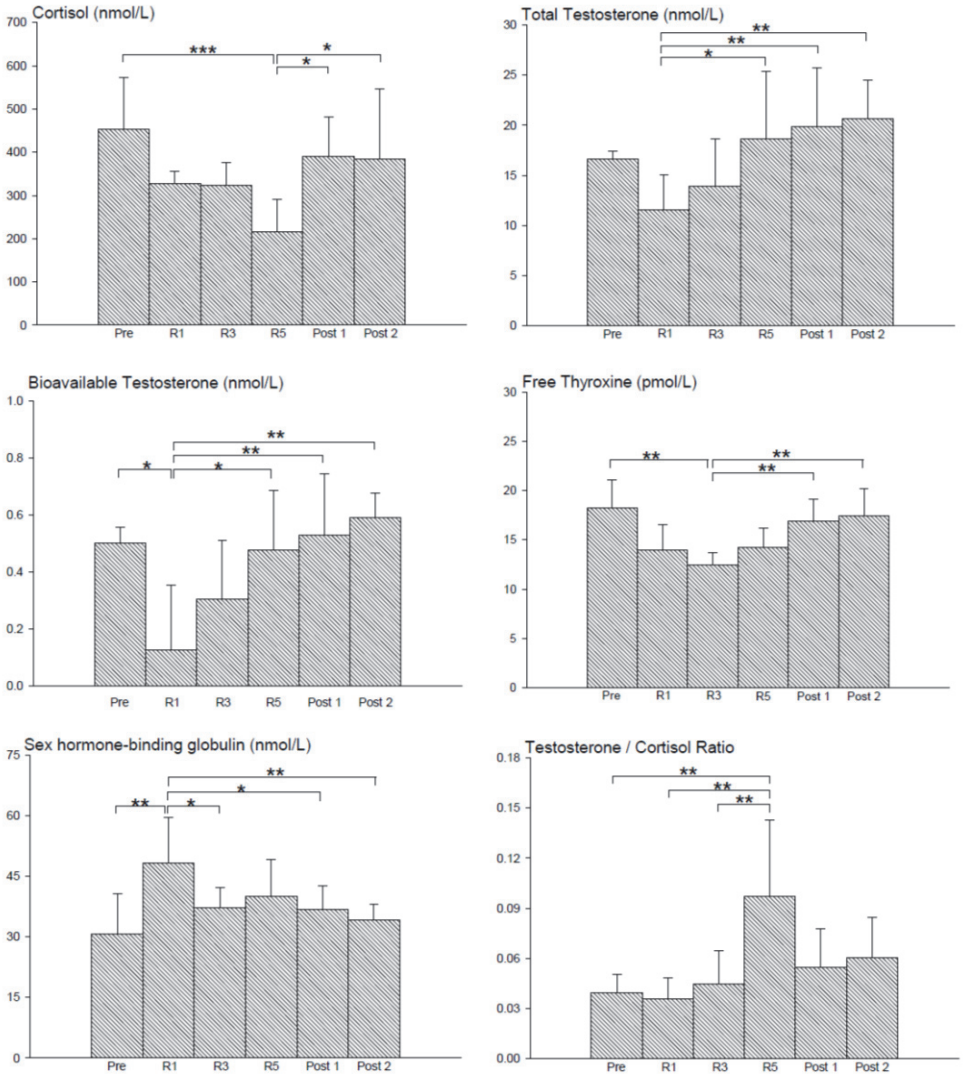

FIGURE 10 Changes in serum hormonal concentrations in the days (R1 – R5), 2 weeks (Post 1) and months (Post 2) following a North Pole expedition. * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

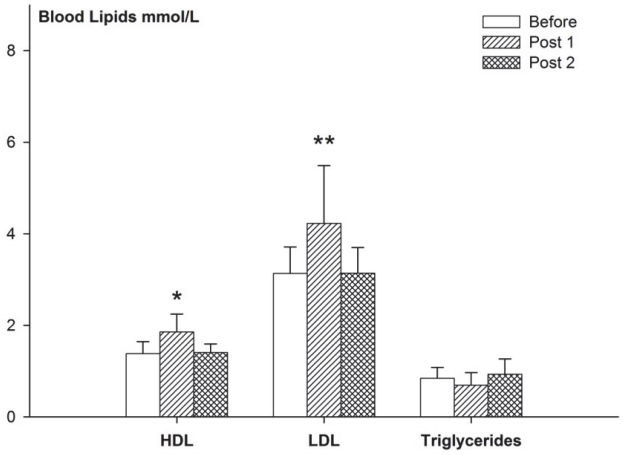

FIGURE 11 Changes in serum blood lipids at 2 weeks (Post 1) and and 2 months (Post 2) following a North Pole expedition. * Significantly greater than before and Post 2 ($P < 0.05$). **($P < 0.01$).

TABLE 1 Hematological components 2 weeks before (Pre) the expedition, 2 weeks (Post 1) and 2 months after (Post 2) for white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), lymphocytes (LYM), and neutrophils (NEUT). * Significantly different from Pre (P<0.05). † Significantly different from Pre and Post 2 ($P < 0.05$). Mean ± SD

Hematological Components	**Pre (n=7)**	**Post 1 (n=7)**	**Post 2 (n=5)**
WBC ($x10^9$)	5.33 ± 0.69	6.61 ± 0.86*	5.28 ± 0.66
RBC ($x10^{12}$)	4.81 ± 0.44	4.52 ± 0.3*	4.65 ± 0.41
HGB (g/L)	145.14 ± 8.07	135.14 ± 5.27*	140.60 ± 7.29
HCT (%)	43.40 ± 2.19	41.60 ± 0.16*	42.10 ± 2.00
PLT ($x10^3$)	219.00 ± 21.85	270.86 ± 42.01 †	214.80 ± 9.28
LYM ($x10^9$)	1.81 ± 0.37	2.06 ± 0.48	2.14 ± 0.36
NEUT ($x10^9$)	2.93 ± 0.68	3.84 ± 0.77 †	2.62 ± 0.34

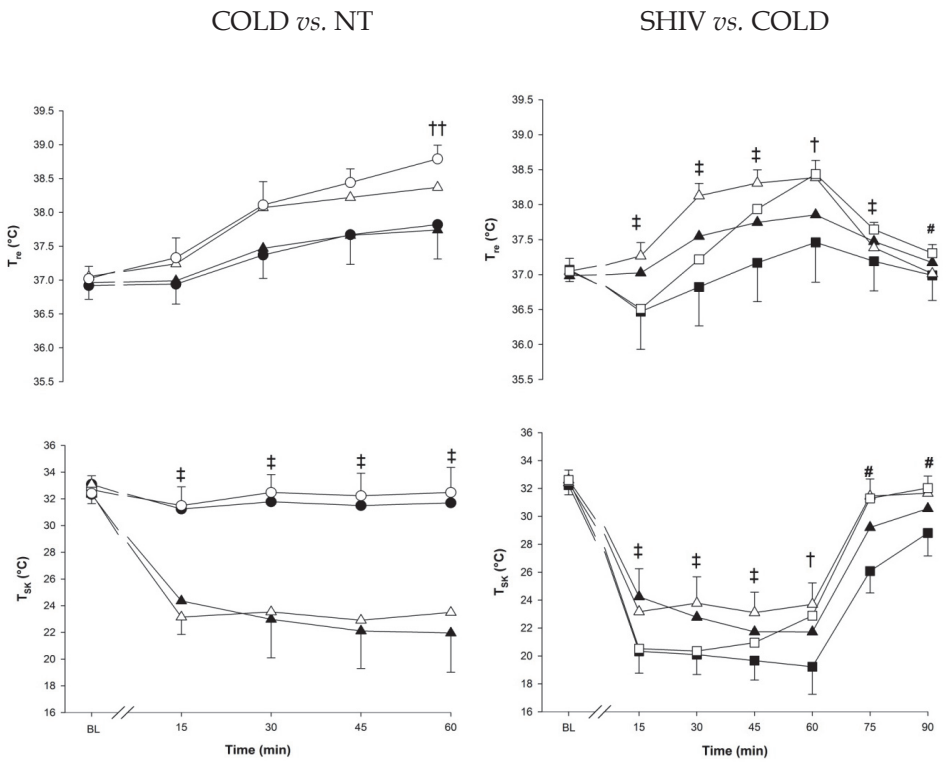

FIGURE 12 Changes in core and skin temperatures at baseline, 60 min of exercise and 30 min of recovery (SHIV vs. COLD only; 90 min total) during LOW and MOD conditions (dark and white symbols respectively) in COLD (▲Δ) (n = 9), SHIV (■ □) (n = 10) and NT (●○) (n = 9). ‡, Significant difference between environmental conditions within both LOW and MOD (P < 0.05). ††, Significant difference between environmental conditions across exercise intensities (p < 0.05). †, Significant difference between environmental conditions within LOW (P < 0.05). #, Significant difference between environmental conditions within MOD (P < 0.05).

The increase in metabolic rate during exercise was greater in MOD compared to LOW by 337 ± 44 W (P < 0.001). Metabolic rate at 30 min was lower in LOW SHIV compared to LOW COLD (P < 0.05). The heat loss response increased during exercise in all conditions (P < 0.001). Heat loss was greater during exercise in MOD compared to LOW (695 ± 28 W *vs.* 577 ± 24 W) (P < 0.001) and COLD compared to SHIV at 15 and 30 min, and in MOD only at 45 and 60 min (P < 0.001). No differences were seen during recovery.

5.2.2 Oxygen consumption, heart rate, (Papers II-III-V) and treadmill speed (Papers II-IV)

Oxygen consumption was not different between conditions according to protocol (see Appendix 3). Heart rate and treadmill speed values for the COLD, SHIV and NT conditions in LOW and MOD are presented in Table 2.

5.2.3 Effects of pre-exercise shivering protocol (Paper III)

Thermal inter-subject responses variability demonstrated a wide time frame to reach 15% VO_{2peak} from low-intensity shivering ranging from ~40 - 120 min. Table 3 presents thermal, neuromuscular, cardiovascular, and energetic parameters at baseline and at the end of the pre-exercise shivering period.

5.2.4 Electromyography (Paper III)

Greater aEMG activity in the LOW SHIV condition was observed at recovery *vs.* baseline (9 ± 2 *vs.* 3 ± 1 μV) ($P < 0.01$). The main effect of time demonstrated lower values in MPF in recovery compared to baseline as well (73 ± 5 Hz *vs.* 95 ± 6 Hz, respectively) ($P < 0.011$). The main effect of time also modulated MF with lower values during recovery compared to baseline (45 ± 4 Hz *vs.* 56 ± 3 Hz) ($P < 0.001$).

5.2.5 Respiratory exchange ratio, fuel oxidation, energy expenditure, and relative contribution (Papers II-III)

RER and CHO and fat oxidation data is presented in Figure 13. Regarding the effects of temperature on fuel selection between COLD and NT, the RER response that was lower in COLD (0.85 ± 0.03) compared to NT (0.88 ± 0.03; $P < 0.05$). From 15 min to the end of exercise, a higher RER response was seen in NT vs. COLD ($p < 0.01$), and in MOD *vs.* LOW ($P < 0.05$).

TABLE 2 Heart rate and treadmill speed during exercise in cold (COLD) (n = 10), in the cold following a pre-exercise low-intensity shivering protocol (SHIV) (n = 9) and neutral (NT) (n = 10) environments at baseline, during exercise and during recovery. *, Significantly higher HR in NT compared to COLD within times ($P < 0.05$). †, Significant difference between SHIV and COLD within LOW ($P < 0.05$). .**, Significantly lower in SHIV compared to COLD and NT within times ($P < 0.05$). Values are represented as mean ± SD.

		50% VO_{2peak} (LOW)			70% VO_{2peak} (MOD)		
		COLD	SHIV	NT	COLD	SHIV	NT
Heart Rate (beats·min^{-1})							
Baseline		68 ± 16	69 ± 9	67 ± 14	70 ± 12	71 ± 15	69 ± 13
Exercise	15 min	121 ± 18	111 ± 12†	128 ± 7	152 ± 9	148 ± 14	159 ± 11*
	30min	122 ± 14	112 ± 17	133 ± 9	159 ± 13	159 ± 17	170 ± 13*
	45min	124 ± 12	119 ± 21	136 ± 10	162 ± 14	163 ± 15	176 ± 12*
	60 min	126 ± 14	130 ± 20	140 ± 9	163 ± 16	165 ± 14	178 ± 13*
Recovery	15 min	77 ± 22	71 ± 15	85 ± 15	88 ± 15	91 ± 13	107 ± 13
	30 min	73 ± 12	72 ± 13	82 ± 15	80 ± 14	87 ± 12	101 ± 10
Treadmill Speed (km·h^{-1})							
Baseline		--	--	--	--	--	--
Exercise	15 min	6.0 ± 0.3	5.4 ± 0.8**	6.5 ± 0.7	8.1 ± 0.8	7.6 ± 1.1**	8.4 ± 1.1
	30min	6.5 ± 0.4	5.9 ± 0.8**	6.8 ± 0.1	8.9 ± 1.5	9.1 ± 1.8	9.1 ± 0.1
	45min	6.6 ± 0.4	6.3 ± 0.8	6.8 ± 0.1	8.9 ± 1.6	9.1 ± 1.8	8.7 ± 0.1
	60 min	6.6 ± 0.4	6.5 ± 0.8	6.8 ± 0.8	8.8 ± 1.7	9.2 ± 1.9	8.8 ± 0.1
Recovery	15 min	--	--	--	--	--	--
	30 min	--	--	--	--	--	--

TABLE 3 Core temperature, (T_{re}), mean skin temperature (T_{skin}), averaged EMG (aEMG), percentage (VO_2%and relative peak O2 consumption (VO_{2peak}), respiratory exchange ratio (RER), CHO (CHO_{oxi}) and fat oxidation (fat_{oxi}), and percentage to the energy yield for CHO (%En CHO) , fat (%En fat) and protein (%En Pro). * Significantly different ($P < 0.05$). Mean ± SD.

	Baseline	*Pre-exercise Shivering*
T_{re} (°C)	37.1 ± 0.2	36.8 ± 0.1*
T_{skin} (°C)	32.5 ± 0.7	22.7 ± 0.4*
aEMG (μV)	3 ± 2	29 ± 6*
VO_2% (%)	8.5 ± 2.0	14.9 ± 3.3*
VO_{2peak} ($ml \cdot kg \cdot min^{-1}$)	4.4 ± 0.8	7.5 ± 1.7*
RER	0.81 ± 0.01	0.79 ± 0.01*
CHO_{oxi} ($kcal \cdot min^{-1}$)	0.62 ± 0.20	0.86 ± 0.33*
Fat_{oxi} ($kcal \cdot min^{-1}$)	0.88 ± 0.30	1.90 ± 0.58*
%En CHO (%)	35 ± 9	27 ± 5*
%En Fat (%)	50 ± 11	63 ± 11*
%En Pro (%)	15	10*

All main factors independently modulated the oxidation responses for both CHO and fat ($P < 0.05$). From 30 min to the end of exercise, CHO oxidation was greater in NT, while fat oxidation was greater in COLD ($P < 0.05$). CHO oxidation reliance was greater at each exercise time point during running ($P < 0.001$). This was not observed with fat oxidation ($P = 0.259$). Total energy expenditure over the 60-min period had a greater reliance of energy derived from fat in COLD conditions (+1.62 ± 1.99 $kcal \cdot min^{-1}$) and a greater reliance of CHO in NT conditions (+1.38 ± 1.09 $kcal \cdot min^{-1}$) ($P < 0.05$). Exercise intensity only modulated the CHO contribution to total energy expenditure, which was greater during running (+4.05 ± 1.20 $kcal \cdot min^{-1}$, $P < 0.001$).

Concerning the effects of pre-exercise shivering on fuel selection, RER showed no differences between COLD and SHIV conditions during exercise ($P = 0.154$). No statistically significant differences were seen due to shivering on CHO or fat oxidation ($P = 0.358$). Relative contributions of energy substrates demonstrated a shift towards greater reliance in CHO to the energy yield in MOD compared to LOW but no significant differences were observed due to shivering ($P = 0.696$).

5.2.6 Catecholamines (Papers II-III-V)

When comparing COLD *vs.* NT, Epi concentration response was greater at 30-min (0.08 ± 0.02 $ng \cdot ml^{-1}$) and 60-min (0.09 ± 0.04 $ng \cdot ml^{-1}$) compared to baseline (0.04 ± 0.02 $ng \cdot ml^{-1}$) ($P < 0.001$) (Figure 14). No effects were observed due to temperature ($P > 0.05$) or exercise intensity ($P > 0.05$). A greater NE concentration response was seen in NT *vs.* COLD at 60 min (1.78 vs. 1.07 $ng \cdot ml^{-1}$, $p < 0.05$).

For SHIV *vs.* COLD, Epi concentration was greater at 30 and 60 min of exercise compared to baseline and recovery ($P < 0.01$) but the main effects of shivering ($P = 0.487$) or exercise intensity had no effects ($P = 0.246$). Norepinephrine concentration values at 30 min were significantly greater compared to COLD ($P < 0.001$). Norepinephrine in the SHIV condition was also greater compared to COLD across times ($P < 0.009$). Finally, NE values at 30 min were greater than all other times ($P < 0.001$).

5.2.7 Serum metabolites (Papers II-III) and lipids (Paper II)

Serum concentration changes for NEFA, glycerol, lactate, glucose and BHB are presented in Figures 15 and 16. Regarding the effects of COLD *vs.* NT, temperature did not affect NEFA ($p = 0.630$) nor glycerol responses ($P = 0.109$). NEFA concentration was greater at 30 min (769 ± 203 μmol · L^{-1}) compared to baseline (472 ± 123 μmol · L^{-1}) ($P < 0.001$), and 60 min (1275 ± 230 μmol · L^{-1}) was greater than both 30 min and baseline ($P < 0.001$). Glycerol concentration increased from 30 min (+0.17 ± 0.02 mmol · L^{-1}) to the end of exercise (+0.27 ± 0.03 mmol · L^{-1}) ($p < 0.001$) and was greater in MOD *vs.* LOW ($P < 0.005$). Glucose concentration was greater at the end of exercise (i.e. 60 min) (+0.34 ± 0. 11 mmol · L^{-1}, $P < 0.05$) compared to baseline and was greater in MOD *vs.* LOW ($P < 0.001$). No change was observed due to temperature on the glucose response ($P = 0.417$). An increase of 0.18 ± 0.10 mmol · L^{-1} in the BHB response was seen over time as 60 min was significantly greater than baseline ($P < 0.001$). Neither temperature nor exercise intensity affected the BHB response ($P = 0.531$ and; $P = 0.754$ respectively). Finally, time and exercise intensity both modulated the lactate response ($P < 0.001$ and $P < 0.01$, respectively) but no differences were seen between COLD and NT (1.79 ± 0.48 mmol · L^{-1} *vs.* 1.92± 0.48 mmol · L^{-1}; $P = 0.439$).

NEFA concentration increased during exercise ($P < 0.001$) and was greater in SHIV compared to COLD within MOD across time ($P < 0.05$). Glycerol concentration also increased during exercise ($P < 0.001$) and was greater in SHIV compared to COLD within MOD across time ($P < 0.05$). In addition, glycerol concentration in SHIV was greater than COLD at 30 min ($P < 0.05$). The glucose response showed no significant differences based on either shivering ($P = 0.950$) or exercise intensity ($P = 0.222$) but was affected by time as recovery values were lower than 30 and 60 min ($P < 0.001$). The BHB values in SHIV were greater than those in COLD within MOD ($P = 0.022$) and greater in MOD compared to LOW and at 60 and 90 min compared to other times ($P = 0.033$). Finally, lactate values were greater in SHIV (2.03 ± 0.15 mmol · L^{-1}) compared to COLD (1.69 ± 0.15 mmol · L^{-1}) over time and at 30 min ($P < 0.05$). No effect was seen from exercise intensity ($P = 0.063$).

Serum lipid concentrations during baseline, exercise and recovery are presented in Table 4.

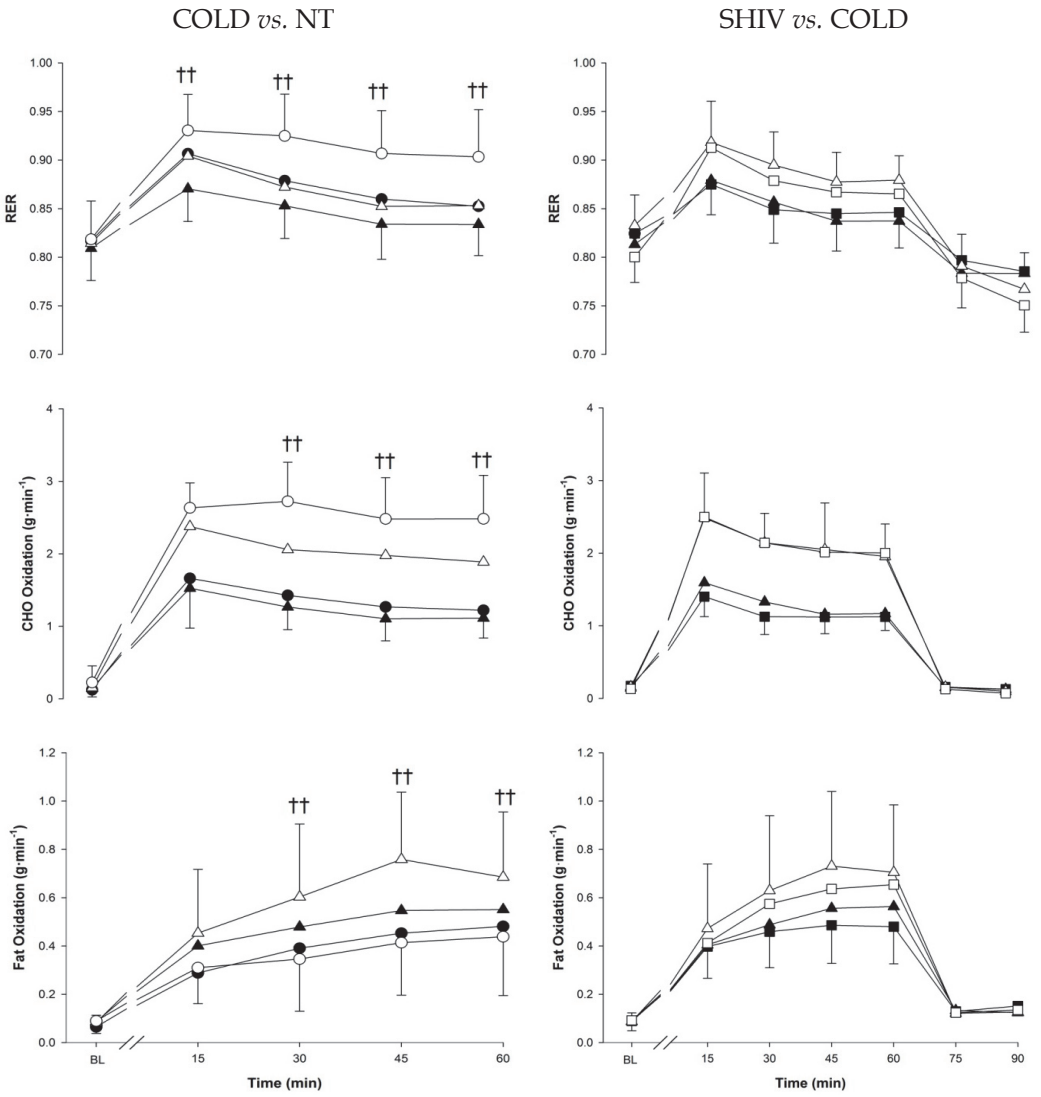

FIGURE 13 RER, CHO and fat oxidation at baseline, 60 min of exercise and 30 min of recovery (SHIV vs. COLD only; 90 min total) during LOW and MOD conditions (dark and white symbols respectively) in COLD (▲Δ) (n = 9), SHIV (■ □) (n = 10) and NT (●○) (n = 9). ††, Significant difference between environmental conditions across exercise intensities ($P < 0.05$).

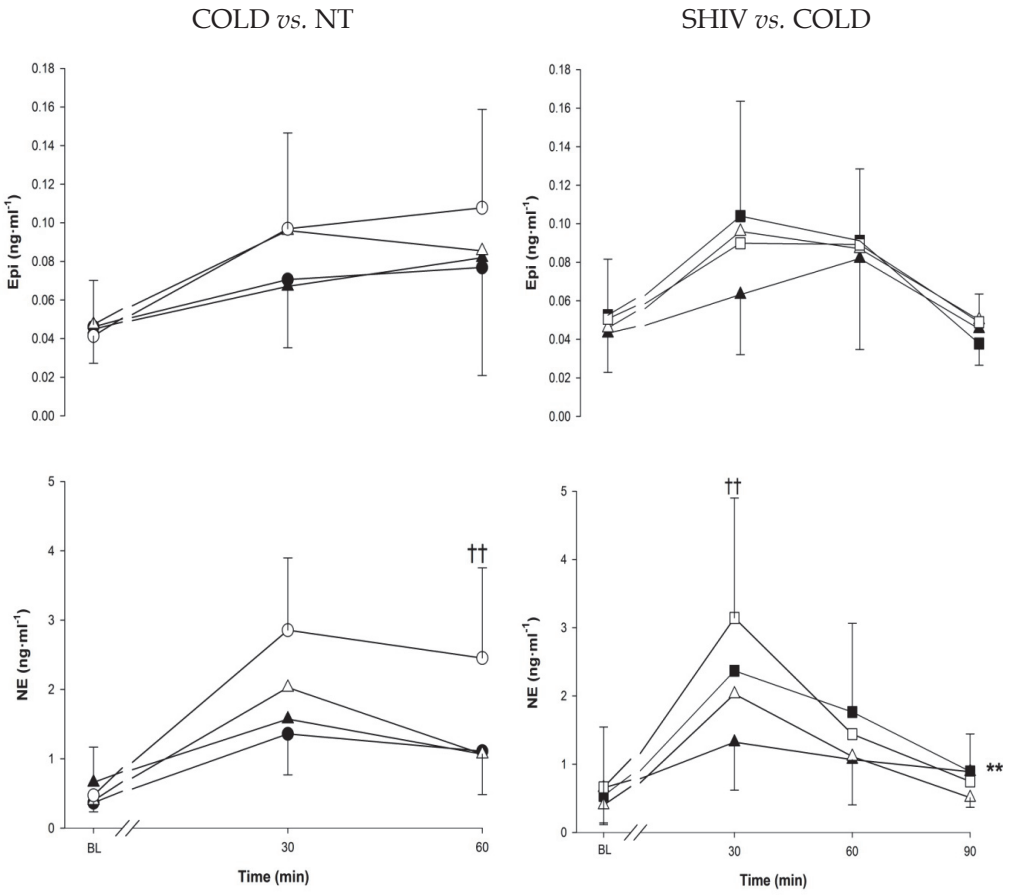

FIGURE 14 Epi and NE at baseline, 60 min of exercise and 30 min of recovery (SHIV vs. COLD only; 90 min total) during LOW and MOD conditions (dark and white symbols respectively) in COLD (▲Δ) (n = 9), SHIV (■ □) (n = 10) and NT (●○) (n = 9). ††, Significant difference between environmental conditions across exercise intensities ($p < 0.05$). **, Significant difference between environmental conditions across exercise intensities and time ($p < 0.05$).

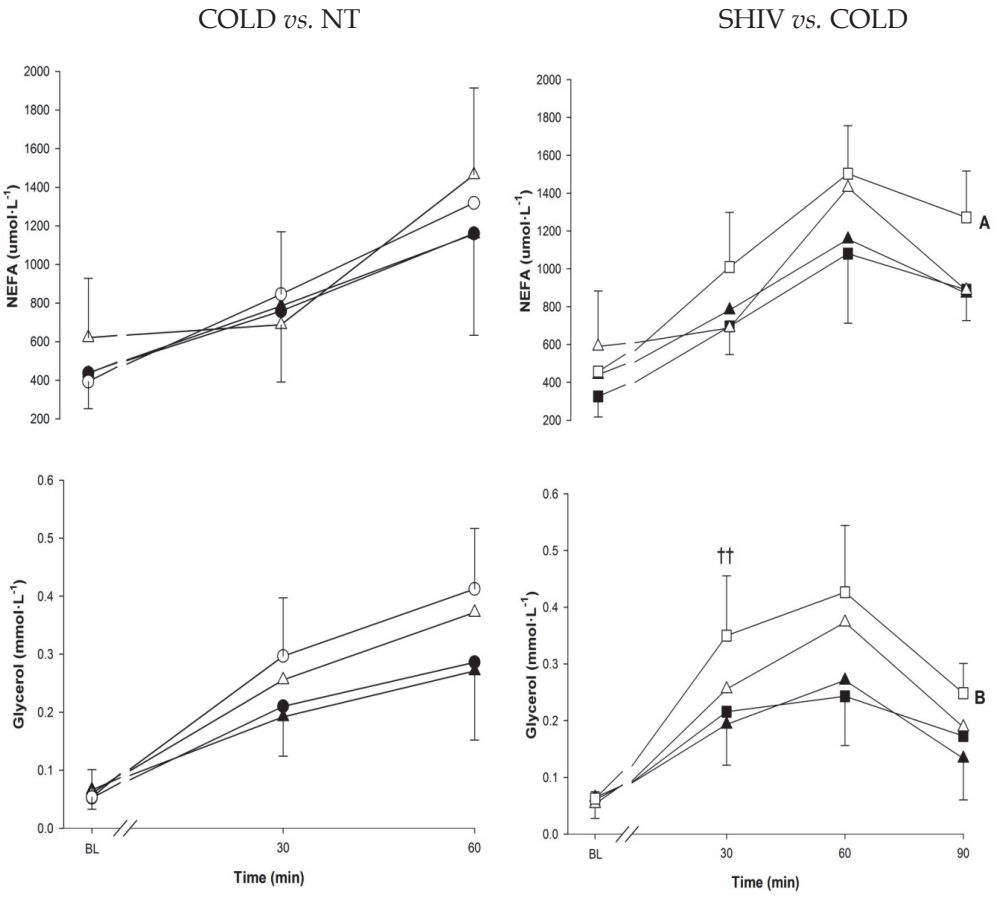

FIGURE 15 NEFA and glycerol at baseline, 60 min of exercise and 30 min of recovery (SHIV vs. COLD only; 90 min total) during LOW and MOD conditions (dark and white symbols respectively) in COLD (▲Δ) (n = 9), SHIV (■ □) (n = 10) and NT (●○) (n = 9). ††, Significant difference between environmental conditions across exercise intensities (P < 0.05). A, Significant difference between COLD and SHIV across time within LOW (P < 0.05).B, Significant difference between COLD and SHIV across time within MOD (P < 0.05).

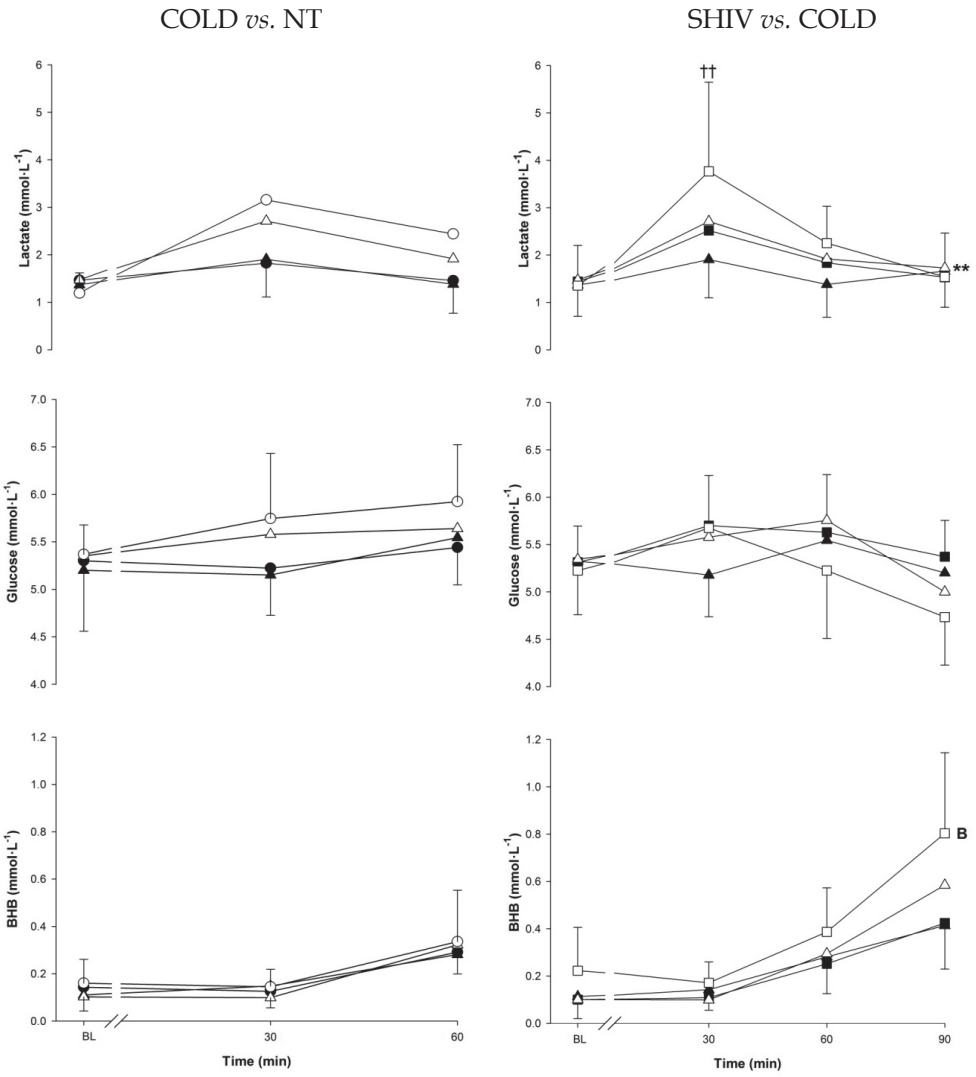

FIGURE 16 Glucose and BHB at baseline, 60 min of exercise and 30 min of recovery (SHIV vs. COLD only; 90 min total) during LOW and MOD conditions (dark and white symbols respectively) in COLD (▲Δ) (n = 9), SHIV (■ □) (n = 10) and NT (●○) (n = 9). ††, Significant difference between environmental conditions across exercise intensities ($P < 0.05$). ^{B}, Significant difference between COLD and SHIV across time within MOD ($P < 0.05$). **, Significant difference between environmental conditions across exercise intensities and time ($P < 0.05$).

TABLE 4 Serum lipids during walking and running in the cold (Cold) (n = 10), in the cold following a low-intensity shivering protocol (SHIV) (n = 9) and a neutral (NT) (n =10) environmental at baseline and during exercise. $CHOL_{tot}$, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides. [A] Significantly lower from 30 and 60 min within exercise modality ($P < 0.05$).[B] Significantly greater during running compared to walking within times ($P < 0.001$). Values are represented as mean ± SD.

		50% VO_{2peak} (LOW)			70% VO_{2peak} (MOD)		
		COLD	SHIV	NT	COLD	SHIV	NT
$CHOL_{tot}$ (mmol/L)							
	Baseline	4.57 ± 0.73	4.45 ± 0.61	4.43 ± 0.75[A]	4.41 ± 0.90	4.51 ± 0.76	4.51 ± 0.84[A]
	30min	4.72 ± 0.67	4.53 ± 0.45	4.59 ± 0.93	4.73 ± 0.90	4.90 ± 0.86	4.82 ± 0.82
	60min	4.67 ± 0.73	4.49 ± 0.51	4.73 ± 0.97	4.64 ± 0.82	4.69 ± 0.78	4.84 ± 0.85
	Recovery	4.58 ± 0.75	4.59 ± 0.74	455 ± 0.86	4.47 ± 0.83	4.58 ± 0.47	4.67 ± 0.85
HDL(mmol/L)							
	Baseline	1.60 ± 0.45	1.63 ± 0.35	1.60 ± 0.37	1.58 ± 0.40	1.53 ± 0.37	1.62 ± 0.46[A]
	30min	1.66 ± 0.46	1.68 ± 0.27	1.71 ± 0.43	1.69 ± 0.40	1.68 ± 0.40	0.72 ± 0.45
	60min	1.70 ± 0.52	1.66 ± 0.24	1.72 ± 0.45	1.69 ± 0.42	1.65 ± 0.40	1.73 ± 0.47
	Recovery	1.67 ± 0.48	1.74 ± 0.36	1.71 ± 0.45	1.70 ± 0.37	1.73 ± 0.38	1.70 ± 0.46
LDL(mmol/L)							
	Baseline	2.56 ± 0.70	2.35 ± 0.57	2.52 ± 0.70	2.45 ± 0.87	2.49 ± 0.73	2.49 ± 0.63
	30min	2.57 ± 0.70	2.33 ± 0.50	2.48 ± 0.81	2.53 ± 0.92	2.65 ± 0.77	2.59 ± 0.62
	60min	2.47 ± 0.75	2.27 ± 0.54	2.57 ± 0.84	2.40 ± 0.91	2.45 ± 0.72	2.56 ± 0.63
	Recovery	2.52 ± 0.76	2.41 ± 0.61	2.51 ± 0.77	2.39 ± 0.83	2.40 ± 0.46	2.58 ± 0.64
TG(mmol/L)							
	Baseline	0.88 ± 0.37	1.00 ± 0.50	0.76 ± 0.27[A]	0.85 ± 0.36	1.08 ± 0.61	0.89 ± 0.37[A]
	30min	1.04 ± 0.39	1.22 ± 0.20	0.92 ± 0.30	1.17 ± 0.38	1.24 ± 0.33	1.13 ± 0.36[B]
	60min	1.10 ± 0.40	1.24 ± 0.24	0.98 ± 0.20	1.27 ± 0.31	1.28 ± 0.35	1.20 ± 0.34[B]
	Recovery	0.90 ± 0.39	1.00 ± 0.28	0.73 ± 0.21	0.85 ± 0.27	1.01 ± 0.33	0.88 ± 0.37

5.3 Acute Cold Exposure and Exercise: Skeletal Muscle Oxygenation

5.3.1 Near-infrared signals: O_2Hb, HHb, tHb, and diffHb (Paper IV)

Oxygenated, deoxygenated, total and difference in hemoglobin content is presented in Figures 17 and 18 both in the gastrocnemius (GAS) and vastus lateralis (VL) muscles in the LOW (walking) and MOD (running) conditions.

Near-infrared spectroscopy derived signal for hemoglobin changes in GAS during walking demonstrated no changes in all parameters between conditions during exercise or recovery (Fig. 17, left panel). In VL during walking (Fig. 17, right panel), the decrease in O_2Hb at Ex10 was greater in SHIV (-13.0 ± 1.7 μM) *vs.* CO (-4.1 ± 2.9 μM) and NT (-2.1 ± 0.7 μM). The same response was observed in tHb but at Ex60 only. Moreover, diffHb at Ex10 indicated a greater deoxygenation in SHIV (-15.9 ± 2.9 μM) *vs.* CO (-4.2 ± 4.2 μM) and NT (-0.4 ± 1.4 μM). During recovery, a greater reoxygenation was seen in diffHb in COLD (12.8 ± 2.6 μM) *vs.* SHIV (0.1 ± 2.5 μM). A greater change was observed in HHb in SHIV (-8.1 ± 1.3 μM) *vs.* COLD (-0.7 ± 3.1 μM) at Ex60. At Rec10, HHb was stronger in SHIV (4.7 ± 2.1 μM) compared to COLD (-2.7 ± 2.7 μM).

During running, hemoglobin changes in GAS (Fig. 18, left panel) indicated that O_2Hb was greater in NT (-27.2 ± 2.1 μM) vs. SHIV (-18.6 ± 1.7 μM) at 10 min while O_2Hb at Rec10 was greater in NT (43.3 ± 2.2 μM) *vs.* COLD (32.9 ± 3.0 μM). There was no difference between conditions in tHb at Ex10. However, tHb had a greater increase in COLD (10.1 ± 5.4 μM) and SHIV (3.9 ± 4.1 μM) *vs.* NT (-7.8 ± 8.7 μM) at Rec10. Tissue reoxygenation at Rec10 was greater in NT *vs.* COLD as seen in HHb (-51.1 ± 10.3 μM *vs.* -22.8 ± 3.35 μM) and diffHb (90.1 ± 15.1 μM *vs.* -53.6 ± 4.1 μM). Hemodynamic responses in VL during running demonstrated a greater decrease in O_2Hb and tHb in SHIV *vs.* the other conditions at Ex10 (Fig. 18, right panel). Additionally, O_2Hb was greater at Rec10 in NT compared to COLD (17.6 ± 2.0 μM *vs.* 12.9 ± 0.9 μM). There were no differences in muscle deoxygenation during exercise or recovery between conditions as seen in HHb or diffHb.

5.4 Acute Cold Exposure and Exercise: Immuno-Endocrine Responses

5.4.1 Endocrine changes (Paper V)

Table 5 presents changes in measures hormones in all conditions, at BL and 60 min and in LOW and MOD. Testosterone measures (TES_{tot} and TES_{bio}) tended to increase only in the NT condition from exercise and demonstrated greater

values compared to SHIV in LOW (TES_{tot}: 19.77 ± 7.53 vs. 15.10 ± 4.41 nmol · L^{-1}) and MOD (19.97 ± 7.76 vs. 16.10 ± 6.45 nmol · L^{-1}) ($p < 0.05$). While there was a tendency of TES_{tot} and TES_{bio} to decrease in SHIV, statistical significance was not achieved. The response of IGF-1 demonstrated an increase at 60 min in all conditions in MOD only with values greater in NT (40.66 ± 12.54 nmol · L^{-1}) greater than COLD (35.51 ± 10.81 nmol · L^{-1}) ($p < 0.05$). ACTH and COR responses exhibited no changes from exercise in LOW. In MOD, however, ACTH and COR values were increased in NT from BL and were greater compared to COLD and SHIV conditions ($p < 0.05$). Regarding catecholamines, both NE and Epi demonstrated increases at 60 min. Additionally, NE concentration in COLD condition was lower compared to SHIV and NT across exercise intensities ($p < 0.05$). Lastly, thyroid hormones (TSH, T_3, T_{3free}, and T_{4free}) concentrations demonstrated increases at 60 min ($p < 0.05$). Nonetheless, only T_{3free} concentration showed a condition difference as the SHIV condition demonstrated lower concentration (2.69 ± 0.47 pg · ml^{-1}) compared to NT (2.30 ± 0.65 pg · ml^{-1}) ($p < 0.05$).

5.4.2 Leukocyte count and subsets (Paper V)

Total leukocytes increased at 60 min compared to BL in LOW SHIV while in MOD, an increase over time was seen in NT and COLD ($p < 0.05$) (Figure 19). The increase in NT in MOD was also greater than COLD ($p < 0.05$). Lymphocytes only increase over time in the NT condition in MOD which was greater compared to both COLD and SHIV conditions (Fig. 21). Granulocytes demonstrated increases at 60 min in all conditions in MOD but only in SHIV in MOD ($p < 0.05$) (Fig. 21). No differences between conditions were observed in granulocytes or monocytes (Figure 19). Correlation coefficients for leukocytes, lymphocytes, granulocytes and monocytes with HR, T_{re}, $\bar{T}_{sk}$, and hormones in all environmental conditions are presented in appendix 4.

5.4.3 Cytokines (Paper V)

Changes and statistical differences for pro-inflammatory, anti-inflammatory as well as growth factor cytokines are presented in Tables 6-9. When weighted and indexed through a z score (Fig. 20), the immune response regarding growth factors, indicated a greater response in SHIV MOD compared to LOW ($p < 0.05$). Furthermore, within MOD, score for COLD was significantly lower than both NT and SHIV ($p < 0.05$). The pro-inflammatory response also indicated a greater response of SHIV in MOD compared to LOW. Score for SHIV in LOW was also lower than NT while score for COLD in MOD was lower than both SHIV and NT ($p < 0.05$). Correlation coefficients between cytokines and HR, T_{re}, $\bar{T}_{sk}$, and hormones in all environmental conditions are presented in appendix 4.

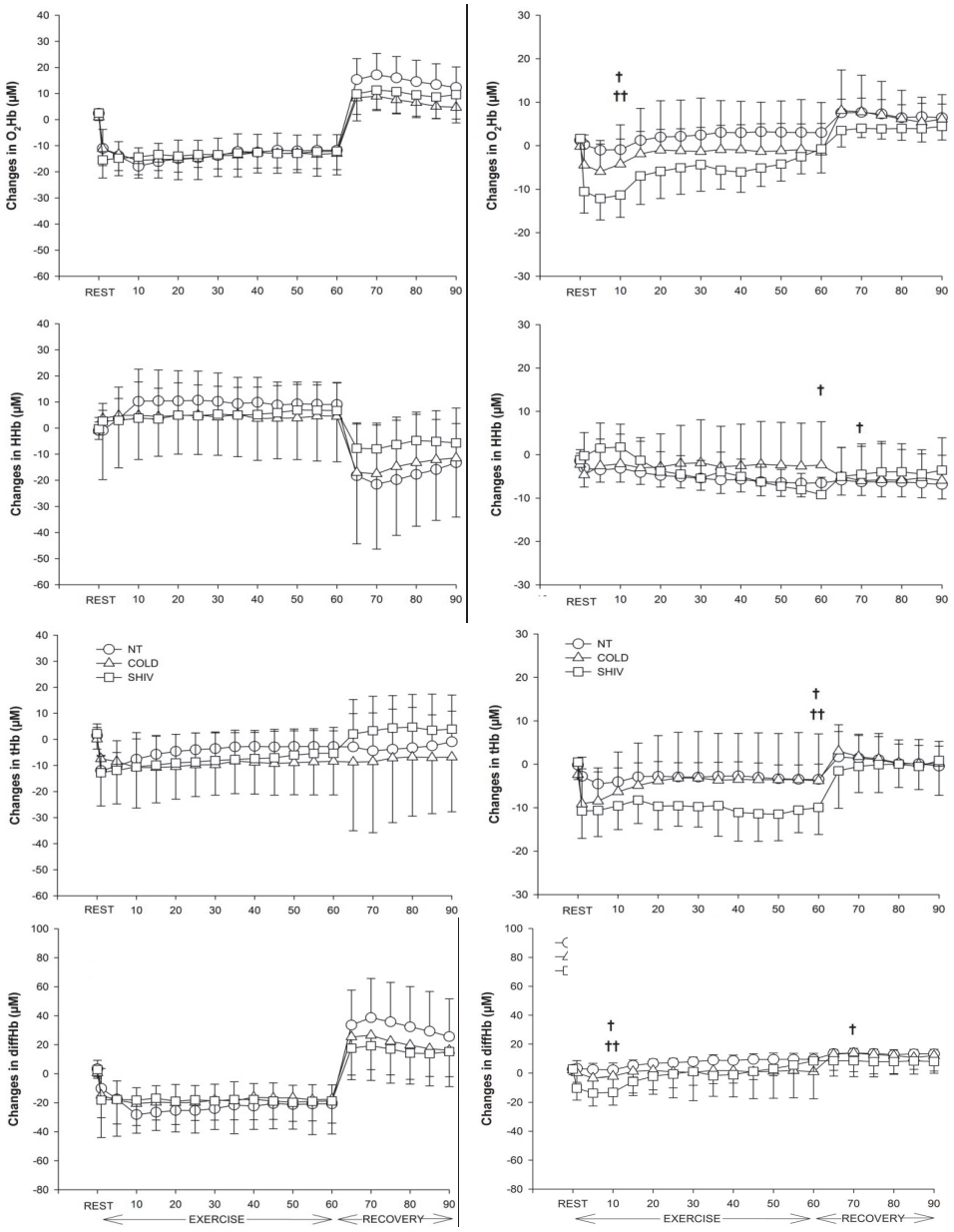

FIGURE 17 Changes in O2Hb, HHb, tHb, and diffHb, during rest, 60 min of walking and 30 min of recovery in GAS (right) and VL (left) in NT, COLD, and SHIV conditions. Significant difference between NT and CO (*), CO and PC (†), and NT and PC (††) ($p < 0.05$).

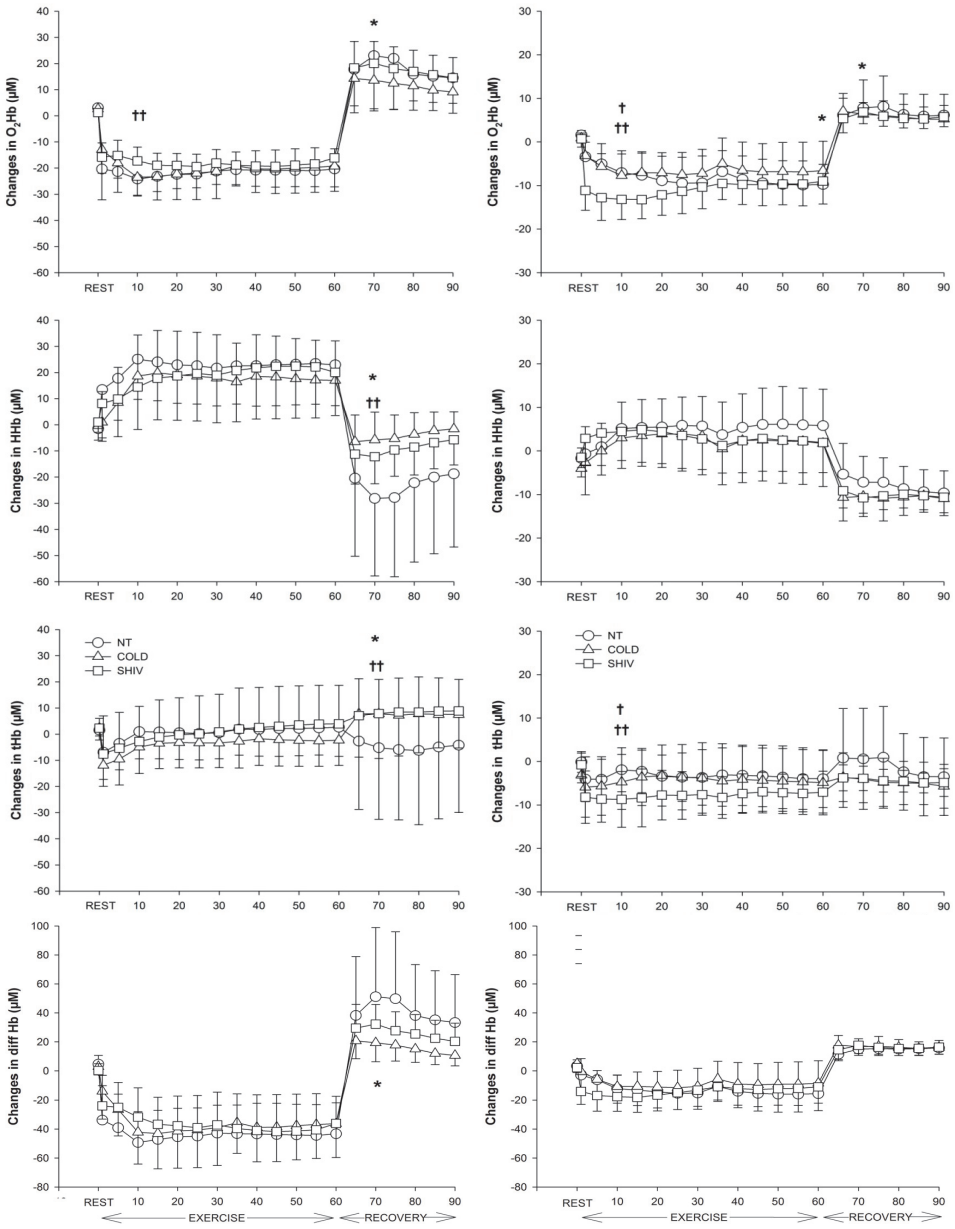

FIGURE 18 Changes in O2Hb, HHb, tHb, and diffHb, during rest, 60 min of running and 30 min of recovery in GAS (right) and VL (left) in NT, COLD, and SHIV conditions. Significant difference between NT and CO (*), CO and PC (†), and NT and PC (††) ($p < 0.05$).

TABLE 5 Mean ± SD changes in hormonal concentrations following 60 min of exercise in a NT, COLD and SHIV. *, Significant difference between BL and 60 min ($p < 0.05$). †, Significant difference between BL and 60 min across environmental conditions. ‡ difference between BL and 60 min across environmental and exercise conditions [A,] Significantly different from SHIV ($p < 0.05$). [B,] Significantly different from COLD ($p < 0.05$). [C,] Significantly different from NT ($p < 0.05$). [D,] Significantly different from COLD across exercise intensities ($p < 0.05$). [E,] Significantly different from NT across exercise intensities ($p < 0.05$). [F,] Significantly different from SHIV across exercise intensities ($p < 0.05$).

HORMONES		LOW			MOD		
		NT	COLD	SHIV	NT	COLD	SHIV
TES_{tot} (nmol ·L^{-1})	BL	16.17±3.99	16.85±3.76	15.63±4.44	14.67±4.02	14.71±4.01	16.28±3.69
	60 min	19.77±7.53[A,B]	15.93±4.62	15.10±4.41	19.97±7.76 [A,B,*]	15.73±5.91	16.10±6.45
TES_{bio} (nmol ·L^{-1})	BL	0.71±0.17	0.74±0.15	0.68±0.18	0.65±0.23	0.66±0.17	0.72±0.14
	60 min	0.76±0.23 [A]	0.68±0.19	0.66±0.17	0.77±0.25 [A]	0.67±0.20	0.65±0.31
SHBG (nmol ·L^{-1})	BL	39.84±12.66	36.61±11.49	40.24±7.54	38.46±12.98	41.53±9.36	41.89±11.93
	60 min	42.94±13.56	39.15±10.89	46.37±8.32[B,*]	40.86±12.10	43.267±10.83	42.04±11.17‡
IGF-1 (nmol ·L^{-1})	BL	31.34±8.82	31.23±11.07	30.22±6.14	35.23±10.53	29.59±11.04	32.50±8.46
	60 min	35.16±9.57	35.42±9.24	32.51±6.38	40.66±12.54[B,*]	35.51±10.81*	37.59±9.58*
ACTH (nmol ·L^{-1})	BL	16.80±6.99	21.61±8.29	22.39±18.19	22.30±13.31	17.07±9.25	17.78±8.88
	60 min	22.2±7.63	20.12±12.46	17.04±9.74	45.50±19.05 [A,B,*]	21.99±5.13	18.73±6.94
COR (nmol ·L^{-1})	BL	413±116	468±150	444±166	493±149	433±109	459±142
	60 min	413±110	453±204	457±143	593±133 [A,B,*]	444±104	464±162
NE (ng ·ml^{-1})	BL	0.37±0.13	0.66±0.54	0.54±0.23	0.45±0.15	0.40±0.26	0.66±0.89
	60 min	1.18±0.48	1.07±0.66 [E,F]	1.76±1.30†	2.46±1.30*	1.11±0.60	1.44±0.80†
Epi (ng ·ml^{-1})	BL	0.05±0.02	0.04±0.02	0.06±0.03	0.04±0.01	0.05±0.02	0.05±0.03
	60 min	0.08±0.06	0.08±0.05	0.09±0.04†	0.11±0.05	0.09±0.07	0.09±0.06†
TSH (mU ·L^{-1})	BL	1.46±0.73	1.51±0.96	1.47±0.58	1.45±0.47	1.44±0.81	1.43±0.65
	60 min	1.63±0.88	1.48±1.06	1.57±0.79	1.76±0.69	1.82±1.43	1.54±0.82†
T_3 (nmol ·L^{-1})	BL	1.28±0.36	1.33±0.40	1.33±0.29	1.30±0.25	1.26±0.29	1.38±0.35
	60 min	1.35±0.34	1.41±0.37	1.47±0.23	1.38±0.33	1.32±0.30	1.35±0.33‡
T_{3free} (pg ·ml^{-1})	BL	2.18±0.63	2.32±0.65	2.40±0.68	2.09±0.52	2.02±0.46	2.32±0.71
	60 min	2.30±0.65	2.55±0.43	2.69±0.47[C,*]	2.31±0.55	2.20±0.55	2.27±0.62‡
T_{4free} (pmol ·L^{-1})	BL	15.87±2.83	14.47±2.12	14.35±1.93	15.29±2.17	14.31±1.97	15.19±2.51
	60 min	15.90±3.07	15.02±2.37	15.00±1.94	15.71±2.41	15.07±1.45	16.08±2.03‡

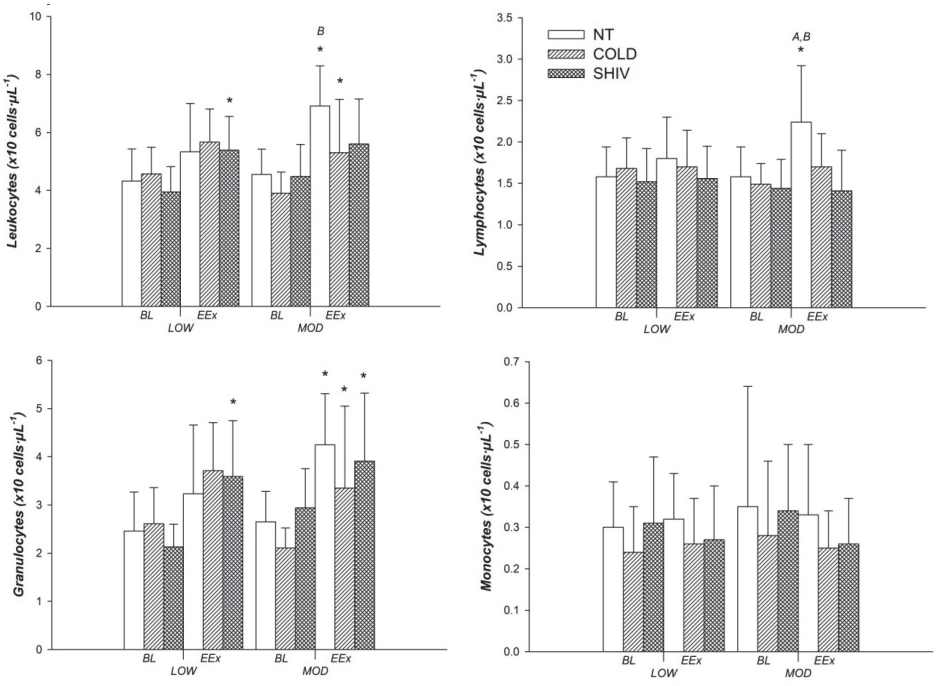

FIGURE 19 Mean changes in leukocytes, lymphocytes, granulocytes, and monocytes between pre and post exercise (N = 9). *Significantly different between pre and post ($p < 0.05$). [A] Significantly different from SHIV ($p < 0.05$). [B] Significantly different from COLD ($p < 0.05$). Bars represent SD.

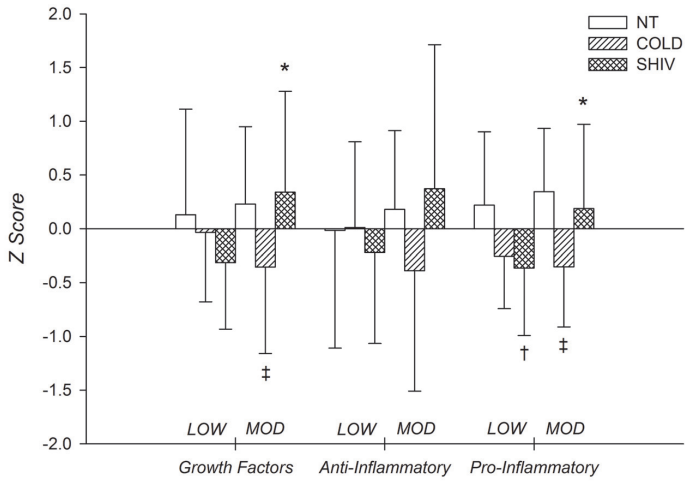

FIGURE 20 Mean Z scores for growth factors, anti-inflammatory and pro-inflammatory cytokines in NT, COLD and SHIV within LOW and MOD. *Significantly different between LOW and MOD ($p < 0.05$). † Significantly different from NT ($p < 0.05$). ‡ Significantly different from NT and SHIV ($p < 0.05$). Bars represent SD.

TABLE 6 Mean ± SD changes (pg · ml^{-1}) in pro-inflammatory cytokines following 60 min of low and moderate exercise in NT, COLD and SHIV. *, Significant difference between BL and 60 min ($p < 0.05$). †, Significant difference between BL and 60 min across environmental conditions. ‡ difference between BL and 60 min across environmental and exercise conditions [A,] Significantly different from SHIV ($p < 0.05$). [B,] Significantly different from COLD ($p < 0.05$). [C,] Significantly different from NT ($p < 0.05$). [D,] Significantly different from COLD across exercise intensities ($p < 0.05$). [E,] Significantly different from NT across exercise intensities ($p < 0.05$). [F], Significantly different from SHIV across exercise intensities ($p < 0.05$). ($n = 8$)

PRO-INFLAMMATORY CYTOKINES		*LOW*			*MOD*		
(1/2)		*NT*	*COLD*	*SHIV*	*NT*	*COLD*	*SHIV*
IL-1β	BL	1.55±0.97	1.92±0.82	1.68±0.86	1.63±0.93	1.67±0.71	1.83±1.12
	60 min	3.30±1.41	2.47±0.85 [E,F]	2.26±1.09†	3.13±0.92	2.11±1.06	3.53±1.44 *†‡
IL-2	BL	3.33±2.33	4.79±1.97	4.09±2.29	4.50±2.34	3.37±2.00	4.44±2.81
	60 min	8.04±3.28[D,]*	6.39±2.21	5.39±2.51	8.30±3.28*	5.63±2.82	8.36±3.39*‡
IL-5	BL	3.64±1.27	3.79±1.11	3.49±0.97	3.96±0.87	3.29±1.10	3.77±1.82
	60 min	5.34±1.38	5.24±1.55	4.97±1.48	5.32±1.24	4.74±1.57	5.47±1.94‡
IL-6	BL	9.32±3.06	10.58±3.26	8.98±3.43	9.90±2.68	8.82±2.90	10.20±4.44
	60 min	12.28±3.49*	12.75±2.51	11.94±4.04*	13.51±3.22*	11.53±4.03*	13.79±3.89*‡
IL-7	BL	16.63±6.25	19.42±6.12	16.11±6.89	17.86±8.59	16.36±7.35	16.16±6.09
	60 min	22.40±7.67 [D,F]	16.62±5.75	14.43±3.56	22.89±8.9	14.95±5.57	22.61±11.01‡
IL-8	BL	7.12±4.81	7.90±5.07	6.69±4.44	7.60±5.44	6.11±4.86	6.97±5.12
	60 min	8.04±4.20	8.28±5.11	7.43±5.33	8.61±5.02	7.77±5.38	8.70±4.95†
IL-9	BL	23.12±22.52	27.53±21.78	23.27±16.06	19.99±13.04	17.56±16.22	28.28±19.54
	60 min	34.53±24.69	30.19±16.36	26.10±18.46	34.38±23.93*	25.65±13.89	35.55±22.23†‡
IL-12	BL	33.47±18.38	37.38±19.47	34.08±18.83	39.44±20.93	29.07±19.83	33.64±22.73
	60 min	26.76±13.24[D]	41.56±14.75	40.50±17.48	26.89±12.45	36.85±22.26	28.86±14.31

TABLE 7 Mean ± SD (pg · ml^{-1}) changes in pro-inflammatory cytokines following 60 min of low and moderate exercise in NT, COLD and SHIV. *, Significant difference between BL and 60 min ($p < 0.05$). †, Significant difference between BL and 60 min across environmental conditions. ‡ difference between BL and 60 min across environmental and exercise conditions [A,] Significantly different from SHIV ($p < 0.05$). [B,] Significantly different from COLD ($p < 0.05$). [C,] Significantly different from NT ($p < 0.05$). [D,] Significantly different from COLD across exercise intensities ($p < 0.05$). [E,] Significantly different from NT across exercise intensities ($p < 0.05$). F, Significantly different from SHIV across exercise intensities ($p < 0.05$). ($n = 8$)

PRO-INFLAMMATORY CYTOKINES		*LOW*			*MOD*		
(2/2)		*NT*	*COLD*	*SHIV*	*NT*	*COLD*	*SHIV*
IL-15	BL	5.83±2.96	6.74±2.93	5.34±3.07	5.78±2.81	5.08±3.00	5.75±3.79
	60 min	7.54±2.56	7.23±1.94	6.60±2.86	7.79±2.55*	6.65±2.76	7.85±3.62‡
IL-17	BL	67.10±64.19	71.04±61.28	62.63±67.06	72.64±62.29	54.43±63.61	60.47±65.17
	60 min	83.68±67.28	82.78±54.85	70.19±66.66	93.65±78.92[B]	70.21±62.39	91.16±69.63*†‡
IFN-γ	BL	110.26±38.55	118.49±29.05	110.34±46.30	116.46±41.21	104.68±48.91	115.81±53.89
	60 min	173.87±54.16 [A,B,D,F,*]	132.36±35.01	124.74±43.49	175.85±55.46 [B,*]	125.37±43.84	172.50±64.22 [B,*] ‡
Rantes	BL	818±201	847±160	912±122	895±135	932±109	897±141
	60 min	1041±209[A,B,D,*]	740±160	785±75*	1078±130*	832±160[A,C]	1029±302*
Eotaxin	BL	83.74±19.36	87.78±12.08	76.16±16.20	78.69±15.98	77.74±8.68	80.05±12.82
	60 min	99.25±20.20 [A,B,D,F]	75.75±11.41	77.10±12.21	96.70±17.35	78.64±11.50	86.09±19.70‡
IP-10	BL	854±348	709±233	674±229	861±720	656±172	866±550
	60 min	887±294 [D,F]	605±219	593±171	1150±798 [A,B]	656±193	645±223
MIP-1β	BL	31.27±8.24	32.81±6.48	30.97±7.58	33.45±7.92	27.98±7.42	34.17±12.81
	60 min	36.48±9.19 [A,B,D,F]	29.59±6.28	27.56±7.79 [D]	38.65±8.59 [B]	29.24±7.38	39.01±11.92 [B]
MCP-1	BL	12.71±4.43	12.37±3.40	12.29±3.68	11.82±3.39	12.09±3.11	13.90±6.31
	60 min	14.94±4.33 [A,B, D,F]	9.33±2.24	9.43±1.92	16.49±5.07 [B,*]	11.33±3.49	15.79±7.27 [B,]

TABLE 8 Mean ± SD changes ($pg \cdot ml^{-1}$) in anti-inflammatory cytokines following 60 min of low and moderate exercise in NT, COLD and SHIV. *, Significant difference between BL and 60 min ($p < 0.05$). †, Significant difference between BL and 60 min across environmental conditions. ‡ difference between BL and 60 min across environmental and exercise conditions [A,] Significantly different from SHIV ($p < 0.05$). [B,] Significantly different from COLD ($p < 0.05$). [C,] Significantly different from NT ($p < 0.05$). [D,] Significantly different from COLD across exercise intensities ($p < 0.05$). [E,] Significantly different from NT across exercise intensities ($p < 0.05$). F, Significantly different from SHIV across exercise intensities ($p < 0.05$). ($n = 8$)

ANTI-INFLAMMATORY CYTOKINES		*LOW*			*MOD*		
		NT	*COLD*	*SHIV*	*NT*	*COLD*	*SHIV*
IL-1ra	BL	300±119	298±80	262±83	287±74	242±69	287±119
	60 min	437±167*	401±113	386±109	442±119*	347±144	471±154*‡
IL-4	BL	2.73±0.76	2.97±0.83	2.71±0.85	2.93±0.81	2.49±0.60	2.84±1.11
	60 min	3.45±1.13	3.74±0.80	3.43±1.01	3.49±0.56	3.37±1.15	3.74±1.38‡
IL-10	BL	2.42±1.16	2.45±0.93	2.47±0.57	2.54±0.86	1.44±0.98	3.34±1.69
	60 min	3.13±1.29	3.34±1.21	2.98±1.34	4.31±1.82 [B,*]	3.05±1.80*	4.47±2.22 [B,*] ‡

TABLE 9 Mean ± SD changes (pg · ml^{-1}) in growth factors following 60 min of low and moderate exercise in NT, COLD and SHIV. *, Significant difference between BL and 60 min ($p < 0.05$). †, Significant difference between BL and 60 min across environmental conditions. ‡ difference between BL and 60 min across environmental and exercise conditions [A,] Significantly different from SHIV ($p < 0.05$). [B,] Significantly different from COLD ($p < 0.05$). [C,] Significantly different from NT ($p < 0.05$). [D,] Significantly different from COLD across exercise intensities ($p < 0.05$). [E,] Significantly different from NT across exercise intensities ($p < 0.05$). F, Significantly different from SHIV across exercise intensities ($p < 0.05$). ($n = 8$)

GROWTH MEDIATES AND COLONY-STIMULATING FACTOR		*LOW*			*MOD*		
		NT	*COLD*	*SHIV*	*NT*	*COLD*	*SHIV*
VEGF	BL	27.02±12.76	31.84±16.95	30.11±18.20	33.61±15.50	23.96±15.90	26.84±19.24
	60 min	44.23±21.41 [D,*]	38.70±17.20	34.35±17.56	50.52±24.91 [B,*]	34.95±20.58*	51.57±22.33 [B,*‡]
FGF	BL	34.66±30.63	39.72±33.71	32.95±32.95	37.18±30.55	30.58±35.52	32.33±35.96
	60 min	47.22±32.62	40.86±26.68	33.43±29.21	48.67±35.23	36.48±31.62	49.26±33.09†‡
PDGF	BL	478±248	409±251	390±198	482±271	294±126	417±269
	60 min	1398±922 [A,B,*]	775±366 [E,F]	680±199	1353±563 [B,*]	709±292	1567±1012 [B,*‡]
G-CSF	BL	31.56±8.66	34.28±5.06	28.93±9.85	32.48±5.85	27.89±7.09	32.03±8.61
	60 min	38.13±11.29	45.22±8.41 [E,F]	40.09±10.06	39.21±9.17 [B]	36.77±11.85	39.87±10.78‡

6 DISCUSSION

This study demonstrated that prolonged strenuous physical loading and cold exposure had minimal influences on hormonal recovery. Changes in blood lipids and hematological parameters (hemoglobin, hematocrit, platelets and neutrophils) from the expedition, however, did not return to baseline values as rapidly as hormones. When observing acute changes in energy metabolism, exercising in the cold at low and moderate intensities did increase fat reliance as an energy source. When preceded by a pre-exercise shivering protocol induced greater fat utilization, no changes in energy substrate preferences were seen during subsequent exercise in the cold. These findings support a dissociation between energy substrates availability and utilization during exercise in the cold and suggest a concomitant shift in intramuscular triglycerides use. Skeletal muscle tissue deoxygenation in the gastrocnemius and vastus lateralis muscles indicated no significant changes in tissue deoxygenation during exercise in the cold compared to a thermoneutral environment. A pre-exercise cooling period, inducing skin and deep tissue cooling, did increase tissue deoxygenation during exercise but in the vastus lateralis muscle only. The gastrocnemius muscle, despite being greatly affected by the pre-exercise cooling period, did not show further deoxygenation. This could be explained by the degree of muscle recruitment and thermogenic potential for heat production in reestablishing thermal balance in active muscle, in addition to muscle fiber composition (type I *vs.* type II) that is known to differ in cold-induced sympathetic stimulation. Finally, immuno-endocrine responses in a cold environment may be reduced as pro-inflammatory cytokines and growth factors responded more strongly in a thermoneutral environment. Furthermore, the presence of shivering, in addition to a cold environment, demonstrated immuno-stimulatory effects compared to cold only, mainly during moderate-intensity exercise. A greater response in norepinephrine in the thermoneutral environment seems to be involved in these results in addition to greater free triiodothyronine concentrations when shivering is present. Studies examining the effects of cold exposure on physiological functions should consider the variability in thermal responses between subjects as a standardized thermal

stimulus (i.e. same cooling time across individuals) will not generate a fixed metabolic effect across subjects.

6.1 Metabolic and Endocrine Responses from a North Pole Expedition

We examine the hormonal, blood lipid and hematological profiles recovery at multiple periods following a 56-day North Pole expedition with a relatively large sample size (n = 7) compared to previous studies (i.e. 2 - 4 subjects) (Stroud et al. 1997; Helge et al. 2003). During the course of the expedition, participants had an average estimated energy deficit of ~6.6 MJ $\cdot d^{-1}$. A return to baseline values in serum hormone concentrations within 2 weeks was observed following prolonged strenuous physical loading, ED, and extreme cold exposure associated with the expedition. Blood lipids and hematological changes also return to baseline values but within 2 weeks to 2 months, demonstrating the fact that they did convey more long-term effects compared to hormones. These findings are in agreement with previous results indicating large hormonal and metabolic changes following prolonged strenuous physical loading, ED, and cold exposure with a recovery timeframe similar to other strenuous events (Stroud et al 1997; Friedl et al. 2000; Helge et al. 2008).

Physiological Mechanisms

Prolonged exercise, ED and cold exposure are known to increase cortisol concentrations (Opstad 1994; Friedl et al. 2000; Pääkönen and Leppäluoto 2002). An Antarctic study by Farrace et al. (1999), oppositely, showed a decrease in COR levels, similarly to ours. They concluded that an exhaustive effect on hormonal responses induced a downregulation of the stress-associated HPA hormonal axis, as the stressor effects were exerted for such a long period of time (two-month expedition). A 95-day Antarctic expedition (Stroud et al. 1997) observed a trend for an increase in COR when both participants were clearly underweight and undernourishment. Our subjects also had noticeable ED throughout the expedition (6 MJ $\cdot d^{-1}$), but not to the same severity compared to the South Pole study and not maintained as long either (56 days *vs.* 95 days). A likely mechanism explaining our results may be that immediately after the expedition, physical and psychological sources of stress were removed, in addition to adequate food intake. Recovery point R5 demonstrated the lowest COR and significantly high TES, consequently the highest TES/COR ratio, indicating anabolic state in subjects. It would seem possible that a reduction in COR post expedition may be related to a mechanism to enhance whole-body anabolism following an event in which catabolism was observed with an important decrease in body mass of 10 kg.

Regarding changes in testosterone, the training and preparation period for the expedition included large amounts of endurance type training, generally

associated with decreases in TES_{tot} levels. TES_{tot} and TES_{bio} demonstrated similar responses during recovery, although TES_{tot} was not significantly lower immediately upon return from the expedition compared to pre-expedition values. Pre-expedition training could account for this lack of change in TES_{tot}. R5, Post 1 and Post 2 were greater than R1, indicating a return to the real baseline values. Concerning TES_{bio} concentration levels, its higher sensitivity to the stimuli compared to TES_{tot} can explain its significant decrease immediately at R1 compared to the pre-expedition values. Furthermore, SHBG, an important binding protein for testosterone in addition to albumin, peaked during the lowest value of TES_{bio}, an expected response to a combined loading of ED and physical stress (Nindl et al. 1997). The combination of TES_{tot}, TES_{bio} and SHBG responses in the first days of recovery is indicative of an important reduction in hypothalamic-pitiutary-gonadal axis activity which subsequently recovered debuting 5 days post-event.

Thyroxine results, on the other hand, are more ambiguous. Previous studies, in association with ours, confirm that prolonged ED and cold exposure are associated with decreases in FT_4 concentrations (Leppäluoto et al. 1998; Hassi et al. 2001; Helge et al. 2006) as the disposal of thyroid hormones is accelerated (Leppäluoto et al. 1998; Hassi et al. 2001). A greater use of thyroid hormones from cold exposure activates uncoupling protein 1 (UCP1), found in mitochondria of BAT, and is responsible for heat production via non-shivering thermogenesis (Ribeiro et al. 2001). The implication of non-shivering thermogenesis activity to maintain heat production during the expedition would have likely continued in the first few days of recovery (removal from cold environment) since the lowest FT_4 level was recorded at R3. As we know, seasonal changes bring adaptation in BAT activity with the greatest level of activity in the winter time and a progressive reduction as the summer months are approaching (Au-Yong 2009). Adaption of BAT activity from the expedition would thus not be lost in a few days only. Moreover, the refeeding state of the subjects post-expedition could reduce circulating free fatty acid levels as the primary source of energy. Free fatty acids and FT_4 share the same protein carrier, albumin, thus competing for binding sites and the ability to be transported (Stockigt and Lim 2009). Free fatty acid concentrations in the blood may have interfered with the measured outcome of FT_4. A reduction in circulating free fatty acids would consequently increase binding site availability, likely causing a decrease in assessed circulating FT_4.

The food intake of the expedition's diet contained on average 55% of fat over a period of 56 days, thus changes in blood lipid content was expected. The addition of prolonged strenuous exercise, which tends to improve the blood lipid profile by decreasing the LDL/HDL ratio (Pelkman et al. 2004), further provided a large window of expected change. Our results demonstrated not only an increase in HDL, considered as a positive blood lipid change, but also in LDL and TC, which has also been seen in other ED studies combined with strenuous exercise (Stroud et al. 1997; Friedl et al. 2000). While one of the Greenland Cap crossings (Helge et al. 2003) observed a decrease in LDL and TC

levels, their fat intake was about 40% for 32 days. ED was nonetheless greater in our study as seen by a greater weight loss upon return (i.e. 10 kg body mass loss). The combination of important ED and of a high-fat diet during the expedition likely contributed to the increase in LDL and TC despite prolonged exercise, indicating the importance of energy availability during prolonged strenuous events.

Within our hematological parameters measured, we were particularly interested in RBC and RBC derivatives, involved in blood flow dynamics. Our observations in RBC and HCT are consistent with other findings when subjects are exposed to a cold environment for a prolonged period of time with a significant decrease at Post 1 (D'Alesandro et al. 1992), to facilitate blood flow in vasoconstricted areas, especially in hands and feet, where severe cold exposure can lead to cell death and amputation. Moreover, an increase in plasma volume from a decrease in HCT has been suggested to explain the maintenance mechanism for oxygen delivery in vasoconstricted blood vessels during cold exposure. Among other hematological changes, hemoglobin concentration decreased post-expedition. The expedition included large amounts of skiing, translating into an endurance training effect that can be observed as lower hemoglobin content (Spodaryk 1993). Finally, mean corpuscular hemoglobin concentration (MCHC) showed a decrease at Post 1. Although changes in MCHC can be attributed to cold (D'Alesandro et al. 1992), possible anemia from long-term ED and malnutrition could also explain this change.

White blood cells (WBC) are part of the immune system and respond acutely to infection and pathogens. We measured elevated levels of WBC and neutrophils at Post 1 despite previous findings indicating no change following prolonged cold exposure and physical stress (D'Alesandro et al. 1992). Immediately upon return, three subjects presented symptoms of infections with elevated core temperatures of 39-40°C. One subject also suffered a burning incident on day 45. WBC changes cannot fully explain the mechanism behind these results but the current indications seem to point out towards an acute response from intra-subject variability and not from an adaptation of the expedition itself. As lymphocytes were not increased, which include acute responses natural killer cells, T helper cells and B cells, and only neutrophils were increased, the elevation in WBC seemed to be attributable mainly to neutrophils as they are the most abundant type of WBC.

The circulating hormones assessed in our study returned to pre-expedition baseline values within a period of 2 weeks and thus parallelled other multi-stressor long-term events. An 8-week US army ranger course (Friedl et al. 2000) that included severe ED, prolonged physical loading and intermittent cold exposures, demonstrated a hormonal recovery of about 1 week for all hormones measured. A 5-day military course concurrently demonstrated hormonal values back to their normal range within 4 to 5 days after the event (Opstad 1994). Importantly and despite what we expected, shorter periods of physical stress such as marathon style races or sporting matches have demonstrated similar recovery time (Lac and Berthon 2000; Elloumi et al. 2003. Following heavy

physical loading events, time of recovery for most hormones is not proportionally affected by the severity of the physical stress.

Practical Implications

The study brings up two important practical implications. First, despite severe multi-factorial stresses for a prolonged period, hormonal therapy following an expedition would not be necessary, as hormonal concentrations seem to return to normal range within two weeks. Second, although we assessed hormonal concentrations, we did not assess potential changes in hormonal circadian cycles, which are known to be disrupted from much more limited stressful environments and influence sleep and cognitive patterns (i.e. 5-day military training camp) (Opstad 1994). Future studies should focus on this important hormonal component to further understand its variability from a multistressor environment on various physiological functions.

6.2 Substrate Availability and Utilization in the Cold: Effects of Cold *vs.* Thermoneutral

Substrate availability and utilization during exercise is an important determinant of performance. We are aware of many factors affecting fuel selection (i.e. diet, gender, exercise intensity, training status, etc.), but the effects of environmental conditions are still unclear. This portion of the study attempted to elucidate some changes in fuel dynamics between a cold and a neutral environment. Our results indicated that during our exercise protocol of walking at 50% and running at 70% VO_{2peak}, a greater energetic reliance from derived fat sources occurs in a cold compared to a neutral environment (i.e. 0˚C vs. 22˚C), despite the absence of a concurrent increase in NEFA availability or in glycerol or catecholamine concentrations. Our observations leading to this conclusion were based on a lower RER, lower CHO oxidation, and higher fat oxidation level in cold conditions while VO_2 and core temperature were maintained.

Studies examining fuel selection at different ambient temperatures have often observed an increase in VO_2 and/or a decrease in core temperature in the cold, both affecting substrate utilization, thus limiting data interpretation. Our experimental design consisted of the maintenance of VO_2 throughout the entire exercise session to differentiate the metabolic effects of cold exposure without the potential interference of a change in VO_2. The experimental protocol also attempted to maintain, with adequate heat production from exercise, core temperature as it is well known that central cooling is associated to whole-body lipolysis (Hurley and Haymes 1982; Clavert et al. 1972). Nonetheless, core temperature in COLD was slightly below NT at 60 min of exercise, indicating that it had yet to be stabilized as core temperature can be predicted from exercise intensity (Nielsen 1938; Astrand 2003). As the RER and fuel selection

responses were similar throughout the 60 min, the difference in core temperature at the end of exercise likely did not affect the energetic outcomes.

Physiological Mechanisms

Previous work has shown an association with substrate availability and utilization in thermoneutral (see Review by Hawley 2002) but not in cold environments. Vallerand et al. (1999) showed evidence of an uncoupling between fatty acid availability and oxidation in the cold at rest. The main finding of our work is the disparity between energy availability and oxidation when both core temperature and VO_2 are maintained during treadmill exercise. We demonstrated a rise in fat reliance in the cold with no concurrent increase in serum levels of NEFA, glycerol, glucose, BHB, or in plasma lipids. Layden et al. (2002, 2004a) developed an experimental design including cycling subjects in the cold at 65% VO_{2max}, an intensity sufficient to maintain core temperature. Their main findings included an increase in CHO and a decrease in glycerol availability in the cold. While the work of Layden et al. (2002, 2004a) has great value to the literature for cycling exercise, the greater muscular stress imposed on a smaller muscle mass to perform exercise (cycling as opposed to treadmill exercise) at a similar relative intensity may explain the difference in results (Hermansen and Saltin 1969). The muscle mass for walking and running would involve more muscle groups to perform the same physical load, therefore each muscle working at a lower intensity. A larger muscle mass activation has been associated with greater blood flow (Matsui et al. 1978) and catecholamine release (Savard et a. 1989; Kjaer et al. 1991) which could account for the differences in results. The greater muscular activation during walking and running, in combination with an increase in sympatho-adrenergic activity from cold exposure, would have enhanced lipolytic activity, known to be very active in the upper body adipose tissue beds (Arner et al. 1990; Horowitz et al. 2000).

A decrease in core and skin temperature induces important increase in both Epi and NE (Galbo et al. 1979; Weller et al. 1997a; 1997b; Frank et al. 2002). Our experimental protocol unsuccessfully generated significant differences in catecholamine concentrations from cold exposure as exercise seemed to be the main modulator. Increases in catecholamines related to heat stress have also been documented (Hargreaves et al. 1996). Skin cooling is a strong activator of the SNS but since exercise debuted immediately upon entering the climatic chamber the exercise-related activation of the SNS likely limited the influence of skin cooling. Heat production from exercise most likely balanced the thermal response, and even produced low-level heat stress at 60 min during running in NT, causing an increase in the NE response. Norepinephrine is a strong vasoconstrictive agent. It is possible that during running, the increase in NE potentially induced vasoconstriction in adipose tissue beds, reducing NEFA transport during thermoneutral conditions. Since the fluctuation in catecholamines and the lack of change in substrate availability cannot clearly explain our results, other factors need to be explored.

Layden et al. (2002) previously suggested that a reduction in subcutaneous adipose tissue blood flow (ATBF) from vasoconstriction would limit non-esterified fatty acids and glycerol transport. Skin temperature decreased markedly in the cold and as we know, peripheral capillaries may vasoconstrict to a point where skin blood flow can be almost zero. We did not observe a decrease in NEFA or glycerol in COLD conditions but reduced ATBF may have limited some lipolytic effects in adipocytes from cold exposure. Although skin temperature represents surface cooling, we cannot ignore the fact that deeper tissues (i.e., subcutaneous fat and muscles) could have been cooled to some extent (Oksa et al. 1995). Due to the cylindrical shape of the limbs, even a minimally extended cooling from the skin towards deeper tissues may result in high total cooled limb volume. Therefore, the role of deeper tissues may have contributed to our results. Kiens et al. (1993) suggested that lower muscle blood flow was linked to a greater affinity of working muscles to extract free fatty acids (FFA) as an energy source. An increase in muscle capillary density and a decreased blood flow in trained vs. non-trained participants was subject to induce a longer mean capillary transit time, thereby providing greater FFA extraction capability by the muscles. The repeated measure design of our protocol nullified any potential training effects between our subjects and isolated the effects of cold exposure and the possible changes in skeletal muscle capillary density between COLD and NT from limb cooling. The present vasoconstriction and peripheral tissue cooling (as indicated by lower skin temperature) could have provided a greater FFA uptake capability. Walking and running require a large muscle mass performing at low intensity providing an adequate environment for limb cooling (compared to cycling with all the work performed by the quadriceps muscle group), and thus reduced muscle blood flow. This potential mechanism and its effects on FFA uptake, however, need to be explored further.

Two other intra-cellular mechanisms might support the present findings and rely on intra-muscular energy sources, namely glycogen and intra-muscular triglycerides (IMTG). Studies examining the effects of cold exposure on muscle metabolism have observed reduced glycogenolysis in the working muscle, with and without changes in Epi, a modulator the glycogen response during exercise (Febbraio et al. 1996; Parkin et al. 1999; Starkie et al. 1999). A compensatory mechanism by additional oxidation of IMTG could partially support reduced glycogenolysis in the cold. Romijn et al. (1993) determined that maximal contribution of IMTG during sub-maximal exercise is established at ~65% VO_{2max}. This was validated by comparing intra-muscular and circulating fuel selection at 25%, 65% and 85% of VO_{2max}. Our experimental design included exercise intensities of 50 and 70% VO_{2max}, near the maximal contribution of IMTG. The reduction in certain energy substrates requires the increase of others at relative intensities, which, in the present study, could have likely originated from IMTG since no difference in NEFA was observed between conditions.

Practical Implications

Energy intake and expenditure are key factors in body weight and composition. The novelty of this study brings to light the possibility of including environmental factors in exercise recommendations to burn more fat. Although cold exposure can be detrimental to performance with strength and power type exercise, it would seem that submaximal aerobic exercise in the cold could also be aimed to increase fat utilization. A possible counter effect of exercise in the cold, however, is the greater post-exercise energy intake compared to a neutral environment. White et al. (2005), examined energy expenditure and intake during and after water-immersion, respectively. While energy expenditure was similar for the cold and neutral water conditions, energy intake after the cold condition was 44% and 41% higher compared to neutral and resting conditions, respectively, indicating that cold-water temperature significantly stimulated post-exercise energy intake.

6.3 Substrate Availability and Utilization in the Cold: Effects of Pre-Exercise Low-Intensity Shivering (SHIV *vs.* COLD)

Beyond examining the effects of a cold environment on substrate availability and oxidation during exercise, we also examined if a pre-exercise low-intensity shivering protocol would offset fuel selection during subsequent exercise. The use of energy substrates between exercise and shivering is not considered analogous as the crossover point at which the balance between CHO and fat contribution occurs is seen at a much lower VO_2 during shivering compared to exercise. We determined that a pre-exercise low-intensity protocol did not influence fuel selection during subsequent short-term submaximal treadmill exercise in the cold at both 50% and 70% of VO_{2peak}. This finding was interesting as we also observed an increase in fat utilization due to pre-exercise shivering and greater concentrations of NE, NEFA, glycerol, and BHB in the shivering condition at 70% of VO_{2peak}. The importance of these results reside in the fact that our experimental included: *i*) using the same ambient temperature in all trials (0˚C), *ii*) oxygen consumption clamping between trials to avoid the interference of a change in total VO_2 originating from additional thermal stress, and *iii*) standardizing the pre-exercise shivering state across subjects at 40% $Shiv_{peak}$ (equivalent to 15% VO_{2peak}).

There was, as expected, important variation in the pre-exercise shivering protocol which lasted between 40 and 120 min depending on subjects' characteristics (i.e. age, % body fat, BMI, fitness) (Eyolfson et al. 2001; Xu et al. 2005). The variation is associated to the time necessary to achieve steady shivering state from cold exposure (i.e. 60 min) (Blondin et al. 2010). Among certain physical characteristic to achieve steady shivering state, peak oxygen consumption and body surface area would be important contributors; although the variability in peak VO_2 (52.9 ± 5.2 $ml \cdot kg \cdot min^{-1}$) between subject was not

large. Initial body heat content, partly determined by body surface area (2.04 ± 0.18 m^2) was also likely not significantly different between subjects.

The current literature is scarce regarding the effects of shivering on exercise. The few reports on whole-body cooling prior to exercise have, despite indicating a greater reliance on fat during exercise, unfortunately provided a limited window of time to induce a steady state shivering (i.e. 20 – 30 min only) and relied on subjective identification of the presence of shivering (unmeasured signs of shivering considered shivering onset) (Hurley and Haymes 1982; Kruk et al. 1991). The present results indicated no significant changes in RER, CHO and fat oxidation or relative contribution to the energy yield when comparing control to the shivering condition within both low and moderate exercise intensities. Tipton et al. (1997) demonstrated higher plasma concentrations of FFA and BHB and lower glucose disposal during increasing shivering activity compared to exercise when matched for oxygen consumption. Our pre-exercise cooling protocol induced an increase in sympathetic activity, as demonstrated by higher NE concentrations (Weller et al. 1997), increased whole-body lipolysis by higher glycerol concentrations (Romijn et al. 1993), increased NEFA availability, and higher BHB concentrations inhibiting glucose uptake (Balasse et al. 1978) during exercise. These changes were expected to increase fat reliance during exercise but instead provided further evidence suggesting an uncoupling or dissociation between substrate availability and oxidation in the cold.

Peripheral vasoconstriction affected heat loss, which tended to be greater in COLD as subjects in SHIV proceeded to exercise with already vasoconstricted limbs and cooled skin, limiting heat loss during exercise. This difference was temporary in the low intensity condition, until the thermoregulatory response of the control condition would match the shivering condition, but was carried on for 60 min by the moderate intensity condition. In this condition, running required greater limb movements, inducing greater convective heat loss. Nonetheless, the metabolic response associated in the MOD condition with greater heat production compared to the LOW condition likely limited peripheral vasoconstriction due to increased core temperature.

Physiological Mechanisms

The pre-exercise low-intensity shivering protocol indicated a preferential use of lipids prior to exercise. This was observed by an increase in aEMG (29 *vs.* 3 μV), oxygen consumption (15 *vs.* 8 %VO_{2peak}) and fat contribution to the energy yield (63 *vs.* 50%) and significantly lowered RER (0.79 *vs.* 0.81) and CHO contribution to the energy yield (27 *vs.* 35%). Previous work on shivering and exercise demonstrated an increase in VO_{2peak} when both processes were combined as opposed to exercise alone (Nadel et al. 1973; Weller et al. 1997). To eliminate the effects of a change in VO_2 on fuel preference during exercise, we maintained the relative physical load constant so that potential metabolic and fuel selection changes would be linked to pre-exercise shivering and/or the potential combined presence of exercise and shivering without the interference of a

change in VO_2 between conditions. The LOW SHIV conditions demonstrated shivering activity both during baseline and recovery but showed no alteration in fuel selection during exercise. This suggests that shivering drive and its associated metabolic outcomes were suppressed by voluntary muscle activation during exercise. It is, however, difficult to explain the basis of these findings as muscle motor unit recruitment during combined shivering and exercise is poorly understood. Whether motor units of different types or different motor units of the same type would be involved in the response is unknown (Meigal 2002). Based on the thermogenic potential between low-intensity shivering and exercise, the larger contribution of exercise towards oxygen consumption and heat production could have been the main fuel selection modulator. Previous findings, nonetheless, have hypothesized that core temperature and not the thermogenic effect of exercise is the main modulator of shivering activity during exercise (Meigal 2002). Core temperature data in the present study was lower in the SHIV condition at 15 min of exercise, but returned to near baseline values at 30 min. Consequently, the combined presence of both shivering and exercise, early during MOD and LOW conditions, likely occurred.

Furthermore, shivering in the early stages of exercise was seen as treadmill speed was lower in the first 30 min in LOW and 15 min in MOD. This reduction can be partly explained by a lower muscle mechanical efficiency due to muscle cooling (Oksa et al. 2000, 2002). Reduced muscular efficiency, including a decrease in contraction velocity during dynamic exercise (Oksa 2000), will increase VO_2 to perform the same work at a given workload (Weller et al. 1997). To compensate for an increase in oxygen requirements for shivering thermoregulatory purposes and reduced muscle efficiency, reducing treadmill speed was necessary to maintain stable VO_2. The positive heat balance from exercise (heat gain minus heat loss) increased body heat content and consequently core temperature. With a thermal balance achieve between conditions, speed became similar between COLD and SHIV. Fuel selection, however, was unaffected by treadmill speed or shivering. The contribution of exercise-related muscular activity towards VO_2 and thus heat production was much more than shivering which, by itself, could explain shivering suppression or its lack of effects on fuel selection.

We previously suggested a shift in ATBF as a potential mechanism at play during exercise in the cold vs. neutral environment (Layden et al. 2002). When shivering was involved, we measured an increase in lipolytic markers (NE, NEFA, glycerol and BHB) in the MOD condition only but not in LOW. As exercise intensity increased (from 50% to 70% VO_{2peak}), the influence of greater physical stress likely increased NE concentrations. In combination from a increased blood flow from greater exercise intensity in MOD *vs.* LOW, the effects of greater circulating NE on well perfused adipose tissue would increase NEFA and glycerol release and transport. In the LOW condition, despite greater NE concentrations at 30 min, lower ATBF compared to MOD could explain the lack of changes in NEFA or glycerol.

Similarly to the COLD *vs.* NT portion of this study, intra-cellular mechanisms may need to be explored to fully understand the present results as the dissociation between substrate availability and utilization in the cold remain unexplained. Layden et al. (2004b) administered acipimox, an inhibitor of lipolysis (targeting HM74 receptors expressed in adipose tissue but not in skeletal muscles) (Tunaru et al. 2003) and a known suppressor of fat oxidation from circulating NEFA, in exercising subjects in the cold. Despite the clear reduction in NEFA from acipimox injection, no difference in total fat or CHO oxidation in 0°C compared to 20°C was observed. Unfortunately, they did not measure IMTG directly in the muscle or by imaging. They nonetheless concluded that IMTG functions remained unaffected by cold exposure. In a thermoneutral environment, extensive work by van Loon et al. (2004, 2005) and by Watt et al. (2002) have provided valuable information on IMTG utilization and its balance with NEFA. Watt et al. (2002) observed a decrease in IMTG contribution to fat oxidation following 2 and 4 h of cycling at 55% VO_{2max} with a concomitant increase in plasma FFA. With a lipolysis-inhibiting acipimox-associated decrease in NEFA during cycling at 50% VO_{2max} for 120 min, van Loon et al. (2005) observed compensation in fat oxidation by an increase in IMTG oxidation. The current theory suggests an inverse relationship between IMTG and plasma FFA availability/oxidation work as an energetic buffer during exercise. This adaptation to changes in circulating NEFA concentrations seems to be modulated by intramuscular hormone sensitive lipase (HSL) (Langforst et al. 1998; van Loon 2004). Our measured increase in circulating NEFA could systematically reduce IMTG contribution to energy expenditure, as fat oxidation was similar between conditions, and therefore suppress HSL activity to rely mainly on circulating fat sources to fuel exercise. Accumulation of unused circulating NEFA could, however, also translate into a greater use of IMTG.

Practical Implications

Many occupational groups including outdoor workers and military personnel are often exposed for long periods of time in a cold environment without enough physical activity to prevent a decrease in thermal balance by metabolic heat production. Although body cooling prior to performing physical task can be rather difficult from a muscular point of view with the decrease in strength and speed associated with muscle cooling, it would seem that from an energetic standpoint, pre-exercise body cooling does not modulate a shift towards either fat or carbohydrates. Importantly, periods of inactivity in the cold nonetheless increase oxygen consumption and heat production and energy expenditure. It would consequently be essential to consider an increase in energy intake, as fuel availability could be impeded from long duration of inactivity in the cold that are sufficient to increase energy demand.

6.4 Skeletal Muscle Tissue Oxygenation during Exercise in the Cold

The present portion of the study attempted to determine the effects of exercising in a cold environment on hemoglobin content of active skeletal muscles, namely the gastrocnemius and vastus lateralis muscles during walking and running. The experimental design included three environmental conditions including a neutral (25˚C) (NT), a cold (0˚C) (COLD), and a cold with whole-body pre-cooling (0˚C) (SHIV) condition that would be conducive of not only skin but deep tissue cooling. The effects of ambient temperature alone (0˚C *vs.* 22˚C), without pre-exercise cooling, demonstrated no detrimental changes in skeletal muscle tissue blood volume or oxygenation in the assessed muscles during submaximal exercise. Nonetheless, following pre-exercise whole-body cooling, greater tissue deoxygenation was observed in VL compared to cold and thermoneutral conditions at 10 min of steady-state walking, with a similar trend during running. Tissue blood volume was also affected by pre-cooling in VL. Although tHb remained lower in SHIV *vs.* COLD and NT throughout the 60 min of exercise, diffHb indicated greater tissue deoxygenation in the VL in the early phase of exercise only. Changes in O_2Hb, HHb, tHb and diffHb all had a tendency to respond in a similar manner in both muscles, although the amplitude of change was greater in GAS. Finally, heat production from greater metabolic rate in running eliminated the maintained greater deoxygenation in SHIV seen in walking, pointing towards exercise intensity as a modulating factor.

Oxygen consumption was maintained steadily throughout the exercise protocol to control the associated effects of $\dot{V}O_2$ shifts on skeletal tissue oxygenation (Hiroyuki et al. 2002). This created a disparity in muscle function and contribution to total $\dot{V}O_2$ during the earlier portion of exercise as low-intensity shivering accounted for 15% within the 50% and 70% intensities used. To compensate for this change, treadmill speed had to be reduced during the first 10 min of walking in the SHIV condition only (see Table 2). Reduced contribution of muscular activity in SHIV compared to CO and NT early during exercise was expected to limit our ability to match differences between conditions in microvascular dynamics. Nonetheless, lower values in tHb (~9 μM) and diffHb (~18 μM) were observed at 10 min of exercise in VL demonstrating sufficient muscular activity to generate a greater demand on oxygenated hemoglobin content.

Yanagisawa et al. (2007) examined the effects of cold on skeletal tissue hemoglobin content and reported that peripheral cooling is associated with increased in tissue deoxygenation through lower blood volume in the muscle at rest. The pre-exercise whole-body cooling protocol used in the present study induced an immediate drop in all NIRS-derived parameters, indicating rapid peripheral skin vasoconstriction upon cold chamber entry (known to affect deep tissue NIRS signal) (Tew et al. 2010); although the decrease was not as extensive compared to the results of Yanagisawa et al. (2007). This could be due

to a difference in cooling methods. The progressive air whole-body cooling used in the present study likely produced slower deeper tissue cooling compared to the use of a cooling pad directly on the region of interest. Along the time-course of pre-exercise cooling, GAS demonstrated a progressive deoxygenation (i.e. increased HHb and decreased tHb and diffHb), which was not observed in VL. This could be due to: *i)* the greater skin surface to limb volume of GAS, and *ii)* the cooling gradient between skin temperature and deeper tissues (Webb 1992). A previous report (Webb 1992) showed a decrease of 3.7ºC in the thigh muscle during a 2-hr stay in 15ºC. While calf muscle temperature was not measured, subcutaneous temperature indicated a stronger cooling effect gradient on the lower leg compared to the thigh. The present study showed greater pre-exercise cooling effect on GAS. However, this did not translate into a shift in deoxygenation or blood volume during subsequent exercise, as opposed to VL

Physiological Mechanisms

A possible explanation for this finding would be the degree of muscle recruitment and consequently the thermogenic effect of muscular contraction. The amplitudes of change in all NIRS parameters in GAS were all greater compared to VL, suggesting a more important activity-related and thermogenic contribution of GAS for both walking and running. A previous study examining the neuromuscular contribution of VL and GAS during walking and running at various speeds had a greater EMG activity in GAS (Simonsen and Dyhre-Poulsen 1999). Despite its much greater potential for heat production, the cold-induced vasoconstrictive state of the VL was not adequately compensated due to its lower thermogenic activity, thus limiting hemoglobin resupply and return to NT and CO levels over the exercise period. This important finding also asserts that the muscle pump action during rhythmic exercise is powerful enough, despite initial limb vasoconstriction, to reestablish adequate tissue oxygenation during exercise in the cold as seen in GAS.

Furthermore, recent work by Horiuchi et al. (2013) examined sympathetic activation performing plantar flexion exercise at low intensities during a cold pressor test. Their results suggest that skeletal muscles mainly composed of type II glycolytic fibers are more sensitive to blunting of cold-induced sympathetic vasoconstriction during low-intensity exercise at 20% of MVC. The GAS is composed of approximately 80% of type II muscle fibers (Trappe et al. 2001) as opposed to approximately 50% in VL (Green et al. 1981). The lack of differences in hemoglobin parameters in GAS could therefore also be related to a higher sensitivity to functional sympatholysis in predominantly glycolytic muscles. The change in thermogenic potential between walking and running, as indicated by metabolic rate, may have played a role in the tHb response in VL between Ex10 and Ex60. During walking, lower heat production along the course of exercise did not increase tHb over time, as opposed to running. Exercise intensity therefore seems to be a modulating factor in the microvascular oxygenation response, at least in VL.

The recovery period indicated a tendency for greater skeletal muscle tissue reoxygenation following exercise in a thermoneutral environment compared to cold, mainly in GAS. Both the CO and SHIV conditions maintained lower skin temperature throughout exercise (~22˚C and ~20˚C, respectively vs. ~32˚C in the NT). Similarly to exercise, skin cooling activates the sympathetic nervous system (SNS). Yet, during recovery, skin temperature in the CO and SHIV conditions returned slowly back to baseline values because high SNS stimulation was still present. Consequently, a greater peripheral vasoconstrictive state limited skeletal muscle tissue reoxygenation in CO and SHIV compared to NT. As no deep muscle tissue temperature measurement was collected, it is difficult to assume that cooled limbs were also involved in the response. Based on the work by Yanagisawa et al. (2007), some degree of muscle cooling, despite slight increases in core temperature in CO and SHIV, could have been a factor in post-exercise tissue reoxygenation.

Practical Implications

The early reduction in oxygenated and total hemoglobin as seen in VL, a crucial muscle during many types of exercise, may be a limiting factor during physical performance. A difference ranging approximately 5-10 $\mu mol \cdot L^{-1}$ of oxygenated hemoglobin, however, should not be necessarily taken as a strong marker of potential performance reduction. Winter athletes should nonetheless ensure to maintain skin and muscle temperature high enough prior to events, without overheating, to avoid an early reduction in performance until progressive limb rewarming returns hemoglobin content and tissue blood volume to adequate levels. Although exercise intensity as low as 50% of $\dot{V}O_{2peak}$ was enough to return O_2Hb and tHB levels in SHIV comparable to CO and NT, the reduced treadmill speed at 10 min during steady-state exercise could be indicative of a possible reduction in performance and needs to be further explored. Importantly, walking and running involves many muscles (quadriceps muscles, medial and lateral gastrocnemius heads, soleus, etc.). The short-term and limited changes that we observed in the vastus lateralis may have minimal impact on overall performance outcomes, considering that no changes may occur in other exercise-related muscles. Analysis of other muscles would be necessary to validate a possible change in performance.

6.5 Immuno-Endocrine Responses during Exercise in the Cold

The immuno-endocrine effects of acute submaximal exercise and cold exposure on hormonal concentrations and immunological measures such as leukocyte count and subsets as well as cytokines were investigated. Additionally, we examined the relationships between hormonal concentrations and immunological measures as the endocrine and the immunological systems are bi-directional and influencing each other's responses. Previous work has focus

on the interactions and relationships within the immune-endocrine system through the SNS and HPA axes while this study attempted to extent past results with hormones from the SNS, HPA, HPT and the HPG axes to explain changes in immunological measures. The present experimental protocol elicited a change in all endocrine axes and some immunological measures. The main findings of this study are that: *i*) exercising in a thermoneutral environment elicited a stronger response in a large array of endocrine and immunological measures suggesting a reduction of the exercise-related inflammatory response in the cold, and *ii*) the presence of shivering during exercise in the cold may induce an immune-stimulatory effect during moderate exercise intensities compared to cold alone. The first finding was determined based on the greater responses of total leukocytes, lymphocytes, TES_{tot}, TES_{bio}, ACTH, COR, IGF-1, NE, IL-2, IL-7, IL-12, IL-17, IFN-γ, Rantes, Eotaxin, IP-10, MIP-1β and MCP-1 in a thermoneutral compared to cold environment. Regarding the second finding, increases in some hormones and cytokines in the SHIV condition compared to COLD were also observed. Nonetheless, when cytokines were indexed on a standardized score, both growth factors and pro-inflammatory cytokines were elevated in SHIV in MOD compared to LOW and was greater than COLD at both intensities. Moreover, associations between endocrine and immunological measures suggested weaker relationships in the cold between endocrine and immunological parameters.

McFarlin and Mitchell (2003) suggested that core temperature could be a driving force in immune responses during exercise but their results pointed towards unchanged disturbances in immunity between cycling at 60% VO_{2peak} in 8˚C and 38˚C. Following cycling for 90 min in 18˚C and 32˚C, Peake et al. (2008) observed increases in IL-8, IL-10 and IL-1ra in a hot environment but no change in leukocytes compared to cool. Other reports examining the immunomodulatory effects of temperature used cold environmental protocols of 8°C and above (Mitchell et al. 2002; Niess et al. 2003; Laing et al. 2005), suggesting a limited intensity of the cold stimulus compared to the present study. Rhind et al. (1999, 2001, and 2004) performed a series of well-designed experiments demonstrating that core temperature *per se* does not directly influence immune responses. Rather, responses in HPA and SNS endocrine axes from exercise and environment-related increase in core temperature modulated immune and cytokine responses. The current data seems to support this mechanism between the NT and COLD conditions.

Physiological Mechanisms

The SHIV condition responded in a different manner, irrespective of core temperature changes, suggesting another mechanism. Greater increases in NE and T_{3free} concentrations were observed in SHIV compared to COLD and NT respectively, in addition to strong negative relationships between T_{3free} and many cytokines, including all interleukins. The additional cold stress prior to exercise may have been sufficient to induce the increase in T_{3free}, leading to its implication on cytokine production, mainly interleukins. The greater NE

response seen in SHIV was also present in NT, a condition that offered little relationships with T_{3free}. Although in lower quantity than T_{4free}, T_{3free} is a more potent hormone on targeted cells. Limited work has been done to demonstrate an interaction between the HPT axis and the immune system. Klecha et al. (2006) determined a regulatory role of TSH, T_3, and T_4 on cytokine production in lymphocytes in mice and attributed the interaction via protein kinase C enzymatic pathway between both systems. While the present study does not offer a mechanistic approach to the results, the implication of the HPT axis in regulating cytokine production in the SHIV condition seems likely.

Endocrine modulations were limited in the LOW condition since the increase in core temperature was restricted to 0.9°C in NT, 0.8°C in COLD and 0.4°C in SHIV, and physical demand was limited to 50% of VO_{2peak}. Although Rhind et al. (2004) suggested that an increase of over 0.5°C in core temperature represented a thermal threshold for stress hormones release and subsequent cytokine production, only TES_{tot}, TES_{bio} and NE were modulated in LOW. Increases in TES in a thermoneutral environment, associated with some endurance exercise protocols (Jensen et al. 1991; Fahrner and Hackney 1998), have been reported, but the underlying mechanisms remain to be elucidated. Literature suggests limited to no change in serum testosterone concentration from acute cold air exposure (Leppäluoto et al. 1988). Since luteinizing hormone, a stimulating hormone produce in the anterior pituitary gland, does not seem to change under cold exposure either (Leppäluoto et al. 1988), higher order mechanisms from the hypothalamus and the gonadotropin-releasing hormone could explain our result. Moreover, the HPA axis responded more strongly in the MOD condition with increased ACTH and COR concentrations, similarly to IGF-1, in NT, probably due to elevated heat production at a higher workload with increased stress. The HPT axis demonstrated little to no change in TSH, T3, and T_{4free} from acute thermal or exercise stresses, and is consistent with previous findings (Leppäluoto et al. 2005). Interestingly, T_{3free} demonstrated greater concentrations in SHIV compared to COLD. Although shivering and non-shivering thermogenesis are reversely correlated (Ouellet et al. 2012), our shivering protocol surely induced non-shivering thermogenesis as subjects, in the SHIV condition, were cold-exposed prior to exercise for 40 -120 min. Ribeiro et al. (2001) suggested thyroid hormones-dependent pathways for heat production from uncoupling protein 1 activation in mitochondria found in brown adipose tissue, requiring a greater use in thyroid hormones. As this was observed in LOW only, the heat production from the MOD condition likely attenuated the need for non-shivering thermogenesis during the exercise portion of the trials.

Changes in leukocytosis and leukocyte sub-groups during exercise in various environmental setting have previously been reported (McFarlin and Mitchell 2003; Niess et al. 2003; Laing et al. 2005). The lack of differences in leukocytosis between conditions in previous reports may originate from the use of higher temperatures (i.e. 28-38°C) compare to a limited cold stress (i.e. 8-18°C). The greater leukocytosis in NT in the present study, attributed mainly to

increased lymphocytes, could be associated to the greater catecholamines secretion, known to mobilize leukocytes out of the marginal pools in response to stress. β-adrenergic receptor expression on lymphocytes, particularly T and B cells and natural killer cells, are targeted by catecholamines and induce a cascade of events through the adenyl cyclase system for lymphocyte mobilization (Carlson et al. 1989). Although the degree by which the lymphocyte mobilization response may be determined by cell surface receptor density (Pedersen and Hoffman-Goetz 2000), the greater NE secretion in the SHIV condition compared to COLD did not seem to have the same effect on leukocyte response as it did in the NT condition. As increased testosterone levels, observed in NT, have been linked to T lymphocyte cells apoptosis (McMurray et al. 2001), the difference in lymphocyte and total leukocytes in the NT and SHIV conditions remain unexplained and will require further investigation.

Cortisol and ACTH, part of the HPA axis, generally increase with elevated inflammatory response and systematically act to suppress and control inflammation through changes in glucocorticoid receptor expression on lymphocyte cells. Fragala et al. (2011a) suggested a temporal role of the HPA axis in modulating immune responses during one hour of exercise where despite increases in cortisol levels, glucocorticoid expression was lower in B-lyphocytes before increasing during recovery, indicating possible greater suppression of inflammatory response following exercise. Thereby, the increase in cortisol and ACTH associated with our NT condition may only taken effect during later recovery.

Previous studies have examined a limited array of cytokines in response to exercise and thermal stress which consequently made it difficult to draw accurate interpretations of pro-inflammatory and anti-inflammatory immune responses (Rhind 1999, 2001; Mitchell 2003, Patterson et al. 2008). We combined and indexed on a z score sixteen pro-inflammatory cytokines, three-inflammatory cytokines and four growth factors to examine immune responses during low and moderate-intensity exercise with environmental stress. This was done based on the rationale that previous studies have qualified changes on inflammatory responses based on a limited number of immune parameters. Our experimental design indicated a stronger pro-inflammatory response in NT and SHIV compared to COLD at moderate-intensity exercise. Interestingly, the anti-inflammatory response was not different between conditions, but followed a similar trend. IL-6 has been defined as both a pro- and anti-inflammatory myokine but was included in the pro-inflammatory group as its primary function. The increase in pro-inflammatory response in NT was mainly driven by changes in IL-1β, IL-7, IL-17, Rantes, IFN-γ, Eotaxin, IP-10, MIP-1β, and MCP-1 and in SHIV by IL-1β, IFN-γ, Rantes MIP-1β, and MCP-1. Coefficient correlations indicated a very limited amount of relationships between immune and endocrine parameters in the COLD conditions as opposed to NT and SHIV. Cross et al. (1996) observed a similar dissociation between variables when the rise in core temperature was abolished. The presence of other immune

modulating mechanisms, with absence of thermal stress-related endocrine changes, could offset known immuno-endocrine associations.

Practical Implications

The interest in regulation of the immune system importantly stems from the potential dysregulation leading to disease and health problems. Alteration or changed levels of physical and psychological stress on the HPA, SNS and SAM have been linked to immune-related health issues such as cytokine overproduction, latent virus appearance, viral susceptibility, and others (Yang and Glaser 2002). As controlled physical stress, such as exercise, can modulate endocrine axes in a way to examine the immune system, outcomes on immune measure from physical stress may provide indications on the role of certain cytokine dysregulation on short- and long-term health impacts. The present study aimed at providing a large window of results from the immunomodulatory effects of exercise and cold exposure. Future work should focus on mechanisms of control of individual cytokines and leukocytes sub-types.

6.6 Methodological Considerations

The North Pole expedition was logistically complex and unfortunately forced some limitations in data collection, thus preventing a complete interpretation of certain metabolic functions post-event. Namely, FFM and %fat data on returning and few days immediately post-event would have provided much more insights on energy use and body composition assessment. The recovery period for hormones and blood lipid profile is also difficult to fully interpret as dietary intake post-event was not monitored and could have greatly varied between participants. Positive energy balance was evident (removal of physical stress and unlimited access to food) was the composition of the diet would have been useful. Moreover, the hormones assessed in this study merely represented a fraction of their endocrine axis' functions as additional hormones (i.e., T_3, ACTH, TSH) and cross-factorial measurements (i.e., temporal sampling and carrier binding competitors modified artifacts) would have provided clearer insights for the mechanisms involved in the many changes observed following the expedition. The measured hormones did provide valuable insights of endocrine and metabolic functions during the expedition and have been used by themselves in previous studies as well but complementary information from additional hormones within the same axes would have provide a complete endocrine view the expedition.

The COLD *vs.* NT and SHIV *vs.* COLD bioenergetics portions of this study used two exercise intensities (50% and 70% VO_{2peak}) representing low and moderate intensities. It is without a doubt that using lower intensities (<50% VO_{2peak}) would have provided different thermal results as lower heat

production would have delayed changes in core and skin temperatures during the 60 min of exercise. Although we are confident that fuel selection would not have been modulated by lower of higher exercise intensities, future work should confirm these results. The use of young healthy men only is an important consideration in this study. It is well known that lipolysis activity differs in rates and tissue location between men and women (Horowitz et al. 2000). The crossover concept is also modulated by gender as women tend rely more on fat as an energy source and thus have a crossover point at higher exercise intensity than man (Venables et al. 2005). The bioenergetics conclusions of this study therefore apply mainly to men but since the precise mechanism by which our results occurred are unknown, we cannot discount the possibility that they apply to women as well. More specifically, regarding the SHIV *vs.* COLD portion of the study, the whole-body cooling was done until steady state continuous low-intensity shivering appeared (i.e. 40% of $Shiv_{peak}$). This was performed in the perspective that outdoor workers or winter activity practitioners may be exposed to low temperatures that may induce body cooling while they are performing tasks. Cooling to a point where task completion would be compromised (e.g. high-intensity shivering), seemed less suitable from a practical perspective. Thus, examining the effects of shivering in combination to lower exercise intensities would broaden the perspective and usefulness of our results. Finally, the current results do not provide a clear mechanistic approach. The experimental design of the study was developed to explore and understand previous inconsistent findings during exercise in the cold. We indeed confirmed previous work that there is dissociation between substrate availability and utilization in the cold compared to a thermoneutral environment but during treadmill exercise and, importantly, with maintained oxygen consumption. We nonetheless could not offer clear evidences of the mechanisms at play to explain these findings. Future work in the field of environmental bioenergetics should give relevant information on this topic.

Concerning the NIRS data, although tHb and diffHb demonstrated lower values in VL during exercise in the PC condition, it was not observed simultaneously at the measured times. A trend in reduced tHb and diffHb early in the exercise response can be seen but an unexpected degree of variation, therefore increasing statistical type II error, limited the interpretation of the results. Previous work investigating muscle oxygenation during running and walking has also presented significant variation in the NIRS signal (Hiroyuki et al. 2002). The source for this variation may have emerged from greater limb movement of the lower leg compared to the upper leg resulting in vibrations affecting the NIRS signal. Previous work examining muscle deoxygenation during exercise via NIRS technology, has used cycle ergometers, therefore reducing movement artifacts (Neary et al. 2002; DeLorey et al. 2005; Martin et al. 2009). We maximized stability of the probes with an adjustable restrictive band, and despite greater limb movement from treadmill exercise as opposed to cycling, precision of probe positions were not comprised. Recent work using treadmill exercise has offered valid and replicable results (Rissanen et al. 2012).

Furthermore, we cannot ignore that the lower limb has a much lower mass compared to the upper leg. This difference in mass leads to greater variability in thermal effects between participants from the environmental conditions and between subjects. The individual changes in cooling of various limbs between subjects, however, were not assessed. Tew et al. (2010) and others (Buono et al. 2005; Davis et al. 2006) investigated the effects of skin blood flow on NIRS-derived signal. The use of local and whole-body heating protocols inducing an increase in skin blood flow seems to be associated with an increase in blood volume and tissue oxygenation signal. Their protocol and results were based on the assumption that heating of the skin does not influence muscle temperature or microvascular dynamics. This was based on previous studies with imprecise techniques and in inactive muscles (Detry et al. 1972; Johnson and Rowel 1975). Recent work by Heinonen et al. (2011) demonstrated that both skin and muscle tissue exhibit increases in blood flow from local and whole-body heating. Although considering that the change in skin blood flow was greater that muscle blood flow from a normothermic state, surface tissue temperature change affected both the skin and the muscles. We recognize that the present protocol presented skin temperature differences between conditions but two factors support the validity of our results. First, skin temperature remained unchanged between Ex10 and Ex60 within each condition. In both walking and running, VL clearly exhibits a marked difference at Ex10 but not at Ex60 in O_2Hb and diffHb in walking and in O_2Hb in running. Since no change in skin blood flow occurred, a shift in muscle microvasculature has to be the cause. Second, cold exposure may induce near complete vasoconstriction in certain areas such as the fingers and toes. A report by Liang et al. (2000) measured thigh and calf skin blood flow and temperature. A decrease in environmental condition from only 24.9°C to 17.3°C induced a reduction in mean skin temperature from 31.7°C to 28.7°C which in turn reduced mean skin blood flow of ~50% from central sites including the torso and leg. Taking these elements together, skin blood flow in our study was likely near minimal at both measured sites. The small differences in skin temperature between the CO and PC conditions could not have translated into a significant amount of blood being redirected, thereby having little to no influence in the results.

Finally, although most studies examining immune changes from circulating venous concentrations of leukocytes and other markers, circulating concentrations of leukocytes represent a narrow 0.2 - 2% of total leukocyte mass (Gleeson 2007), thus limiting the scope of interpretation in immune changes from various external stimuli. The examination of leukocytes and its subsets (lymphocytes, granulocytes and monocytes) did not offer specific modulations of lymphocytes subsets in T and B cells or in natural killer cells, the first line of defense of the immune system, which would have offered additional interesting insights.

7 CONCLUSIONS AND MAIN FINDINGS

The main conclusions and findings emerging from this study are presented below:

1. **Effects of prolonged cold exposure and exercise on energy metabolism and endocrine responses from a 56-day North Pole expedition.**

Following a 56-day North Pole expedition with environmental temperatures ranging from -3˚C to -47˚C, daily energy intake was 23 $MJ \cdot d^{-1}$, energy expenditure was 29 $MJ \cdot d^{-1}$ and, serum hormone levels fully recovered well within two weeks post expedition. It would seem that prolonged and extreme physical stress has little impact on hormonal recovery time when compared to much less strenuous events such as marathons or high-intensity sporting events. Blood lipids and hematological parameters tended to carry longer-lasting effects (i.e. from 2 weeks to 2 months) compared to hormones and need to be further considered in post-event recovery health parameters.

2. **Acute changes in energy substrates availability and utilization from exercising in a cold environment compared to a thermoneutral environment.**

During submaximal exercise with core temperature and VO_2 maintained, a greater energetic reliance from derived fat sources occurs in the cold compared to a thermoneutral environment despite the absence of a concurrent increase in NEFA availability or in glycerol or catecholamine concentrations. This underlines the presence of a different energy selection mechanism in the cold compared to thermoneutral during exercise. The balance of thermal and exercise induced metabolic changes is a determining factor in peripheral regulation of energy metabolism in the cold. Two physiological mechanisms may explain our results: *i*) Mean transit time from decreased muscle blood flow could impact substrate uptake mechanisms, and *ii*) a reduction in

glycogenolysis associated with muscle cooling could increase IMTG reliance to supply the energetic demand.

3. Acute changes in energy substrates availability and utilization from exercising in a cold environment with and without a pre-exercise shivering protocol.

Pre-exercise low-intensity shivering did not influence fuel selection during submaximal treadmill exercise in the cold. The pre-exercise cooling protocol induced a decrease in RER and an increase in contribution of fat to the energy yield before exercise. Increases in NE, NEFA, glycerol and BHB at 70% VO_{2peak} during exercise also did not seem to influence the fuel selection response either. This was determined by: 1) using the same ambient temperature in all trials (0˚C), 2) maintaining oxygen consumption similar between trials to avoid the interference of a change in total VO_2 originating from additional thermal stress, and 3) standardizing the pre-exercise shivering state across subjects at 40% $Shiv_{peak}$ (equivalent to 15% VO_{2peak}). Some elements that seem to guide the present findings include: 1) the greater contribution of exercise over shivering on energy production and consequently fuel selection, and 2) the known dissociation between substrate availability and utilization seen during exercise in cold environments, and 3) the potential implication of IMTG as a buffer between NEFA in circulation and fat oxidation.

4. Effects of pre-exercise whole-body cooling during exercise in the cold, and exercising in a cold compared to a thermoneutral environment on skeletal muscle tissue oxygenation during walking and running.

Exercising in a cold environment without a pre-exercise cooling period demonstrated no detrimental changes in skeletal muscle tissue oxygenation in both assessed muscles. However, following a pre-exercise whole-body cooling period inducing a decrease in skin and core temperature (SHIV condition), increased skeletal muscle tissue deoxygenation in the early stages of exercise was observed in the vastus lateralis muscle. This early shift in tissue oxygenation was nullified over time. This deoxygenation response was not seen in the gastrocnemius muscle. The degree of muscle activation, thermogenic potential and muscle fiber composition could explain the results. Changes in O_2Hb, HHb, tHb and diffHb all had a tendency to respond in a similar matter in both muscles, although the amplitude of change was greater in the gastrocnemius muscle which coincidently demonstrated a large variation in the data. During recovery, tissue reoxygenation response seemed to indicate that exercising in a neutral environment stimulate a stronger recovery compared to a cold environment.

5. Effects of combined exercise and cold exposure on hormonal concentrations, leukocyte count and subsets as well as cytokine responses in addition to investigate relationships between

immunological measures (leukocytes and cytokines) and hormonal changes.

Taken together, the protocol of the study elicited a change in all endocrine axes examined and some immunological measures. Our results demonstrated reduced immune responses, more specifically inflammatory responses, during exercise in the cold. This response seemed to be driven by the known modulating effects of endocrine changes on immune responses. We also observed an immuno-stimulatory effect of shivering in pro-inflammatory cytokines and growth factors. Shivering-associated cold stress may be responsible in modulation of the acute hypothalamic-pituitary-thyroid-immune response, involved in energy expenditure, which was not present with a cold environment only.

YHTEENVETO (FINNISH SUMMARY)

Kylmässä suoritetun fyysisen kuormituksen vaikutukset energia-aineenvaihduntaan, luurankolihasten hapettumiseen ja immune-endokriinisiin vasteisiin.

Lisääntynyt kiinnostus arktiseen alueeseen merkitsee sitä, että entistä enemmän ihmisiä asuu ja työskentelee kylmissä ympäristöolosuhteissa, jotka saattavat aiheuttaa haasteita heidän terveydelleen, turvallisuudelleen ja toimintakyvylleen. Tämän tutkimuksen tarkoituksena oli selvittää kylmäaltistuksen vaikutuksia energia-aineenvaihduntaan, luurankolihasten hapettumiseen ja immuno-endokrinologisiin vasteisiin fyysisen kuormituksen aikana. Pohjoisnaparetken aikana, joka kesti 56 vuorokautta ja oli pituudeltaan 850 km, energiansaanti oli 23 MJ · d^{-1}, energiankulutus 29 MJ · d^{-1} ja lämpötila -3˚C - -47˚C, mitkä laskivat retken jälkeen mitattuja seerumin testosteroni-, kortisoli- ja tyroksiinihormonipitoisuuksia verrattuna lähtötilanteeseen. Hormonitasot palautuivat kahden viikon aikana, mutta HDL ja LDL -kolesterolitasot olivat koholla vielä kaksi viikkoa retken päättymisestä. Akuutti submaksimaalinen kuormitus kylmässä (0°C *vs.* 22°C, shortsit ja juoksukengät) 50% ja 70% maksimaalisesta hapenkulutuksesta) ei lisännyt triglyseridien hajotessa syntyvien vapaiden rasvahappojen (NEFA), eikä seerumin glyseroli- ja katekolamiinipitoisuuksia, mutta sen sijaan lisäsi rasvojen hapettumista molemmilla kuormitustasoilla. Lisäksi kylmän aiheuttama lihasvärinä lisäsi rasvojen osuutta kokonaisenergiankulutuksesta, mitattuna levossa ennen kuormitusta. Tämä ennen kuormitusta aiheutettu lihasvärinä ei kuitenkaan aiheuttanut muutoksia energialähteiden käytössä itse kuormituksen aikana, vaikka plasman noradrenaliini-, NEFA-, glyseroli- ja beta-hydroksibyturaattitasot lisääntyivät 70%:n kuormitustasolla. Kuormitusta edeltävä jäähdytys heikensi ulomman reisilihaksen hapettumista. Endokriiniset ja immunologiset vasteet olivat voimakkaampia termoneutraalissa ympäristössä, ja joissakin mittauksissa myös kylmässä, kun kuormitusta edelsi lihasvärinä. Kaiken kaikkiaan nämä tulokset osoittavat, että 1) pitkäkestoinen ja äärimmäinen fyysinen kuormitus vaikuttaa vain vähän hormonitasojen palautumiseen, mutta pidentää merkittävästi veren rasvatasojen palautumista lähtötasolle. 2) Liikuttaessa kylmässä energianvalintamekanismit muuttuvat, kun energialähteiden saatavuuden ja käytön välillä on ristiriita. 3) Ennen kuormitusta aiheutettu matalaintensiteettinen lihasvärinä ei muokkaa ravintoaineiden valintaa submaksimaalisen työn aikana kylmässä. 4) Kudosten jäähdyttäminen saattaa heikentää luurankolihasten hapettumista submaksimaalisen työn alussa. 5) Kylmässä liikkuminen näyttää vähentävän inflammatorisia vasteita, kun taas lihasvärinä saattaa stimuloida kehon immunologista järjestelmää.

Avainsanat: Kylmäaltistus, lihasvärinä, energialähde, lihas, NIRS, hormonit, sytokiini

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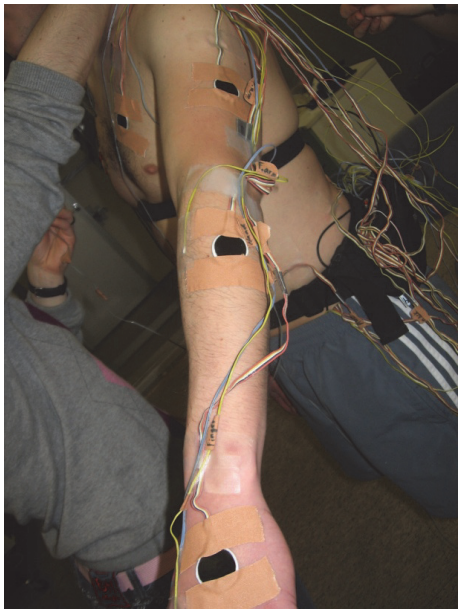
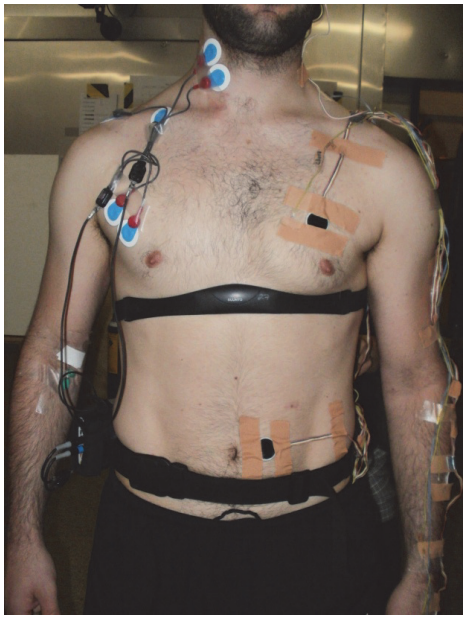
APPENDIX 1. Instrumentation pictures

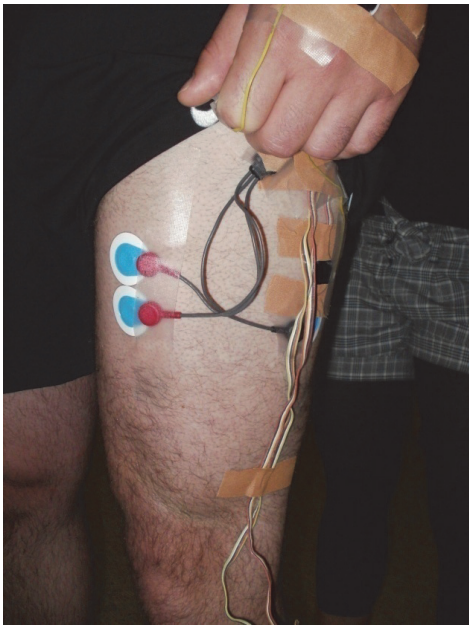
Lateral view of the arm view.



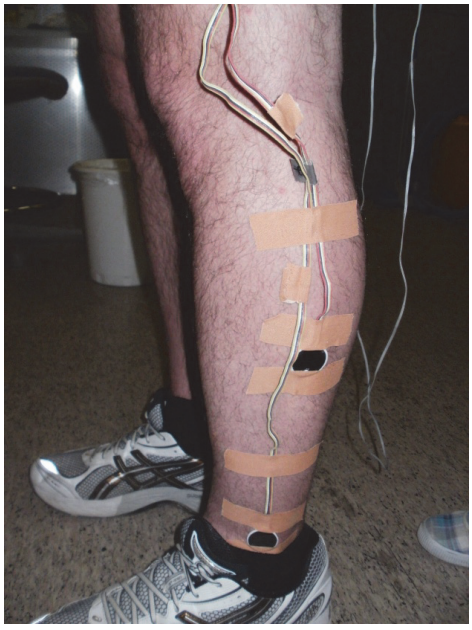
Frontal view of upper body.



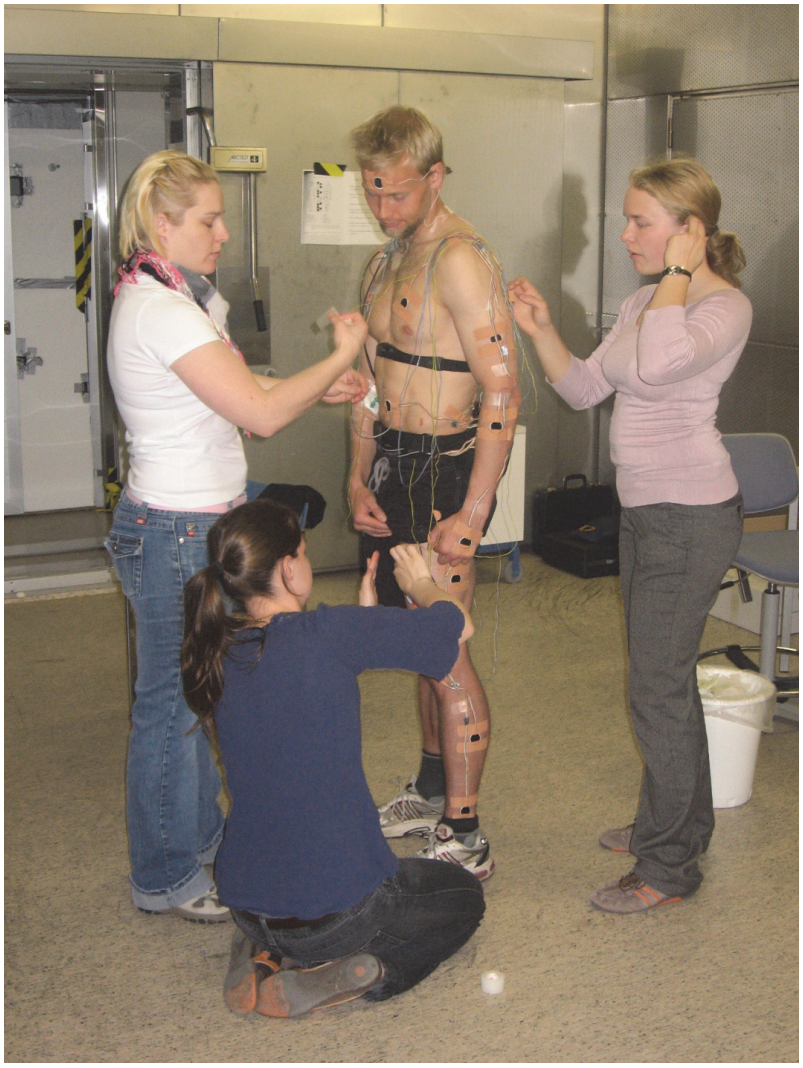
Lateral view of upper leg.



Lateral view of lower leg.

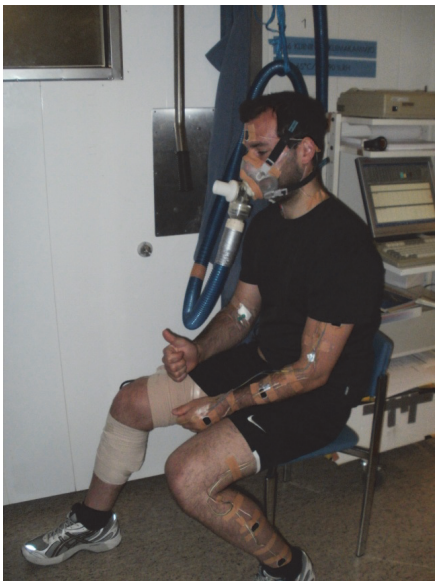


Subject being instrumented by research assistants in a climatic chamber.



APPENDIX 2: Protocol pictures

Fully instrumented subject during baseline.



Exercising subject.

Appendix 3: Oxygen consumption in all conditions.

		50% VO_{2peak} (LOW)			70% VO_{2peak} (MOD)		
		COLD	SHIV	NT	COLD	SHIV	NT
Oxygen Consumption (%)							
Baseline		8.9 (1.8)	9.3 (2.0)	6.6 (1.9)	8.1 (2.0)	8.0 (1.7)	8.4 (2.9)
Exercise	15 min	48.9 (8.8)	47.0 (2.7)	47.4 (5.0)	69.0 (6.7)	66.8 (4.6)	66.3 (7.1)
	30min	46.5 (5.4)	44.5 (3.7)	48.1 (3.3)	69.9 (6.5)	68.1 (0.7)	71.6 (5.0)
	45min	47.2 (3.7)	45.8 (2.7)	47.7 (3.6)	70.8 (6.3)	69.1 (3.0)	71.6 (6.2)
	60 min	47.8 (3.6)	45.7 (2.8)	48.3 (3.4)	70.6 (4.7)	69.7 (3.0)	70.5 (6.8)
Recovery	15 min	10.0 (3.0)	9.4 (1.8)	7.8 (1.6)	9.5 (1.6)	9.7 (1.6)	9.7 (1.8)
	30 min	9.2 (2.5)	9.6 (1.2)	7.4 (1.6)	8.6 (2.0)	9.1 (1.6)	9.2 (1.8)

Appendix 4: Pearson's correlation coefficients between cardiovascular, thermal, endocrine and immune response in all conditions.

Pearson's correlation coefficients from exercising in NT condition. HR, heart rate; t_{re}, rectal temperature; t_{skin}, skin temperature; Epi, epinephrine; NE, norepinephrine; TES_{bio}, bioavailable testosterone, ACTH, adenocorticotropic hormone; IGF-1, insulin-like growth factor-1; TSH, thyroid-stimulating hormone; T_3, triiodothyronine; T_{3free}, free triiodothyronine; T_{4free}, free thyroxine; IL, interleukin; FGF2, basic fibroblast growth factor; G-CSF; granulocyte colony-stimulating factor; IFN- γ; interferon gamma; IP-10, interferon gamma-induced protein 10; PDGF, platelet-derived growth factor; MCP-1, monocyte chemotactis protein-1; MIP-1β, macrophage inflammatory protein 1β; VEGF, vascular endothelial growth factor. NS, non-significant. Correlation coefficients are significant at $p < 0.05$.

	HR	T_{re}	T_{skin}	*Epi*	*NE*	TES_{tot}	TES_{bio}	*SHBG*	*ACTH*	*COR*	*IGF-1*	*TSH*	T_3	T_{3free}	T_{4free}
Leukocytes	0.689	NS	NS	NS	0.544	NS	0.518	NS	0.550	NS	NS	NS	NS	NS	0.529
Lymphocytes	NS	NS	NS	NS	0.580	NS	NS	-0.525	0.701	0.739	NS	0.644	NS	NS	NS
Granulocytes	0.601	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Monocytes	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-1ra	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-1β	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.590	NS	NS	NS	NS
IL-2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.516	NS	NS	NS
IL-5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-6	NS	NS	NS	NS	NS	-0.532	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-7	NS	NS	NS	NS	NS	-0.797	-0.842	NS	NS	NS	NS	NS	-0.758	-0.602	NS
IL-8	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-9	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.566	NS	NS	NS
IL-10	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-12	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.564	NS	NS	NS
IL-15	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-17	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Eotaxin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
FGF2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
G-CFS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.577	NS	NS	NS
IFN-γ	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.656	NS	NS	NS	NS
IP-10	NS	NS	0.689	NS	0.667	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PDGF	NS	NS	NS	NS	NS	NS	-0.842	NS	NS	NS	NS	NS	NS	NS	NS

MCP-1	NS		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.588	0.528	NS
MIP-1β	NS		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.520	NS	NS
Rantes	NS		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
VEGF	NS		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Pearson's correlation coefficients from exercising in COLD condition. See abbreviations above. NS, non-significant. Correlation coefficients are significant at $p < 0.05$.

	HR	T_{re}	T_{skin}	*Epi*	*NE*	TES_{tot}	TES_{bio}	*SHBG*	*ACTH*	*COR*	*IGF-1*	*TSH*	T_3	T_{3free}	T_{4free}
Leukocytes	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Lymphocytes	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Granulocytes	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Monocytes	NS	NS	NS	NS	NS	NS	0.499	NS	NS	NS	NS	NS	NS	NS	NS
IL-1ra	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-1β	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-6	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-7	NS	NS	NS	NS	NS	NS	-0.607	NS	NS	NS	NS	NS	-0.768	NS	NS
IL-8	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-9	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-10	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-12	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-15	NS	NS	NS	NS	0.531	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IL-17	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Eotaxin	NS	NS	0.513	NS	NS	0.532	NS	NS	NS	NS	NS	NS	NS	NS	0.586
FGF2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
G-CFS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IFN-γ	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.499	NS	NS	NS	NS
IP-10	NS	NS	NS	NS	NS	NS	NS	0.535	-0.601	NS	NS	NS	NS	NS	NS
PDGF	NS	NS	NS	NS	NS	0.635	NS	NS	NS	NS	NS	NS	NS	NS	0.554
MCP-1	NS	NS	NS	NS	NS	NS	0.678	NS	NS	NS	NS	NS	NS	NS	NS
MIP-1β	NS	NS	NS	0.514	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Rantes	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
VEGF	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS