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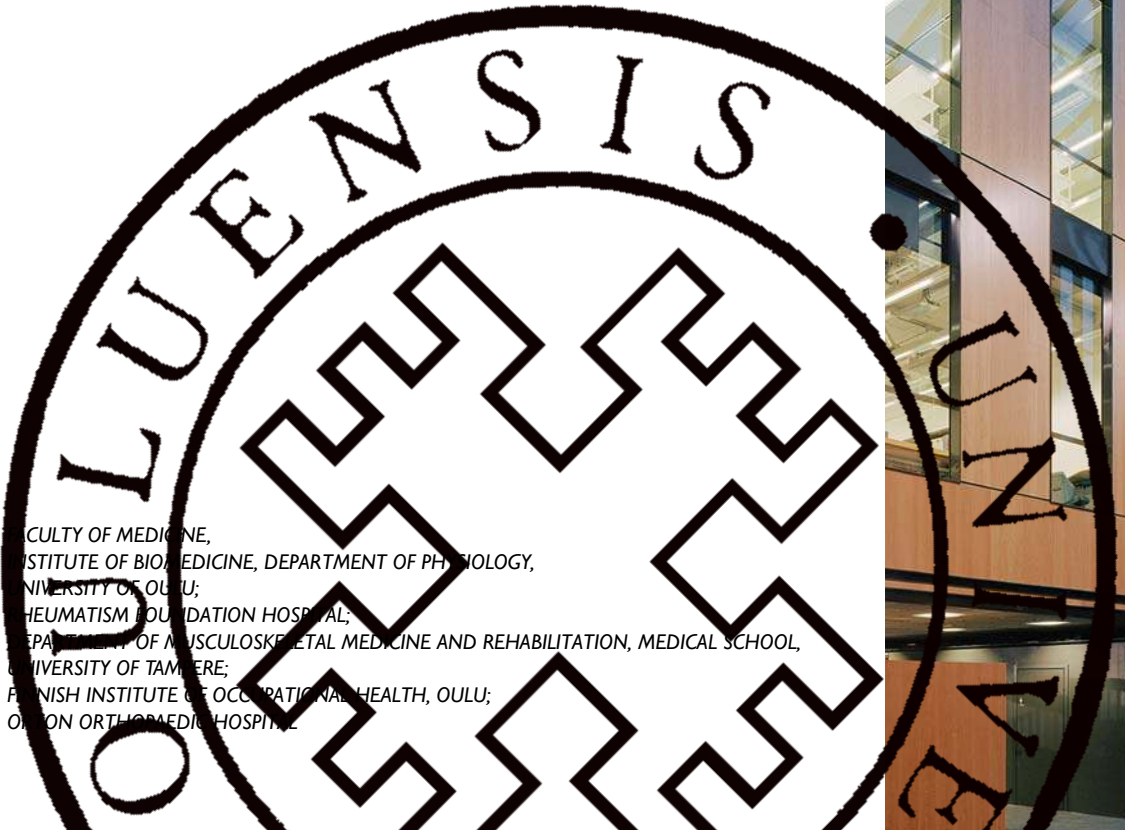
Tarja Westerlund

THERMAL, CIRCULATORY,
AND NEUROMUSCULAR
RESPONSES TO WHOLE-
BODY CRYOTHERAPY

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TARJA WESTERLUND

**THERMAL, CIRCULATORY, AND
NEUROMUSCULAR RESPONSES
TO WHOLE-BODY CRYOTHERAPY**

Academic dissertation to be presented, with the assent of the Faculty of Medicine of the University of Oulu, for public defence in the Auditorium of Kastelli Research Centre (Aapistie 1), on March 27th, 2009, at 12 noon

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Abstract

The purpose of this study was to examine thermal (body temperature, thermal sensation and comfort ratings), circulatory (blood pressure, heart rate variability) and neuromuscular performance responses to whole-body cryotherapy (WBC, -110 °C).

Altogether 66 healthy subjects were exposed to WBC for two minutes. The acute and long-term changes were examined, when the subjects were exposed to WBC three times a week during three months.

Skin temperatures decreased very rapidly during WBC, but remained such a high level that there was no risk for frostbites. The effects on rectal temperature were minimal. Repeated exposures to WBC were mostly well tolerated and comfortable and the subjects became habituated at an early stage of trials. WBC increased both systolic (24 mmHg) and diastolic (5 mmHg) blood pressures temporarily. Adaptation of blood pressure was not found during three months. The acute cooling-related increase in high-frequency power of RR-intervals indicated an increase in cardiac parasympathetic modulation, but after repeated WBC the increase was attenuated. The repeated WBC exposure-related increase in resting low frequency power of RR-intervals resembles the response observed related to exercise training. There are signs of neuromuscular adaptation, especially in dynamic performance. A single WBC decreased flight time in drop-jump exercise, but after repeated WBC these changes were almost vanished. This adaptation was confirmed by the change of the activity of the agonist muscle, which increased more and the change of the activity of antagonist muscle, which increased less/did not change after repeated WBC indicating reduced co-contraction and thus, neuromuscular adaptation.

Keywords: adaptation, blood pressure, body temperature, cold exposure, heart rate variability, muscular performance, thermal comfort, thermal sensation

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List of abbreviations

| | |
|------------------|---|
| A δ fiber | a delta fibre |
| ACTH | adrenocorticotrophin |
| ANOVA | analysis of variance |
| ATP | adenosinetriphosphate |
| BP | blood pressure (mmHg) |
| Ca ²⁺ | calcium |
| CI | confidence interval |
| CIVD | cold-induced vasodilatation |
| ECG | electrocardiography |
| EMG | electromyography |
| aEMG | averaged integrated electromyography (μ V) |
| FDI | first dorsal interosseus muscle |
| FEV1 | forced expiratory volume in 1s |
| FFT | fast fourier transform |
| HFP | high frequency power (ms ²) |
| HRV | heart rate variability |
| LFP | low frequency power (ms ²) |
| MAP | mean arterial pressure (mmHg) |
| MF | median frequency |
| MPF | mean power frequency (Hz) |
| MVC | maximal voluntary contraction |
| ns | non-significant |
| PEF | peak expiratory flow |
| pNN50 | NN50 count, divided by the total number of all NN intervals |
| RMSSD | the root mean square of successive differences in RR-intervals (ms) |
| RRI | R-R-interval (ms) |
| RT | rate of relaxation |
| SD | standard deviation |
| SD1 | instantaneous beat-to-beat variability |
| SD2 | continuous beat-to-beat variability |
| SE | standard error |
| TRAP | total peroxy radical trapping antioxidant capacity of plasma |
| TPT | time for the peak force level |
| WBC | whole-body cryotherapy |
| POST WBC | after whole-body cryotherapy |

PRE WBC before whole-body cryotherapy
WS winter swimming

List of original papers

The present thesis is based on the following papers, which are referred to in the text by their Roman numerals:

- I Westerlund T, Oksa J, Smolander J & Mikkelsen M (2003) Thermal responses during and after whole-body cryotherapy. *J Therm Biol* 28: 601–608.
- II Smolander J, Mikkelsen M, Oksa J, Westerlund T, Leppäluoto J & Huttunen P (2004) Thermal sensation and comfort in women exposed repeatedly to whole-body cryotherapy and winter swimming in ice-cold water. *Physiology & Behavior* 82: 691–695.
- III Westerlund T, Smolander J, Uusitalo-Koskinen A & Mikkelsen M (2004) The blood pressure responses to an acute and long-term whole-body cryotherapy (–110 °C) in men and women. *J Therm Biol* 29: 285–290.
- IV Westerlund T, Uusitalo A, Smolander J & Mikkelsen M (2006) Heart rate variability in women exposed to very cold air (–110 °C) during whole-body cryotherapy. *J Therm Biol* 31:342–346.
- V Westerlund T, Oksa J, Smolander J & Mikkelsen M Neuromuscular adaptation after repeated exposure to whole-body cryotherapy (–110 °C). *J Therm Biol*. In press.

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1 Introduction

Local cold therapy or cryotherapy has been used for centuries to relieve pain symptoms, particularly in inflammatory diseases, injuries and symptoms of overuse. The effects of local cryotherapy have accounted for cold-induced analgesia, a reduction in inflammation and a decrease of the temperature of the joint diminishing the activity of collagenases, which are cartilage destroying enzymes (Harris & McCroskery 1974). Based on these findings, local cryotherapy has been recommended every two to three hours in clinical use for patients with active arthritis.

Yamauchi *et al.* (1981) introduced one form of whole-body cold exposure: whole-body cryotherapy (WBC), which he had modelled for therapeutic purposes in the treatment of rheumatic diseases. WBC has been used in almost 80 places in Germany since 1984. In Finland, WBC began in 2000. In WBC, patients, in minimal clothing, are exposed to very cold air ($-110\text{ }^{\circ}\text{C}$) for a period of one to three minutes. It was originally used for the treatment of inflammatory rheumatic diseases, e.g. rheumatoid arthritis and ankylosing spondylarthritis, to alleviate inflammation and pain (Fricke 1989, Wichmann & Fricke 1997). It has later been used in sports medicine to treat injuries and syndromes of overuse (Zimmer & Zagrobelny, unpublished data, 2000). The performance-enhancing component of WBC is also being increasingly used in athletic sports. New resources are especially the economisation of the cardiovascular system and the utilisation of muscular effects (an increase in muscular perfusion, optimisation of the muscular metabolism, activation of motor units) due to WBC during athletic performance. (Papenfuß 2006). Despite the possible health benefits of WBC, little scientific information is available in literature. The scientific information is based mainly on pilot studies, which have only been published as abstracts or congress reports. Furthermore, only very limited data is available on the safety of WBC and no long-term effects have been reported.

Another form of whole-body cold exposure is winter swimming (WS), i.e. swimming or immersion in ice-cold water. WS is practised in northern countries, where seas, lakes, and rivers freeze during the wintertime. A rough estimate is that there are approximately 120 000 persons in Finland who swim regularly during the wintertime. The reported reasons for WS include an improved general well-being, and self-treatment or body hardening against respiratory tract infections and musculoskeletal pains (Kauppinen & Urponen 1988).

WBC and WS are probably the most severe ambient cold exposures, which are voluntarily practised by lightly dressed humans. Despite such a harsh temperature as -110°C , no health threatening effects have occurred during the eight years of use of WBC in the Rheumatism Foundation Hospital. WBC is not permitted for patients suffering from uncontrolled hypertension, serious coronary disease, arrhythmia, circulatory disorders, Raynaud's phenomenon (white fingers), cold allergies, serious pulmonary disease, or the obstruction of the bronchus caused by the cold. In a randomised controlled trial, Hirvonen *et al.* (2006) reported some adverse effects of WBC, such as respiratory infection, hypertension, urticaria, a long lasting feeling of coldness and malaise. The profile of the side-effects fits well with the clinical experience in the Rheumatism Foundation Hospital and previous reports on WBC (Taghawinejad *et al.* 1989a, Metzger *et al.* 2000).

This review and the summary of five research articles focus on the acute and long-term physiological responses to WBC in healthy volunteers. The primary aim was to locate physiological data, to ensure the safety of WBC for patients and personnel, and also to obtain new data for the basis of the WBC treatment.

2 Review of the literature

The present review concentrates on those aspects which are examined in the separate research articles. However, the studies carried out on WBC have been reviewed in a greater detail.

2.1 Human thermo-regulation

2.1.1 Thermal factors and human thermal balance

The optimal adjustment to a given thermal environment requires that a balance is created and maintained between the metabolic energy produced in the body and the energy that is given off to the environment in terms of heat and mechanical energy. The heat balance is determined by the interaction of climatic factors (air temperature, air humidity, radiation, air velocity), and the activity and clothing of the individual. These six thermal factors dictate the net heat exchange with the environment. Imbalances result in heat either being stored in the body tissues or lost from the tissues.

The human thermo-regulatory system attempts to maintain a constant core temperature (a narrow range around 37 °C; the thermo-neutral zone) and thermal balance. As the core temperature may be very stable despite varying ambient thermal conditions, skin temperatures can vary greatly particularly in the cold.

The heat balance can be described by the following mathematical expression:

$$S = M - (\pm W) \pm E \pm R \pm C \pm K \quad (\text{W/m}^2),$$

where S = the storage of body heat, M = metabolic heat production, W = energy leaving (positive) or entering (negative) the body as external work, E = evaporative heat transfer, R = radiative heat transfer, C = convective heat transfer and K = conductive heat transfer. Additionally, respiratory heat exchange occurs by evaporation (humidifying), and convection (warming of the exhaled air).

The body loses heat by convection, radiation, conduction and evaporation. Convection is dependent on the temperature gradient between the body and the ambient air, and on the air velocity. When standing still in the cold, warm air near to the skin is less dense and flows upwards (free convection). Wind and/or body motions (forced convection) may also ventilate the air layer. Radiation is dependent on the gradient between the body surface and the environmental mean radiant temperature (the temperatures and emissivity of the surfaces), and on the

effective radiation area of the body. Conductive heat transfer is the net rate of heat transfer in a solid material or a non-moving gas or fluid down a thermal gradient. Evaporative heat loss means the heat transfer from the body to the environment through the evaporation of water from the skin and surfaces of the respiratory tract. The main avenues for heat loss in cold air are convection and radiation, whilst the main avenues for heat loss in cold water are convection and conduction (Parsons 2003).

2.1.2 Thermo-reception

The thermo-regulatory system works in a similar way as a thermostat and the temperature regulation is controlled primarily by the hypothalamus. There are two types of thermal sensors/receptors located in the hypothalamus and in the human skin: warm and cold receptors. In the skin, these receptors are free nerve endings. Cold sensors lie closer to the surface than heat receptors. Cold receptors are the endings of the A δ -nerve fibres of the peripheral nervous system. Afferent signals from the peripheral nervous system are transferred by these A δ myelinated fibres to the hypothalamus. At a constant temperature, the receptors have a static discharge. The receptors will increase their firing rate if the temperature changes. When the skin temperature falls, the discharge rate of the cold sensors increases (Papenfuß 2006).

2.1.3 Thermoregulatory responses to cold

When humans are exposed to a cold environment, stimulation of the cold receptors causes cutaneous vasoconstriction. Vasoconstriction begins when the mean skin temperature falls below 34–35 °C (Savage & Brengelmann 1996) and becomes maximal, when the mean skin temperature is about 31 °C or less during whole-body water immersion (Veicsteinas *et al.* 1982) or 26 to 28 °C during localised cooling (Charkoudian *et al.* 1999). The vasoconstrictor response reduces blood flow to the skin and hence, reduces internal core-to-skin convective heat transfer and heat loss. Thus, the vasoconstrictor response to cold exposure protects the core temperature when skin and muscle temperatures are decreasing (Castellani *et al.* 2006).

During a cold exposure, two phases regarding heat production may be found. First, non-shivering thermogenesis will increase heat production by increasing muscle tension, stiffness and metabolism. Second, shivering thermogenesis

begins after a significant loss of body heat. The benefit of shivering can be up to five to six times above the resting levels of the metabolic rate (Keatinge *et al.* 1986). Shivering, which consists of involuntary, repeated, rhythmic muscle contractions may begin immediately or after several minutes of cold exposure. Shivering usually begins within the torso muscles and spreads to the limbs (Bell *et al.* 1992). As the shivering intensity increases and more muscles are involved, the whole-body oxygen uptake increases (Castellani *et al.* 2006).

2.1.4 Human factors affecting cold responses

Individual human factors, such as the amount of subcutaneous fat, body size, fitness level, gender and age affect thermal responses. Leaner subjects have higher skin temperatures and lower muscle temperatures after whole-body cooling than subjects with more subcutaneous fat (Buskirk & Kollias 1969, Oksa *et al.* 1993, Keatinge 1961). Subcutaneous fat provides a relatively high thermal resistance (Toner & McArdle 1996) and persons who have a high fat % tend to maintain their core temperature more than lean people (Glickman-Weiss *et al.* 1999, McArdle *et al.* 1984, Toner & McArdle 1996). There is also some evidence that individuals with a fat % greater than 25% have a higher threshold for vasoconstriction, which enables them to limit their heat loss (Kasai *et al.* 2003). The body surface area-to-mass ratio can have a significant influence on body cooling. Individuals with large surface area-to-mass ratios experience a more rapid fall in core temperature (Buskirk *et al.* 1963, Sloan & Keatinge 1973). Physical fitness allows exercise for a longer period of time at a higher metabolic rate, which may contribute to maintaining a normal core temperature (Castellani *et al.* 2006). Females usually have a greater body fat content, a thicker subcutaneous fat layer, less muscle mass and a higher surface area-to-mass ratio than men (Tikusis *et al.* 2000). Therefore, gender differences are primarily attributable to differences in body composition and anthropometry. Older persons (> 60 yr) are less tolerant to the cold due to blunted physiological and behavioural responses to the cold (Castellani *et al.* 2006).

2.1.5 Human cold adaptation

Short-term (less than 1 h) intensive exposures to the cold, several times a week, will produce the most common form of cold adaptation i.e. habituation. Due to this, physiological responses to the cold become less pronounced than during the

unacclimatized state. Cold-habituated persons not only have blunted shivering and vasoconstrictor responses, but also have less frequent changes in core and skin temperatures during cold exposures. Repeated cold stress on humans leads to physiological adjustments, which can be characterised as 1) hypothermic 2) metabolic or 3) insulative acclimatization. Longer exposures (more than 8 h) on consecutive days over a fairly long period of time (more than two weeks) are required to induce hypothermic habituation, where reduced shivering and more pronounced declines in body temperatures have been found. The metabolic acclimatization is characterised by increased heat production while exposed to the cold. A metabolic pattern may develop a more pronounced increase in shivering or non-shivering thermogenesis. The insulative acclimatization is characterised by enhanced heat conservation mechanisms, in which cold exposure elicits a more pronounced decline in skin temperature and lower thermal conductance of the skin (Young 1996). Local adaptation particularly means the adaptation of the hands: well-adapted subjects have a higher circulation in the hands, as cold induced vasoconstriction is less than in subjects without cold adaptation (LeBlanc *et al.* 1960). Heat loss through the hands is high, but manual performance is good (LeBlanc 1975). Cold adaptation facilitates the Lewis reaction (1930): finger circulation is opened temporarily to prevent the freezing of the fingers during severe cold exposure (CIVD).

2.2 Acute and adaptive subjective and physiological responses to the cold

2.2.1 Thermal sensation and comfort in the cold

Thermal sensation refers to a personal feeling of temperature (warm, neutral, cold). Therefore, it is a sensory experience and a psychological phenomenon. However, it should be noted that a thermal sensation is not how a person would like to feel. Thermal sensation is neither an affective nor value judgement (Parsons 2003). Thermal comfort is defined as “a subjective indifference to the thermal environment” (IUPS Thermal Glossary 2001). According to Fanger (1970), whole-body thermal comfort is based on such facts that 1) the body is in a heat balance 2) the rate of sweat is within comfort limits 3) the mean skin temperature is within comfort limits and 4) there is no local thermal discomfort.

Repeated cold stress on humans, leads to physiological adjustments, which can be characterised as habituation and/or acclimation/acclimatization (Young 1996). The former presents a diminished response and the latter an enhanced or altered response to the cold stimulus. The perceptual and affective adjustments to repeated cold stress have, however, received less attention (Leppäluoto *et al.* 2001). Older studies, carried out during Antarctic expeditions, indicated that in similar outdoor weather conditions at the same level of activity and even with lighter clothing, the individuals felt warmer at the end of the expedition compared with the beginning (Goldsmith 1960, Palmai 1962). Later, in laboratory conditions, cold sensations were decreased through repeated whole-body exposure to cold air (Leppäluoto *et al.* 2001, Bruck *et al.* 1976) or cold water (Jansky *et al.* 1996). Leppäluoto *et al.* (2001) found that both the general thermal sensations and the local thermal sensations became habituated after the first exposure. However, Mäkinen *et al.* (2005) reported (the cold-air exposures were quite similar to the study of Leppäluoto *et al.* 2001) that general thermal sensations became less intense through repeated whole-body exposure, but the local thermal sensations in the hands and feet did not change significantly.

2.2.2 Body temperatures in the cold

In order to maintain an optimal physiological function, the core temperature is regulated to remain around 37 °C. The temperature of superficial tissues varies as a function of thermo-regulation. Therefore, an intensive cold therapy/cold exposure can lead to a freezing of the skin, which can cause frostbite. Hands and feet have a high surface area-to-mass ratio, which makes them particularly susceptible to cold induced injuries (DeGroot *et al.* 2003). In the extremities, the initial sense of cooling begins at skin temperatures of around 28 °C and pain appears at around 20 °C (Heus *et al.* 1995). After cold-induced vasoconstriction, when the skin temperature falls below 12 °C, cold-induced vasodilatation (CIVD) occurs. During CIVD, blood flow to the extremities is increased and shows a cyclic change in skin temperature (O'Brien 2005). CIVD may reduce the risk of local cold injuries.

The first sign of frostbite is numbness, as the skin temperature falls below 10 °C (Provins & Morton 1960). Frostbite occurs when tissue temperatures fall below 0 °C. The freezing point of skin is slightly below the freezing point of water, due to the electrolyte content of the cells and extra-cellular fluid. The skin surface has been reported to freeze from -3.7 to -4.8 °C (Danielsson 1996,

Molnar *et al.* 1973, Wilson *et al.* 1976). Local skin temperatures (e.g. fingers, toes) can be used as “safety limits” upon exposure to cold environments (Parsons 2003).

If the ambient temperature is low enough, the core temperature may fall, and at that time there are practical consequences for the body in terms of health, comfort and performance. The core temperature can fall below 35 °C (hypothermia) relatively rapidly in cold water, but less rapidly in cold air with wet skin and windy circumstances. Such cooling is associated with a range of physiological and pathological responses and dysfunctions (Parsons 2003), which may eventually even lead to death.

2.2.3 Circulatory and respiratory responses to the cold

Acute exposure to a cold environment, either in air or water, causes a stress reaction, which may be a ‘cold shock’ in severe and sudden cases. These responses are under the control of the sympathetic and parasympathetic nervous systems (Finley *et al.* 1979, Heath & Downey 1990). The baroreflex function is vagally mediated and essential in the regulation of blood pressure and fast heart rate changes (Reid 1988).

Earlier works on the cardiovascular responses to cold have been performed using cold pressure tests by immersing the extremities in the cold water (Jansky *et al.* 2003, for review see LeBlanc *et al.* 1975), by exposing the face to wind or by applying ice packs to the face (LeBlanc *et al.* 1976). Facial cooling increases the blood pressure and results in bradycardia in resting subjects, whereas during a cold pressure test, both the heart rate and blood pressure increase (LeBlanc *et al.* 1975). Mourot *et al.* (2008) have found that during a cold pressure test, the heart rate decreased after an initial increase, which indicated the involvement of both the sympathetic and vagal outflow. However, half of the subjects only reacted with an increased heart rate. During a whole-body cold exposure, blood pressure increases markedly in normotensive, healthy persons (Leon *et al.* 1970, Emmett 1995, Gavhed *et al.* 2000, Korhonen 2006). An elevated blood pressure exerts a greater load on the heart and thus increases the cardiac demand for oxygen (Hanna *et al.* 1975, Kitamura *et al.* 1972). Increased blood pressure in cold conditions constitutes an increased risk for patients with cardiac diseases and may be a risk factor for certain diseases in healthy individuals who are regularly exposed to the cold (Lloyd 1991).

A sudden exposure to cold water or air (local or whole-body) may elicit several effects on the respiratory system, such as a gasp response, an increase in ventilation and bronchoconstriction (Keatinge & Nadel, 1965, Josenhans *et al.* 1969, Berk *et al.* 1987, Koskela & Tukiainen, 1995). At low levels of ventilation, stimulation of the face (the trigeminal area) seems to be the main trigger for airway narrowing to a similar degree in healthy subjects and in patients with obstructive lung disease (Berk *et al.* 1987, Koskela & Tukiainen 1995). At elevated levels of ventilation, the bronchoconstriction is much stronger in patients, possibly also due to the direct cooling or drying effect on airway mucosa (Berk *et al.* 1987).

The adaptation of resting blood pressure was seen in winter swimmers during one winter swimming season (Hirvonen *et al.* 2002). Furthermore, a seasonal influence on blood pressure has also been observed (Rose 1961, Brennan *et al.* 1982). The adaptation of lung functions in asthmatic patients was seen as a less pronounced reduction in FEV1 with repeated exposures after three months (Haas *et al.* 1986). The proposed mechanisms were bronchial vascular adaptation, direct airway desensitisation or sensory adaptations (reduced vagal activity). It should however be noted that the patients experienced reduced baseline FEV1 and exacerbation of symptoms indicating a possible airway injury (Haas *et al.* 1986).

2.2.4 Neuromuscular performance in the cold

Body cooling decreases muscular performance, especially during dynamic exercise (Bergh 1980, Davies & Young 1983, Sargeant 1987, Oksa *et al.* 1995). Already slightly lowered muscle temperature is able to deteriorate performance in dynamic exercises (Oksa *et al.* 1997). Some studies (Bawa *et al.* 1987, Oksa *et al.* 1995, 1997) have found that after cooling the co-ordination of muscle contraction changes. Bawa *et al.* (1987) found that the antagonist muscle co-contracted together with the agonist muscle in subjects who exercised and shivered at the same time. Thus, these antagonistic muscles were “fighting” against each other. This occurred only when the subjects were shivering, but not when the subjects were in a thermo-neutral state. Oksa *et al.* (1995, 1997) have reported that during a concentric muscle contraction in a drop-jump (shortening phase) the activity of the agonist muscle decreases and at the same time the activity of the antagonist muscle increases. This phenomenon, named as the ‘braking effect of the muscles due to cooling’, leads to a decreased muscular performance. Static exercise, however, is less sensitive to cooling. It seems that

muscle temperatures below 27 °C are needed before significant changes may be observed (Barnes 1983, Davies *et al.* 1982, Oliver *et al.* 1979).

There are only a few studies regarding the effects of repeated cold exposures on neuromuscular functions. Geurts *et al.* (2005) investigated the effects of cold acclimation on the neuromuscular function of the hand. In their study, the subjects immersed one hand in 8 °C water for 30 minutes, 5 days a week for three weeks. On the first and the last day, the evoked and voluntary force measurements of the first dorsal interosseus muscle (FDI) were performed before and after immersion. Likewise Geurts *et al.* (2006) examined the effects of repeated cold exposure (2 weeks, daily) with an elevated core temperature from exercise (cycling) on the neuromuscular function of the hand. In their studies on the effects of local cold exposure (Geurts *et al.* 2005, 2006), no adaptation in the neuromuscular function was found. Mäkinen *et al.* (2005) investigated how acute and repeated exposures (10 days) to the cold affect the whole body postural control. Sway was assessed at 10 °C after 90 minutes of exposure and as a control at 25 °C. The postural control was impaired in the cold. Repeated exposures decreased the sway by 10–40%, which may be explained by motor learning.

2.3 The physiological and treatment effects of whole-body cryotherapy

2.3.1 Body temperatures in WBC

One purpose of cold therapy is to reduce pain. Cold-induced analgesia is achieved by lowering the skin temperature. The attainment of a clinically optimal physiologic response by using cold therapy requires the skin tissue to be cooled effectively. A number of studies have been performed to establish the critical level of tissue cooling required for specific effects. Localised analgesia requires a skin temperature that is below 13.6 °C, and the explanation for this analgesic effect is that the cold reduces the nerve conduction velocity (Bugaj, 1975).

During WBC, the skin temperature decreases rapidly due to vasoconstriction and direct skin cooling, most profoundly in the extremities. It should be noted that at an extremely cold ambient temperature of –110 °C, frostbite might be anticipated to occur. However, only very limited data is available in regards to actual body temperatures. Taghawinejad *et al.* (1989a) registered a slight decrease (0.38 °C) in oral temperature after WBC (–100 °C, 90 s) and Joch *et al.* (2002)

found that the ear canal temperature changed by 0–0.2 °C from the initial temperature. Savalli *et al.* (2006) observed a decrease (0.63 °C) in ear temperature after 5 minutes of WBC (–110 °C, 4 minutes), but the change was not significant after 20 minutes. The lowest skin temperatures (9.0 °C) were measured on the calf (Savalli *et al.* 2006). According to these few studies, the effect of WBC on the core temperature seems to be minimal. To the best of our knowledge, data related to changes in skin temperatures during or after WBC are lacking.

2.3.2 Circulatory and respiratory responses to WBC

Heart rate. Fricke (1989) has reported an increase in the heart rate by 5–8 beats/min in WBC (30 s to 3 minutes). The subjects were patients with rheumatoid arthritis, who had normal blood pressure. Taghawinejad *et al.* (1989b) found that with healthy subjects, the heart rate increased during WBC by 25 beats/min and after WBC by 13 beats/min and in patients (coronary heart disease, arrhythmias, hypertension) 24 beats/min and 13 beats/min, respectively. In patients (under 70 years) the heart rate after WBC increased by 7 beats/min and in patients (over 70 years) 16 beats/min (Taghawinejad *et al.* 1989a). Details of the methods used in these studies were not available.

Blood pressure. Scant data is available on the effects of WBC on blood pressure, and no long-term effects have been reported. Fricke (1989), Taghawinejad *et al.* (1989a) and Taghawinejad *et al.* (1989b) have reported that systolic blood pressure increased by 9–10 mmHg immediately after WBC, compared with the values before the WBC. In their studies, the exposure time was from 30 s to 3 min (Fricke, 1989; Taghawinejad *et al.*, 1989a) and from 30 s to 90 s (Taghawinejad *et al.* 1989b). Fricke (1989) found that diastolic blood pressure increased by 10 mmHg. Taghawinejad *et al.* (1989a) found that it increased by 5 mmHg after WBC, compared with the values before WBC. However, Taghawinejad *et al.* (1989b) reported no change in the diastolic blood pressure measured after WBC. Zagrobelny *et al.* (1992) have observed that WBC does not affect the heart rate or arterial blood pressure in patients with rheumatoid arthritis. They were treated for 14 days (once a day) with temperatures ranging from –110 degrees °C to –160 degrees °C. Therefore, WBC has been found to influence blood pressure only by a small extent (Fricke 1989, Taghawinejad *et al.* 1989a, b, Zagrobelny *et al.* 1992), when the measurements have been done post-exposure.

No adverse ECG changes have been registered in either patients with stenocardia complaints or arrhythmias or in healthy subjects (Taghawinejad *et al.* 1989b). Neither did the WBC cause arrhythmias or ischemic changes of the heart (Zagrobelny *et al.* 1992).

Precooling (WBC) responses during exercise. Joch *et al.* (2005) investigated how the heart rate behaves in endurance exercise, with and without pre-cooling (WBC). In their study, the heart rate was examined over a five minutes rest period in which the subjects were seated. The subjects were then exposed to WBC (2.5 min), after which there was an additional five minute rest period. The endurance test (ergometer) was followed. The loads were 250 W and 150 W of alternate 2 min phases over 26 min, whilst the blood lactate and heart rate variability (HRV) were measured. The main finding was that after pre-cooling, the heart rate was significantly lower (7–11 beats/min) at rest and throughout the entire 26 min test period than in the condition without pre-cooling. After pre-cooling, the lactate values were also lower at exercise. All of the tested time-domain parameters of HRV (RRI, SD, SD1, SD2, RMSSD, pNN50) were significantly higher after pre-cooling during high-load phases of exercise, compared with the condition without pre-cooling. This finding indicates that parasympathetic activity has increased due to the pre-cooling. In literature, it is assumed that ca 75% of energy is required for cooling the biological system during exercise and only 25% is available for actual muscular activity (Marsh & Sleivert 1999). Joch *et al.* (2005) speculated that this ratio may improve during endurance exercise due to pre-cooling. The researchers also pointed out that cooling may be applied during endurance training to aid recovery (when two training elements must be performed in one day). This would allow either training at a higher intensity or training at the usual intensity with a reduced stress.

Respiratory responses. Yamauchi (1988) has described that very intense cold exposures (up to -175°C), for several weeks in Japan, improved the lung function of asthmatic patients. A transient bronchodilatory effect has been found by Engel *et al.* (1989). In their study, the PEF in rheumatic patients and healthy subjects increased significantly immediately after WBC (with a mask) compared with pre-exposure values. After 3 min, the values were at the baseline level. The study by Smolander *et al.* (2006) did not support the hypothesis that the WBC improves lung functions. At 2 and 30 min, after WBC at all time points (4, 8, 12 weeks), the PEF values were slightly lower when compared with the values before the WBC (Smolander *et al.* 2006). According to this study, the WBC induced minor bronchoconstriction in healthy humans. However, the changes in

lung functions were only minor, and therefore the WBC did not seem to be harmful for lung functions.

2.3.3 Neuromuscular performance and WBC

Information on the effects that WBC has on neuromuscular functions is very limited. Fricke *et al.* (1999) have reported that the isokinetic torque of the knee flexors and extensors improved after WBC. Furthermore, they observed that after WBC, a five minutes pause caused the greatest increase in muscle strength, which can be upkept for the most effective time for conditioning in sporting applications. Later, Fricke *et al.* (2000) reported that after WBC, sprint performances (5 m, 10 m, 15 m) improved. However, these studies have only been published as congress reports and the details of the experiments are unavailable.

2.3.4 Hormonal and antioxidant responses to WBC

In order to understand the mechanisms of the effects of WBC, results related to humoral responses to pain, stress and inflammation have also been reported (Birwe *et al.* 1989, Samborski *et al.* 1992b, Gutenbrunner *et al.* 1999, Metzger *et al.* 2000). After a single therapy session, pro-opiomelanocortin related hormones, ACTH and beta-endorphin, and the adrenal hormones epinephrine and cortisol remained unchanged or decreased. On the other hand, plasma norepinephrine showed a clear increase after the exposure (Stratz *et al.* 1991). A more intensive WBC (from -110 to -160 °C, every day for 2 weeks) has been shown to increase plasma ACTH, beta-endorphin and cortisol in arthritis patients (Zagrobelny *et al.* 1992). After WBC and WS, plasma norepinephrine increased significantly each time for 12 weeks (Leppäluoto *et al.* 2008). However, there was no stimulation in the traditional stress hormones such as epinephrine, ACTH, beta-endorphin and cortisol or in pro-inflammatory cytokines.

Dugué *et al.* (2005) have studied the effects of WBC and WS on the total peroxyl radical trapping antioxidant capacity of plasma (TRAP) over 12 weeks in healthy women. From a physiological point of view, experiencing acute cold temperatures on a regular basis for a period of 12 weeks represents an obvious stress, which may lead to some adaptive mechanisms. This is one of the tentative explanations of increasing the body tolerance after cold treatment. Surprisingly, researchers observed a significant increase – and not a decrease, as expected- in the values of TRAP after acute cold stimuli in both groups at the beginning of the

period of adaptation (during the first 4 weeks). However, all of the changes due to WBC and WS were relatively mild (< 5%). After 4 weeks, no changes in the TRAP values after the cold exposures were noticed and no long-term changes in the basal TRAP values were observed. These striking results are at variance with those of Siems *et al.* (1992, 1994, 1999). Siems *et al.* (1992, 1994, 1999) have shown that acute WS in ice-cold water for a period of 1 to 5 minutes leads to oxidative stress in experienced winter swimmers. However, the winter swimmers had significantly higher baseline values of reducing enzymes in erythrocytes compared with the control subjects (Siems *et al.* 1994). They postulated that this improved antioxidant protection was the result of repeated exposure to non-damaging, mild, oxidative stress.

2.3.5 Treatment effects of WBC

WBC is primarily used to alleviate inflammation, swelling and pain in, e.g., arthritis (Fricke 1989, Wichmann & Fricke 1997) and osteoarthritis (Metzger *et al.* 2000) and to reduce spasticity in clinics for some neurological diseases. A painless period of up to two hours has been observed following one visit to WBC (Metzger *et al.* 2000). A decreased duration of morning stiffness, a feeling of better general health, a decrease in disease activity and an increased pain threshold have been reported in rheumatic patients (Birwe *et al.* 1989, Wichmann & Fricke 1997, Fakhari *et al.* 2000, Ksiezopolska-Pietrzak *et al.* 2000). In a randomised controlled trial, Hirvonen *et al.* (2006) reported that WBC (-110 °C) seems to relieve pain more effectively than other cryotherapies (WBC -60 °C, local cold air -30 °C, cold packs locally) in regards to active rheumatoid arthritis. Disease activity decreased slightly, but there were no significant differences between the therapy groups. Swelling did not change significantly in any therapy groups. Cholewka *et al.* (2006) have used infrared imaging before and after WBC, in patients suffering from low back pain. Thermograms facilitate an accurate localisation of inflammatory and degenerative states. Infrared imaging correlates well with the clinical assessment of the severity of the inflammation (De Silva *et al.* 1986). Infrared imaging revealed a slight decrease in the inflammatory states after 10 exposures to WBC (Cholewka *et al.* 2006). Birwe *et al.* (1989) have reported an improvement in joint functions after WBC. WBC is also used for pain relief in fibromyalgia (Samborski *et al.* 1992). It may have a further sedative effect on psoriasis and neurodermatitis (Fricke 1989). Experience has shown that lung functions have improved in asthmatics after WBC (Fricke

1989). It is believed to positively influence the mental state of patients (Rymaszewska *et al.* 2007) and alleviate symptoms of depression (Rymaszewska *et al.* 2003). In Poland, WBC has been used in sports medicine to treat injuries and syndromes of overuse (Zimmer & Zagrobelny, unpublished data, 2000). However, it should be pointed out that the studies of WBC have not been properly randomised or randomised at all. A portion of them have only been published as abstracts or congress reports and details of these experiments are unavailable.

Savalli *et al.* (2005) have been interested in the effect of WBC on sports traumatology. All subjects had an orthopaedic surgical intervention after which they had experienced a rehabilitation programme. WBC (ca 22 exposures) was included in the rehabilitation programme. The subjects filled out a satisfaction questionnaire. 81% of the subjects were satisfied or very satisfied with the reduction in pain caused by WBC. 86% of them stated that they were satisfied or extremely satisfied with the impact that WBC had on their physical condition, 44% on their sleep and 86% on the recovery of their muscular efforts.

A summary of the studies examining the effects of WBC is given in Table A1 Appendix 1.

2.4 Cold-sensitive diseases

It is known that people with chronic and cold-sensitive diseases are at special risk for exacerbations or complications of their diseases, when exposed to the cold. In most of these conditions, the underlying mechanism is believed to be primarily vascular (Caplan 1999). Patients suffering from cardiovascular diseases, especially arterial hypertension and coronary heart disease, are prone to coronary artery spasm in response to the cold (Houdas *et al.* 1992). In Finland, some 3500 extra deaths occur in winter, of which 900 are certified as being due to coronary heart disease and 500 to strokes (Näyhä 2002). In Raynaud's phenomenon, the blood vessels constrict and the blood supply to these areas is reduced when exposed to the cold. The vasospasm results in several changes in skin colour, from white (ischemic) to blue (cyanotic) and finally to red (hyperaemic) (Caplan 1999). Therefore, patients with Raynaud's phenomenon are most susceptible to frostbite in cold environments. In Finland, 11.6% of adults suffer from Raynaud's disease (Rytkönen *et al.* 2005). Cold-induced urticaria is a form of physical urticaria, where hives or large welts form on the skin after exposure to a cold stimulus. Such exposure may vary from local contact to whole-body exposure. The etiology of cold urticaria is usually unknown (idiopathic), but sometimes a disease or other

factors are associated with it (secondary) (Mahmoudi 2001). Hassi *et al.* (2000) has reported that the incidence of cold-induced urticaria is 4%. The respiratory responses commonly associated with sudden exposure to cold are gasping, an increase in ventilation and bronchoconstriction (Keatinge & Nadel, 1965; Josehans *et al.*, 1969; Berk *et al.*, 1987; Koskela & Tukiainen, 1995). A cold exposure may therefore be harmful for asthmatic patients (Haas *et al.* 1986).

3 The purpose of the study

It is known that cold exposure is a risk factor for injuries and illnesses. However, neither literature nor clinical experience has reported serious cold injuries after WBC. Therefore, the hypothesis was that the responses to a severe, short-term whole-body cold exposure are not harmful for healthy subjects.

The general aim of this thesis was to study the thermal, circulatory, and neuromuscular responses of WBC ($-110\text{ }^{\circ}\text{C}$), primarily to ensure the safety of WBC during short- or long-term use. More specifically, the aims of this thesis can be expressed as follows:

1. To evaluate the acute effects of a single WBC on rectal and skin temperatures.
2. To evaluate thermal sensation and thermal comfort ratings associated with single and repeated WBC (3 months).
3. To evaluate changes in blood pressure induced by an acute cold exposure ($-10\text{ }^{\circ}\text{C}$, $-60\text{ }^{\circ}\text{C}$, $-110\text{ }^{\circ}\text{C}$) and to find out whether repeated WBC causes adaptation in the blood pressure response.
4. To evaluate changes in HRV after a single WBC and to find out if repeated WBC causes adaptation in HRV responses.
5. To examine the acute effects of a single WBC on neuromuscular performance and to find out if repeated WBC is able to induce neuromuscular adaptation.

4 Material and methods

This chapter is a summary of the procedures and methods used, and further details can be found in the separate articles.

4.1 Subjects

The subjects were recruited to the experiments through announcements. All subjects underwent a medical examination and they were declared healthy and not under medication. They had not regularly practised either WBC or WS. They were moderately active and were advised to maintain the same physical activity level during the experiment as prior to it. Before participating, the experimental protocol was explained. Written consent from the subjects was obtained. The experimental protocol and procedures were approved by the Ethics Committee of the Päijät-Häme District. Table 1 shows the physical characteristics of the subjects in different studies.

Table 1. The physical characteristics of the subjects in different studies. Values are represented as means \pm SD. The same subjects participated in study II (WBC group), study III (group 1) and study IV.

| Studies I–V | Age (yr) | Height (cm) | Weight (kg) | BMI |
|--------------------------------|-------------|-------------|-------------|------------|
| Study I (n=10) | 48 \pm 9 | 163 \pm 8 | 67 \pm 10 | 25 \pm 3 |
| Study II (WBC group) | 38 \pm 3 | 167 \pm 7 | 68 \pm 14 | 24 \pm 3 |
| Study III (group1)* | | | | |
| Study IV, (n=10) | | | | |
| Study II (WS group) (n=10) | 39 \pm 2 | 166 \pm 6 | 67 \pm 9 | 24 \pm 3 |
| Study III (group 2)* (n=22) | 40 \pm 12 | 171 \pm 7 | 69 \pm 11 | 24 \pm 2 |
| Study V (n=14) | 33 \pm 9 | 174 \pm 9 | 73 \pm 12 | 24 \pm 2 |

* Group 1 participated in the study, which compared WBC and winter swimming

* Group 2 participated in the WBC study

4.2 Thermal exposures

WBC exposures took place in a specially built, temperature-controlled unit (Zimmer Elektromedizin, Germany), which consists of three rooms with at different temperatures (Fig. 1). The subjects passed through the first pre-room

($-10\text{ }^{\circ}\text{C}$) and the second pre-room ($-60\text{ }^{\circ}\text{C}$) before coming into the therapy-room ($-110\text{ }^{\circ}\text{C}$). Whilst in the therapy-room, the subjects were advised to slightly move their fingers and legs and to avoid holding their breath. The subjects wore a bathing suit, surgical mask, cap, gloves, socks and shoes.

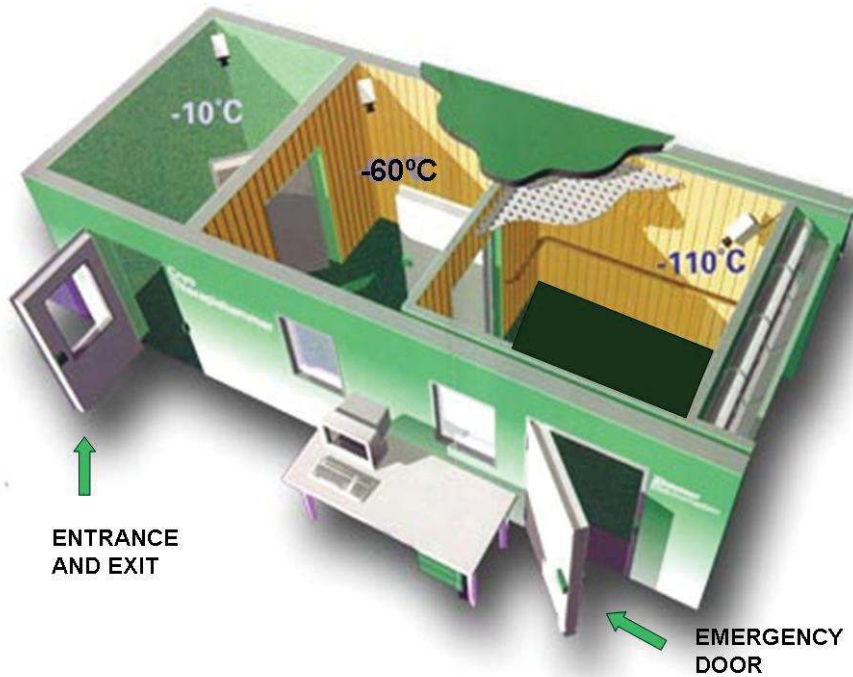


Fig. 1. Whole-body cryotherapy.

In study I, each subject was exposed once to WBC ($-110\text{ }^{\circ}\text{C}$) for a period of 2 minutes. During the 30-minute recovery period, the subjects sat at room temperature ($24\text{ }^{\circ}\text{C}$) and were allowed to dress themselves as warmly as they wished to avoid a subjective sensation of the cold.

In studies II, III, IV, V, the subjects had three 2 minutes periods of WBC exposures per week for three months. During the first visit, each subject was exposed to the first pre-room ($-10\text{ }^{\circ}\text{C}$), at the second visit to the second pre-room ($-60\text{ }^{\circ}\text{C}$) and at the third visit to the therapy-room ($-110\text{ }^{\circ}\text{C}$). After that, the subjects were exposed only to the therapy-room. In study II, the study design was the same for the WBC group, but there was a WS group, too. The WS group had three exposures per week for three months in a small pond in the hospital area.

The WS was performed without a sauna bath. During the first and second visit, the WS group was asked just to take a dip in the water. After that, the subjects were instructed to stay in the water for about 20 s immersed to the neck (“head-out immersion”). In order to do this, they were asked to count slowly from 1 to 20, while immersed in the water. The personnel of the WBC unit of the hospital organised the exposures for the WBC group, but the WS group carried out the exposures by themselves according to the instructions.

4.3 Measurements

4.3.1 Temperature measurements (I)

Rectal temperature (10 cm depth) (Yellow Springs Instrument, YSI 401) and local skin temperatures from nine sites (forehead, chest, lower back, upper arm, extensor side of lower arm, back of the hand, front thigh, calf and foot) were recorded at 5 s intervals with a data logger (Squirrel 1200, Grant, UK) during the WBC and recovery period. The data logger was kept in a foam-filled bag, the leads were well thermally insulated, and the probes were taped (Durapore™ 3M) to the skin. The mean skin temperature was calculated by weighting the nine local skin temperatures by representative skin areas (Mitchell & Wyndham 1969). The reference values for the rectal temperature and skin temperature are the values measured 5 minutes before beginning the WBC. During recovery, the difference between the reference value and the temperature measured at 30 minutes was analysed.

4.3.2 Thermal sensation and comfort (II)

The subjects rated their thermal sensation using a nine-point standard scale (ISO 10051 1995) before and immediately after WBC and WS (‘How are you feeling now?’ 4 = very hot, 3 = hot, 2 = warm, 1 = slightly warm, 0 = neutral, -1 = slightly cool, -2 = cool, -3 = cold, -4 = very cold). The thermal comfort was rated (ISO 10051) immediately after exposures using a five-point scale (‘Do you find this?’ 0 = comfortable, 1 = slightly uncomfortable, 2 = uncomfortable, 3 = very uncomfortable, 4 = extremely uncomfortable). In the dressing rooms, each subject of both the WBC group and the WS group had a diary where they

self-recorded the ratings. The subjects were instructed to relate their sensations to the time of reporting.

4.3.3 Blood pressure measurements (III)

Prior to the measurements, group 1 rested in the supine position for ten minutes, after which they sat for five minutes. Group 2 sat for five minutes or until their blood pressure readings were consistent.

Blood pressure measurements were taken during the first week, in connection with the visit at -10° , -60° and -110°C . After that, they were performed in connection with the visit at -110°C , once a month during three months. Blood pressure was measured just before the cold exposure and immediately after the exposure. The sitting position with the forearm supported in the horizontal position at heart level was used for the measurement. Blood pressure was measured from the right upper arm by the clinically validated automatic blood pressure monitor, Omron 711 Automatic IS (Omron Matsusaka Co. Ltd., Japan). The mean arterial pressure (MAP) was calculated as: $\text{MAP} = \text{diastolic BP} + 1/3 \times \text{pulse pressure}$.

4.3.4 Measurement of heart rate variability (IV)

The subjects first rested at room temperature (24°C) in the supine position for ten minutes. After the measurement, they sat for five minutes before being exposed to the WBC. After the WBC, the subjects rested in the supine position for 30 minutes at thermoneutral conditions, where the measurement was performed.

Both before (PRE WBC) and after (POST WBC) the WBC, a 5-minute supine rest HRV, after 5 minutes lying, was analysed. Additionally, from the two minutes recording during the WBC, a one minute HRV (DURING WBC) in a standing position was analysed. Maximal heart rate areas were not included in the analysis. The measurements were conducted after the first exposure to WBC, and then on monthly for the following three months (at month 1, 2 and 3).

R-R-intervals were measured continuously beat-by-beat with a heart rate monitor (Polar S 810) (Polar Electro, Kempele, Finland). Data transfer was performed with the Polar IR Interface to the Polar Precision Performance SW 3.0 analysis software. HRV was evaluated in time and frequency domains, with the parameters being the following: R-R interval (RRI), standard deviation (SD), the square root of the mean squared differences of RRI (RMSSD), low frequency

power (LFP) (0.04–0.15 Hz), high frequency power (HFP) (0.15–0.40 Hz) and LFP/HFP-ratio.

To study the acute effect of the WBC on HRV, the values of the heart rate variables at PRE WBC and POST WBC were compared. To evaluate adaptation to the WBC, the differences between PRE and POST WBC, the heart rate variables at the start of the experiment and after repeated WBC (3 times a week for 3 months = ‘cold training’) were compared. To evaluate the ‘cold training’ effect on supine resting HRV, the PRE WBC heart rate variables measured at the start of the experiment and at the three-month time point were compared. In addition, DURING WBC HRV at the start of the experiment and at the three-month time point was compared.

4.3.5 Neuromuscular performance (V)

The measurements of neuromuscular performance included a drop-jump exercise and the maximal voluntary contraction force of the wrist flexors (MVC), accompanied with electromyography (EMG) recordings in both. The measurements were performed just before and immediately after the WBC exposures. The single WBC measurements were carried out at the fourth exposure (at the second exposure to -110°C) and the repeated WBC measurements at the last exposure after three months.

Drop-jump exercise (stretch-shortening cycle). The drop-jump exercise was performed from a 40 cm bench. The subjects dropped from the bench two times onto a contact mat (Powertimer, Newtest Inc, Oulu, Finland) and performed an instantaneous maximal rebound jump keeping hands on hips. During contact with the ground, this stretch-shortening cycle performance consists of a stretching phase (downward movement), when *m. gastrocnemius medialis* is stretched and *m. tibialis anterior* is shortened and a shortening phase (upward movement) when *m. gastrocnemius medialis* is shortened and *m. tibialis anterior* is stretched. The subjects were instructed to perform the jump keeping their legs as straight as possible. Beforehand, the subjects thoroughly practised the drop-jumps with an instructor in order to avoid a learning effect during the measurements.

Maximal voluntary contraction (MVC). The subjects’ wrist flexion MVC of the right arm was measured in an upright seated position with their hip and elbow angles adjusted to 90° . The armrest of the seat supported their forearm. The subject held a static handle, so that the palm of the hand was in a vertical position. The subjects were asked to flex their wrist maximally, and as quickly as they

could immediately after a command, and to hold the force for ca. two seconds and to relax their wrist as rapidly as possible after a command. The subject performed two trials and the higher MVC value was used in the analysis. The maximal force level, TPT (the rise of force from resting level to 80% MVC), and RT (restoring the force from 80% MVC to resting level) were analysed from the MVC data.

EMG measurements

To evaluate the level of muscle activity during the drop-jump exercise and MVC surface EMG activity (ME3000P, Mega Electronics, Kuopio, Finland) was measured. EMG signals from the skin above the working muscles were acquired with a sample rate of 1000 Hz with the use of pre-gelled bipolar surface electrodes (M-OO-S, Medicotest, Denmark). The upper filter frequency was set to 500 Hz and the lower to 20 Hz. The measured signal was full wave rectified and averaged (aEMG) with a 10 ms time constant. The electrodes were placed over the belly of the muscle, and the distance between the recording contacts was 2 cm. Ground electrodes were attached above an inactive muscle. During the drop-jump, the activity of the *m. tibialis anterior* and *m. gastrocnemius medialis* was measured. The EMG measurement began with a signal that was given for the subjects to perform the drop-jump. An on-off connector was attached to the sole of the shoe, which gave a signal of the contact phase to the EMG measurement device. The averaged EMG level was analysed during the pre-activity phase (100 ms before the beginning of a stretch phase), and during the eccentric and concentric phase of the stretch-shortening cycle. The stretch-shortening cycle began from the signal of the contact phase and ended at the point where the activity of the *m. tibialis anterior* distinctly increased and the *m. gastrocnemius medialis* decreased. The total contact time was halved for the eccentric (stretch) and concentric (shortening) phases. During the MVC test the EMG activity was measured by the wrist flexor (*m. flexor carpi radialis*) and wrist extensor (*m. brachioradialis*). The averaged EMG was calculated during the total time of the MVC (ca. two seconds). To assess the frequency component of the EMG, the power spectrum was estimated by a moving Fast Fourier Transform (FFT window, 512 points). From the power spectra, the mean power frequency (MPF), median frequency (MF) and zero crossing rate (ZCR) were calculated to describe changes in the frequency component. MPF was chosen to represent the frequency component of the EMG results. Intra-individual comparisons of the mean averaged EMG and MPF results were made between the before and after WBC at

the beginning and at the end of the study. To ensure the accuracy of the relocation of the electrodes on the skin after three months, their locations for each subject were carefully drawn on plastic films with the aid of anatomic marks (e.g. moles, blood vessels) and their distances from the nearest joints were carefully measured with a tape-measure. The test-retest reproducibility in the EMG measurements, expressed as reliability coefficients, has been reported as being rather high: $r = .94$ for aEMG in jumping (Bosco 1982). Oksa (1998) has tested, in a cold environment, the reproducibility of the EMG-measurements during a drop jump exercise from the same muscle as used in this study *m. gastrocnemius medialis*. The results indicated that the coefficient of the variation was less than 7%.

4.4 Statistical methods

In study I, the temperatures are reported as means at 1 minute intervals before WBC, at 5 s intervals during WBC and up to 5 minutes after WBC, at 1 minutes intervals from 5 to 30 minutes after WBC. The mean changes of temperatures (95% confidence interval) were calculated from the reference values to temperatures measured at 30 minutes.

In study II, the data is presented in frequency histograms or as averages \pm SE. For the analysis, the values were averaged for each week (2–13) and compared with the first exposure. The differences in the thermal sensation and the comfort between the two cold exposure groups with time was analysed by the two-way analysis of variance (ANOVA) with repeated measures on time. In addition to the pooled data analysis, the results were separately analysed for both groups by the one-way ANOVA with repeated measures on time. Comparisons of different time points against the first exposure were performed using Duncan's test.

In study III, the mean changes in blood pressures and heart rates (95% confidence interval) were calculated as difference between before and after exposure values.

In study IV, the results were expressed as means and standard deviations (SD). The normality of the variables was evaluated by the Shapiro-Francia test with the Monte Carlo p-value. However, as variable values had a wide dispersion, bootstrap estimation was used to derive a 95% confidence interval; confidence intervals for the means were obtained by bias corrected bootstrapping (5000 replications) (Efron & Tibshirani 1993). Statistical comparisons of the changes in outcome measurements were performed by using a permutation test and

Hommel's adjustments were performed to correct the significance levels for the multiple test.

In study V, to compare differences in measured values between before and after the WBC separately for a time (0 or 3 months), analysis of variance (ANOVA) with repeated measures was used. In addition to, the Student's paired t-test was used to test differences between the after single and after repeated WBC. Statistical analyses were performed using SPSS 15.0 for Windows. The results of the studies II and V were considered as statistically significant, when $p < 0.05$.

5 Results

5.1 The acute effects of a single WBC

5.1.1 Thermal responses

Rectal and skin temperatures .The mean rectal temperature did not change during the 2 minute period of WBC. After WBC, there was a slight continuous decrease in rectal temperature. All skin temperatures decreased very rapidly during WBC. The lowest skin temperatures were observed on the forearm and on the calf (Fig. 2). The highest skin temperatures were recorded on the palm and on the foot, which were areas which were protected (gloves and socks). On unprotected areas, the highest skin temperature was recorded on the forehead. The mean skin temperature (SD) was 12.4 °C (1.8) during WBC. Immediately after WBC, all skin temperatures increased very rapidly for a couple of minutes, after which the increase was slower. None of the temperatures reached the reference values during recovery. The forehead skin temperature reached a plateau after 15 minutes, the upper arm values after 26 minutes, the lower arm values after 25 minutes and the foot values after 12 minutes. The values recorded at the other locations did not reach a plateau. No frostbite was found during and after WBC.

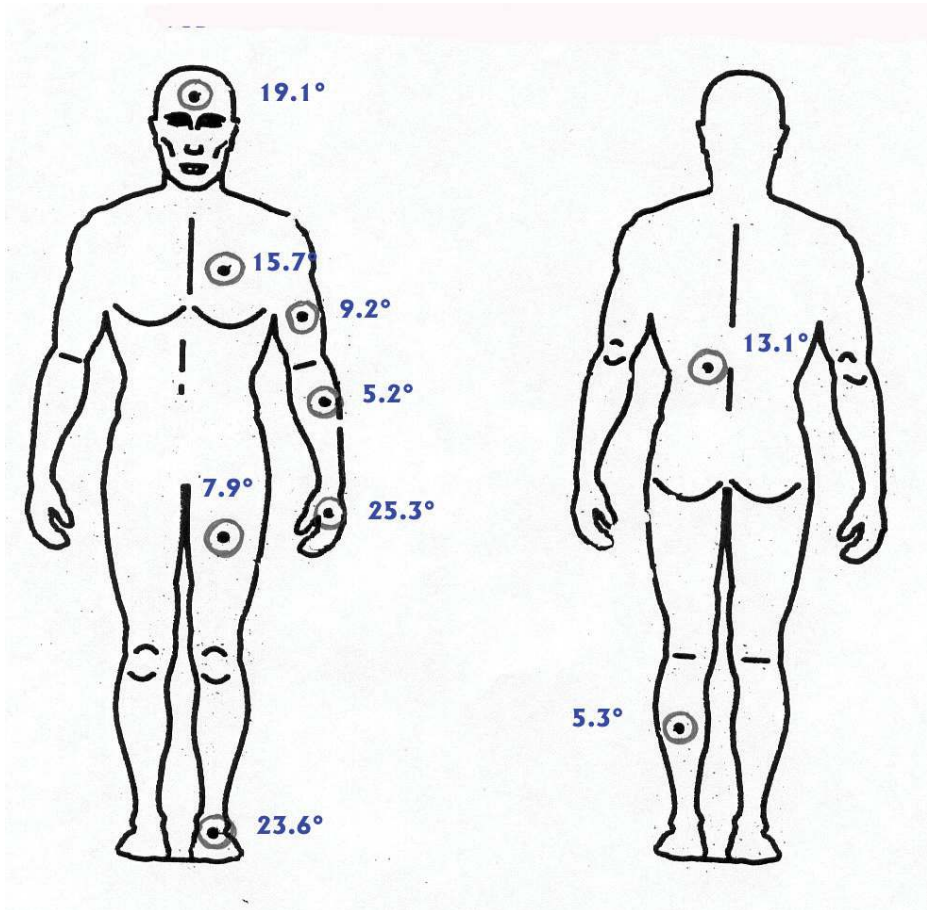


Fig. 2. The lowest skin temperatures (°C) during -110 °C.

Thermal sensation and comfort rating. Primarily, the thermal sensation before the WBC exposures was 'neutral' or higher (90.4%). The thermal sensation after WBC was in most cases (65.4%) 'neutral' or 'slightly cool'. Fewer exposures (16.2%) were cooler after WBC.

When comparing the WBC and the WS group, the WBC group felt cooler ($p < 0.05$ for the group factor) immediately after the exposures compared with the WS group.

The majority of exposures in the WBC group (98.2%) and in the WS group (92.8%) were rated as 'comfortable' or 'slightly uncomfortable'. Only one exposure in the WS group was rated as 'very uncomfortable'.

5.1.2 Blood pressure and heart rate variability responses

During the trials, the blood pressures at rest did not change. After each exposure (−10, −60, −110 °C), both systolic and diastolic blood pressures were on average significantly higher than the pre-exposure values (Table 2). Systolic blood pressure increased significantly more in connection with the −110 °C than with the −10 °C ($p < 0.01$) and −60 °C ($p < 0.001$) cold exposures. However, the increase was similar in connection with the −10 and −60 °C cold exposures. Diastolic blood pressure increased similarly during all of the cold exposures. The inter-individual variation was wide in the systolic and particularly in the diastolic blood pressure responses, and the variation tended to be largest in the coldest exposure. There were no differences in the response of MAP to the different cold exposures. MAP (SD) before and after −10 °C were 98 (8) and 106 (8) mmHg, before and after −60 °C they were 97 (7) and 104 (9) mmHg and before and after −110 °C 96 (8) and 107 (9) mmHg, respectively.

Table 2. Blood pressures at rest, before the cold exposures and the change from pre-exposure values.

| Temperature (°C) | Systolic (mmHg) | | Diastolic (mmHg) | |
|------------------|-----------------|---------------------|------------------|------------------|
| | Before | Change | Before | Change |
| | Mean (SD) | Mean (95% CI) | Mean (SD) | Mean (95% CI) |
| −10 | 130 (11) | 14.4 (10.4 to 18.4) | 81 (8) | 4.8 (1.7 to 7.9) |
| −60 | 129 (12) | 15.0 (11.1 to 19.0) | 81 (7) | 2.9 (0.5 to 5.3) |
| −110 | 128 (12) | 23.6 (19.6 to 27.6) | 81 (9) | 4.8 (1.7 to 7.9) |

The average blood pressure responses to the acute WBC were not significantly different between the men and women. However, wider variability was observed in the response of men to WBC compared with women, indicating that at an individual level some men had a more intense blood pressure reaction.

In an acute experiment, the RRI, the RMSSD and the HFP of RRI increased significantly from PRE WBC to POST WBC, but the LFP of RRI and the LFP/HFP-ratio did not change (Table 3).

Table 3. Changes in HRV induced by an acute cold exposure (−110°) during the first time and at 3 months.

| HRV | First time | | 3 months | |
|------------------------|-----------------------|--|-----------------------|--|
| | Baseline Mean (SD) | Change from baseline after the exposure Mean (95% CI)† | Baseline Mean (SD) | Change from baseline after the exposure Mean (95% CI)† |
| RRI (ms) | 852 (147) | 131 (103 to 160)*** | 890 (171) | 95 (52 to 138)** |
| SD (ms) | 53 (17) | 25 (12 to 34)* | 64 (12) | 16 (7 to 25) |
| RMSSD (ms) | 36 (16) | 19 (12 to 26)** | 46 (17) | 13 (7 to 18)** |
| LFP (ms ²) | 972 (505) | 804 (292 to 1331) | 1479 (583) | 302 (−232 to 837) |
| LFP (%) | 68 (17) | −4 (−11 to 4) | 67 (18) | −3 (−14 to 8) |
| HFP (ms ²) | 528 (388) | 572 (304 to 928)* | 773 (622) | 339 (52 to 625) |
| HFP (%) | 32 (17) | 8 (0 to 13) | 33 (18) | 3 (−8 to 14) |
| LFP/HFP | 340 (276) | −134 (−264 to −20) | 313 (234) | −102 (−229 to 23) |

RRI, R-R-interval; SD, standard deviation; RMSSD, the square root of the mean squared differences of RRI; LFP, low frequency power; HFP, high frequency power

†95% confidence interval obtained by bias corrected bootstrapping (100000 replications)

*p < 0.05, **p < 0.01, ***p < 0.001

5.2 Effects of repeated WBC

5.2.1 Thermal sensation and comfort ratings

In the WBC group, thermal sensation immediately after the exposures was significantly higher during each week (2–13), compared with the first exposure (Fig. 3). A further analysis of the first 10 exposures in the WBC group showed that a cold sensation was less intense already after the second cold exposure ($p < 0.05$). A similar habituation response was also observed in the WS group, although it was weaker.

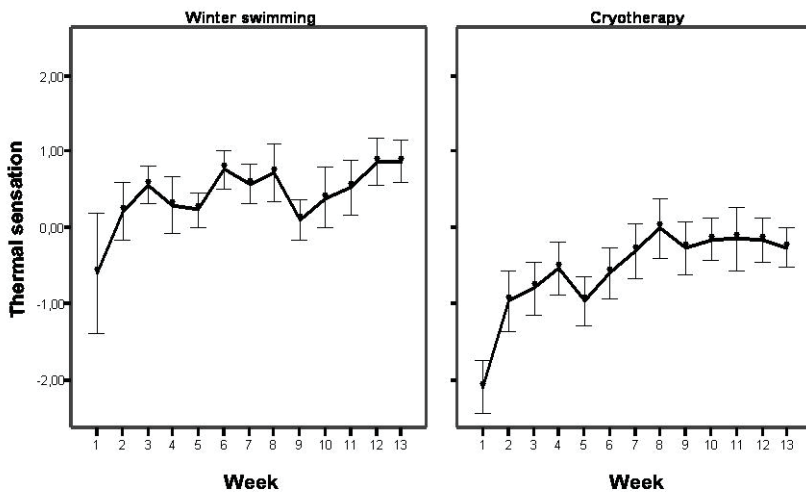


Fig. 3. The average (\pm SE) thermal sensation scores immediately after exposures during each week over 3 months in the whole-body cryotherapy and winter swimming group.

Analysis of the pooled data showed that thermal comfort, immediately after exposures, increased with successive weeks ($p > 0.05$), commencing already during the second week ($p < 0.01$). There was no difference between the WBC and WS groups.

5.2.2 Blood pressure and heart rate variability responses

The average response in systolic, diastolic and MAP was similar to the repeated $-110\text{ }^{\circ}\text{C}$ cold exposures during the three months. However, the individual blood pressure responses to the repeated WBC differed from the average results. 44% of the subjects had a decreasing trend in the systolic blood pressure response, 44% had a variable reaction or there was no change and 12% had a rising trend during the three months. The subjects with the most remarkable increases in systolic blood pressure after the first time at $-110\text{ }^{\circ}\text{C}$ reacted similarly or their trend was decreasing. In diastolic blood pressure, 50% of the subjects had a variable

reaction or there was no change, 28% had a decreasing trend and 22% had a rising trend. There were no significant differences in blood pressure responses to the long-term WBC between the men and women.

After the three month 'cold training', only the RRI and the RMSSD increased significantly from PRE WBC to POST WBC (Table 3). Initially, the RRI increased by 15.4% as a response to acute WBC, while after the three months, it increased only by 10.7%.

Repeated WBC exposures – for the three months – increased the PRE WBC LFP ($p < 0.05$), but the PRE WBC RRI, the RMSSD, the HFP and the LFP/HFP-ratio did not change. HRV during the WBC also showed no significant change during the three months.

5.2.3 Neuromuscular performance

Drop-jump exercise. After a single WBC, the flight time decreased significantly ($p < 0.05$). However, after repeated WBC, only a similar tendency was found (Fig. 4). During the shortening phase, the averaged EMG activity of the agonist muscle (*m. gastrocnemius medialis*) increased by $95 \pm 108 \mu\text{V}$ after a single WBC. The increase was $259 \pm 102 \mu\text{V}$ ($p < 0.05$) after repeated WBC. These changes differed significantly ($p < 0.05$) from each other. The averaged EMG activity of the antagonist muscle (*m. tibialis anterior*) after a single WBC increased by $26 \pm 25 \mu\text{V}$, but after repeated WBC it decreased by $2 \pm 14 \mu\text{V}$. However, these changes did not differ significantly from each other. Fig. 5 shows the percentile difference of these activities after single and repeated WBC. During the stretch phase, no significant changes were found in the averaged EMG of the agonist (*m. tibialis anterior*) and antagonist (*m. gastrocnemius medialis*) muscles, neither after a single nor repeated WBC. During the pre-activity phase after a single WBC, the averaged EMG activity of the agonist muscle increased by $66 \pm 57 \mu\text{V}$ and after repeated WBC by $99 \pm 27 \mu\text{V}$ ($p < 0.05$), but these changes did not differ significantly from each other. During the different phases of the drop-jump, no significant changes were found in the MPF of the agonist and antagonist muscles, neither after a single nor repeated WBC.

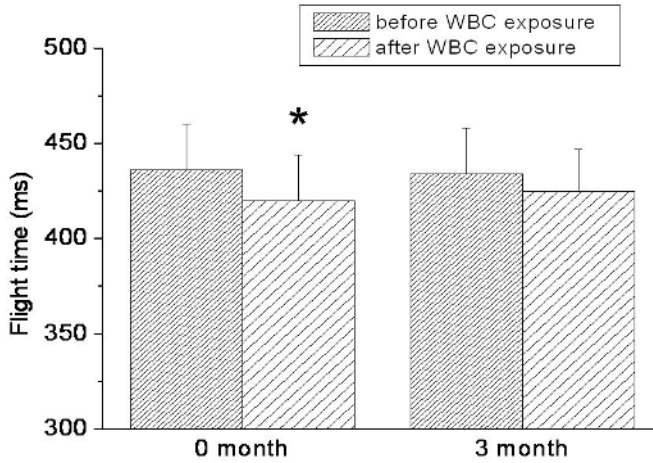


Fig. 4. The flight time of the drop-jump before and after WBC at the beginning (0 month) and at the end (3 month) of the three-month study period. The values are means (SE) and *denotes $p < 0.05$.

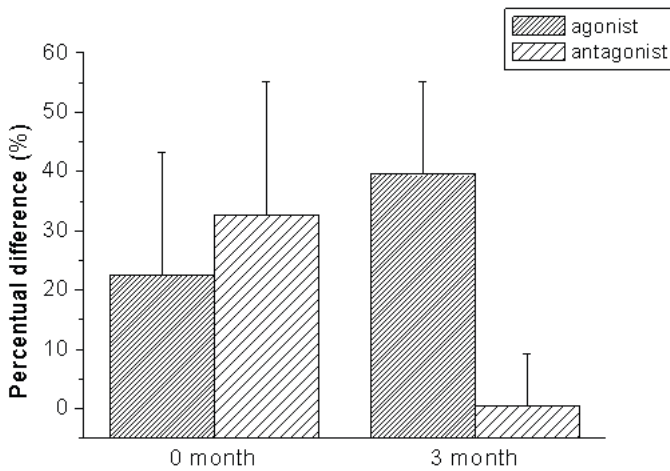


Fig. 5. The percentual difference of the averaged EMG-activity of the agonist (m. gastrocnemius medialis) and antagonist (m. tibialis anterior) muscle before and after WBC, during the shortening phase of the drop-jump at the beginning (0 month) and at the end (3 month) of the three-month study period. The values are means (SE).

MVC. The maximal force level, TPT and RT did not change significantly either after a single or after repeated WBC. However, the averaged EMG activity of the agonist after a single WBC increased only by $84 \pm 37 \mu\text{V}$, but after repeated WBC it increased by $188 \pm 60 \mu\text{V}$ ($p < 0.05$). The averaged EMG activity of the antagonist increased by $50 \pm 24 \mu\text{V}$ and $41 \pm 17 \mu\text{V}$ ($p < 0.05$), respectively. However, these changes did not differ significantly from each other. The percentual differences of these activities after single and repeated WBC are shown in Fig. 6. After a single WBC, the MPF of the agonist decreased by $18 \pm 3 \text{ Hz}$ ($p < 0.05$) and after repeated WBC it decreased by $14 \pm 4 \text{ Hz}$ ($p < 0.05$). There were no significant differences between the changes of the MPF at the beginning and at 3 months. The MPF of the antagonist showed no significant change.

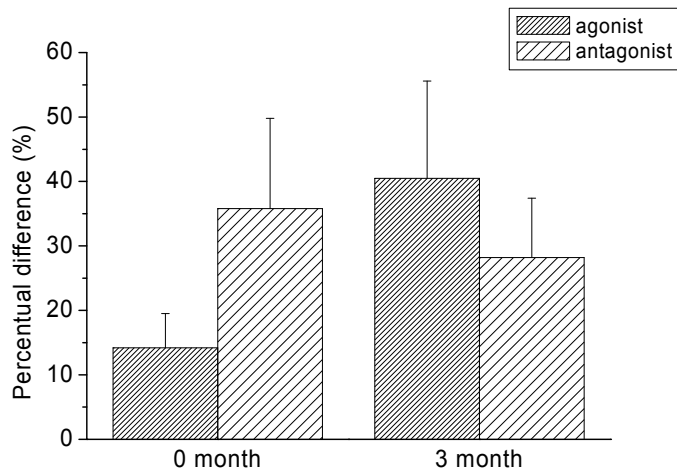


Fig. 6. The percentual difference of the averaged EMG-activity of the agonist (wrist flexor) and antagonist (wrist extensor) muscle, before and after WBC in the MVC. The values are means (SE).

6 Discussion

6.1 Methodological considerations

In study I, rectal and skin temperatures were measured during very cold air as -110°C . Therefore, a great deal of pilot tests had to be performed to ensure a maximal insulation of the probes. Previously, it has been found that if the temperature probes are in direct contact with cold air, they may yield a lower temperature reading compared with a similar probe that is well protected. Therefore, extra precautions were taken to ensure the maximal insulation of the probes, by taping them well to the skin. Further, to detect the cooling of the whole body, the skin temperatures were measured over the belly of the muscles and weighed by their representative areas (Mitchell and Wyndham 1969). We would have most probably found lower skin temperatures if they were measured over the joints and this could have resulted in a slightly lower mean skin temperature than found now. To measure the core temperature, rectal temperature measurements were chosen in this study, as it is more reliable than the oral temperature measurements, for instance, which Taghawinejad *et al.* (1989a) used. When oral temperature is used, ambient conditions may have a great influence on the result.

In study III, there were two groups with different protocols. However, there were no statistically significant differences in blood pressure responses between the two groups. Therefore, the results of the two groups were combined and analysed as a single population.

In study IV, there were few limitations. Breathing frequency was not measured, which is considered modulating the HFP. However, Stemper *et al.* (2002) have reported that bradycardia after cold exposure may also result from central vagal activation. Further, the effect of different phases of menstrual cycles was not controlled, but the repeated measurements were made every 4 weeks and so, individually, at about the same time of the cycle. Therefore, the effect of the menstrual cycle was probably minimal at an individual level. Furthermore, it should be noted that the maximal heart rate area DURING WBC was not included in the analysis, due to the technical reasons in considering HRV analysis.

All the WBC trials were controlled. In study II, the WBC was compared with the WS and the outdoor conditions were variable during the WS. The air and water surface temperatures were higher during the last weeks of the WS intervention.

In study V, the drop-jump exercise was chosen since it had been found to be very sensitive to body cooling (Oksa *et al.* 1997). The cooling of the skin and muscles as well as the electrodes may increase the possibility of methodological errors while using a surface EMG. The cooled tissues may act as a low pass-filter for the EMG signal (Winkel & Jørgensen 1991). However, since the changes in the EMG-activity of the agonist and antagonist muscles in both tests (drop-jump, MVC) did not change in a similar manner, the observed changes can not be explained by the methodological effects of cooling on the EMG.

Finally, it should be pointed out that the results of all these studies can be mainly generalised to apply to healthy people. Therefore, extra precautions should be taken with patients.

6.2 The acute effects of single WBC

6.2.1 Thermal responses

In WBC, the human body is exposed to very cold air ($-110\text{ }^{\circ}\text{C}$) in minimal clothing. Therefore, frostbites might be anticipated to occur. However, neither literature nor clinical experience has reported frostbite occurring during WBC.

In this study, the main observations were that skin temperatures decreased very rapidly, especially in unprotected extremities. However, they remained at such a high level that there was no risk of frostbite. Because literature contains no reported data on skin temperatures during WBC, the results of the present study can be compared with studies on local cold therapy (e.g. frozen gel, dry ice, ice massage). The comparison indicates that WBC is a more intensive exposure than local therapies. The most effective local cold therapy of the lower extremities is ice massage (Chesterton *et al.* 2002, Belitsky et al 1987, Bugaj 1975). In the present study, the calf skin temperature decreased from $31.2\text{ }^{\circ}\text{C}$ to $5.3\text{ }^{\circ}\text{C}$ in 2 minutes. The drop in the skin temperature was approximately the same magnitude as that by Bugaj (1975) with ice massage in 10 minutes. Bugaj (1975) has proposed that localised analgesia requires a skin temperature below $13.6\text{ }^{\circ}\text{C}$, when nerve conduction becomes suppressed. In the present study, skin temperatures dropped below $13.6\text{ }^{\circ}\text{C}$ on the back and in the extremities, excluding the hands and feet that were covered by gloves and socks. To estimate the cooling risk, the criteria for three levels have been determined: $15\text{ }^{\circ}\text{C}$ corresponding to

pain (Havenith *et al.* 1992, Geng *et al.* 2001), 7 °C for numbness (Provins & Morton 1960) and 0 °C for frostbite (Danielsson 1996).

Hollander and Horvath (1950) and Hollander *et al.* (1951) demonstrated that normal knee joints had temperatures of 30.5 to 33 °C, whereas joints with active synovitis had temperatures which were between 34 and 37.6 °C. Oosterveld *et al.* (1992) and Oosterveld and Rasker (1994) have reported skin and intra-articular temperatures of the knee joint, both in healthy subjects and in patients with arthritis, after cold therapy with ice chips (0 °C, 30 minutes) and nitrogen-cooled air (-160 °C, 6.5 minutes). The skin temperatures in the extremities in the present study were lower than those observed by Oosterveld and his colleagues (1992, 1994) in the knee area. It is not easy to compare their results with the results of this study, due to the differences between the measuring sites. It however seems probable that, in the present study, intra-articular temperatures decreased at least as equally as in their study. Furthermore, Hirvonen *et al.* (2006) have reported that WBC seems to relieve pain in patients with rheumatoid arthritis more effectively than local cold therapy (cold packs, cold air -30 °C). According to Leppälüoto *et al.* (2008), plasma norepinephrine showed significant 2 to 3 fold increases after each WBC and WS period for 12 weeks, which could have a role in pain alleviation for patients.

The recovery of skin temperatures after WBC was quick, within a couple of minutes. In 30 minutes, the skin temperatures had not yet reached the reference value, but they were higher than those required for an analgesic effect. In clinical applications (e.g. as therapy for rheumatoid arthritis), WBC is used to alleviate pain, so that the patients can do therapeutic exercises after WBC. Therefore, exercises and mobilisation should be started as soon as possible after the exposure, as Belitsky *et al.* (1987) have also suggested concerning local cryotherapy.

Rectal temperature was unaffected by cooling, probably due to the short duration of WBC. A slight, but continuous decrease in rectal temperature was noted at the beginning of the recovery period, indicating a small after-drop, which is defined as continued cooling following the removal of cold stress. However, rectal temperature remained within the thermoneutral zone. Similar small changes in the core temperature have also been observed by others (Taghawinejad *et al.* 1989a, Joch *et al.* 2002).

Although skin temperatures decreased rapidly, the majority of WBC exposures were well-tolerated and comfortable, as were the WS exposures, too. One possible explanation is that the core temperature does not change during

WBC, and thus autonomic effector responses (e.g. shivering) are not particularly evoked. Similar data is not available on WS, but the skin temperatures (except the head) probably approximate water temperatures very rapidly. Further, motivational factors may partly be involved in thermal sensation and comfort ratings, since the subjects were very interested in trying the cold treatments.

It is important to note the large variation in the individual body temperature responses to the cold in the present study. Chesterton *et al.* (2002) found the same phenomenon in their study with local cold therapy. A great many individual factors may account for this variability (e.g. fitness, fatness). It should be noted that generic application protocols and times do not always ensure a clinically effective cooling of tissue (Chesterton *et al.* 2002). This should also be considered when using WBC as a clinical treatment method.

6.2.2 Blood pressure and heart rate variability responses

It is known that an elevated blood pressure is one of the risk factors for cardiovascular diseases. Cold increases blood pressure and elevated blood pressure may even be a risk factor in healthy persons, who are regularly exposed to the cold (Lloyd 1991). Furthermore, men are more sensitive to cold stress, having earlier and faster cardiovascular and metabolic adjustments than women (Gerra *et al.* 1992, Walsh & Graham 1986, Stevens *et al.* 1987, Graham *et al.* 1989).

In the present study, acute cold exposure increased the average blood pressure levels. However, it is known that short peaks in blood pressure cause much less harm than continuous states of raised blood pressure (Sokolow *et al.* 1966). In this study, systolic blood pressure increased more during $-110\text{ }^{\circ}\text{C}$ cold exposure than at lower temperatures (-10 , $-60\text{ }^{\circ}\text{C}$). The increase in systolic blood pressure, in connection with the $-110\text{ }^{\circ}\text{C}$ cold exposure, was large (24 mmHg) immediately after the WBC, compared with the values before the WBC in the present study, and more than in previous studies. Fricke (1989), Taghawinejad *et al.* (1989a) and Taghawinejad *et al.* (1989b) have reported systolic blood pressure to increase by 9–10 mmHg. The increase in diastolic blood pressure was about the same (5 mmHg) in the present study as that achieved by Taghawinejad *et al.* (1989a). Fricke (1989) found diastolic blood pressure to have increased by 10 mmHg, although Taghawinejad *et al.* (1989b) reported no change in diastolic blood pressure measured after WBC. In literature, there is no data related to blood pressures after the pre-rooms (-10 , $-60\text{ }^{\circ}\text{C}$).

After swimming in ice-cold water, Zenner *et al.* (1980) reported systolic blood pressure to increase by 43 mmHg and diastolic blood pressure by 14 mmHg. In the present study, blood pressure responses were lower compared with WS. Either, the changes in the blood pressures in this study were not as much as they can be during sub-maximal dynamic and isometric exercises in physical rehabilitation therapy (Iellamo *et al.* 1997). They compared blood pressure responses to isokinetic, isotonic and isometric exercises (knee extension/flexion repetitions) in healthy subjects. In their study, the changes of systolic and diastolic pressures were 60 and 39 mmHg during isokinetic exercise, 70 and 50 mmHg during isotonic exercise and 37 and 23 mmHg during isometric exercise. It is however important to note that we measured blood pressure after WBC, and not during WBC. Most probably, blood pressures were higher during the exposure, but the pressures could not be measured by the auscultatory technique.

In the present study, the men had slightly stronger reactions to WBC than the women, but the difference was not significant. However, the physiological mechanisms associated with gender related differences in cardiovascular function are not well understood. The sympathetic nervous system is known to be a major cardiovascular regulating system.

Individual differences in blood pressure responses to cold exposures were however large. This finding is in concordance with the previous reports, where considerable individual differences in cardiovascular responses to cooling have been found (Buskirk *et al.* 1963, Davis 1961, Hardy *et al.* 1970, Mannino & Washburn 1987). Although many studies have demonstrated that cardiovascular responses to overall body cooling is significantly related to the percentage of body fat, metabolic rate and fitness level (Daniels & Baker 1961, LeBlanc 1975), there have also been wide individual differences observed in response to the cold between individuals matched in age, level of fitness, previous exposure to the cold and percentage of body fat (Buskirk *et al.* 1963, Davis 1961, Hardy *et al.* 1970). Zenner *et al.* (1980) reported that after WS, some remarkable decreases in systolic pressure (changes of systolic pressures were 40 mmHg in one subject and 36 mmHg in another) and significant increases in systolic and diastolic pressure (changes of systolic/diastolic pressures were 50/50 mmHg in one subject and 49/10 mmHg in another). Because of striking individual differences in response to the cold, Hines (1940) has divided subjects into hyporeactors and hyperreactors.

Although there were large individual differences in blood pressure in the present study, no untoward clinical signs were noted in any subjects. In clinical use, in the Rheumatism Foundation Hospital, WBC is not allowed for patients

with cardiac disease and blood pressure has to be in stable and not over 160/100 mmHg. In Germany, patients with cardiac diseases are allowed to use WBC 6 months after a cardiac infarction.

Previously, the effect of autonomic regulations on cardiovascular functions has mostly only been characterised by changes in heart rate and blood pressure. In recent works, HRV has been used as an indirect indicator of the activity of the autonomic nervous system. Although the present study was part of a project undertaken to ensure the safety of WBC, it also allowed us to look at how extreme cold air exposure (WBC) influences cardiac autonomic regulation.

In the present study, an acute effect of the WBC demonstrated an increase in supine resting RRI, resting RMSSD and resting HFP, the indices of cardiac parasympathetic modulation. Similar findings have been observed by Joch *et al.* (2005) during exercise with pre-cooling (-110°C). Physiological explanations for the increased parasympathetic tone induced by the WBC could be due to a cold stimulation of the face. Increased activity of the peripheral sympathetic system was however also observed after cold stimulation of the face (LeBlanc *et al.* 1976). The combined activation of both components of the autonomous nervous system might induce peripheral vasoconstriction and then bradycardia via baroreflexes (Friedman *et al.* 1996, Heath & Downey 1990). The finding of this study that the parasympathetic tone increases as a response to WBC could be interpreted as a vagal rebound effect, which also occurs after strong exercise stress (Arai *et al.* 1989, Hautala *et al.* 2001). The mechanism of the vagal rebound effect is unknown.

Since there is very little data on HRV after the whole-body cold stress, the results of the present study can be also compared with that obtained in exercise stress studies. Arai *et al.* (1989) found a marked reduction in the absolute HFP during a maximal bicycle exercise test with a return to the normal level within few minutes of the exercise. Additionally, Pober *et al.* (2004) has shown that a single bout of moderate exercise changes the balance of the autonomic nervous system by increasing parasympathetic activity. This is generally considered as a healthy situation. Thus, the finding of this study that the parasympathetic tone increases as a response to WBC mimics the response induced by physical exercise stress. If the mechanism behind the increase in the HFP of the RRI after physical training is assumed to be similar to that after WBC, the WBC could be considered as beneficial for human health.

6.3 The effects of repeated WBC

6.3.1 Thermal sensations and comfort

Thermal sensations and comfort became habituated in the WBC group at an early stage of the trials. The response pattern of the WS group was rather similar to WBC group. A habituation response may be based on a decrease in synaptic transmission in the limbic system.

Leppäluoto *et al.* (2001) exposed healthy men to cold air (2 h in a 10 °C room) daily for 11 days, demonstrating that thermal sensations already became habituated after the first or second exposure. Jansky *et al.* (1996) exposed young men to cold water (1 h in 14 °C water) 3 times per week for 4–6 weeks. They observed attenuation in cold sensations after the fourth exposure, and further immersions increased this response. Though the scales in these studies were somewhat different from the present study, the average change in the habituation of thermal sensations was rather similar in all of these studies.

6.3.2 Blood pressure and heart rate variability responses

Although a habituation response was observed in thermal sensation and comfort, no adaptive changes in blood pressures were found in the present study. Leppäluoto *et al.* (2008) found no habituation in a norepinephrine response after WBC and WS either.

Hirvonen *et al.* (2002) reported that the mean resting systolic blood pressure of winter swimmers fell from 134 to 128 mmHg ($p < 0.05$) over one winter swimming season (an average of five to six times a week) and a slight but non-significant drop was also seen in the controls not winter swimming. The mean diastolic pressure did not change in either of the groups during the winter. We do not know, if a longer experimental period or more frequent cold exposures per week would have caused adaptation in blood pressure in the present study, too. However, a seasonal influence on blood pressure (Rose 1961, Brennan *et al.* 1982) and on humoral factors (Huttunen *et al.* 2001 & Hirvonen *et al.* 2002) has been observed. Brennan *et al.* (1982) have reported that systolic blood pressure was higher in winter than in summer for mild hypertensives. Our experimental periods were in autumn and in spring.

However, the WBC-related acute increases in resting RRI and resting HFP and RMSSD appeared to be less prominent after the period of three months of

‘cold training’. Additionally, the resting LFP measured before the WBC was found to have increased. The enhanced bradycardia (lower heart rates) after ‘cold training’ has been observed by others (LeBlanc *et al.* 1975, Kauppinen & Vuori 1988), too.

In the present study, the finding of the increased resting supine LFP of RRI before the WBC exposure is in agreement with the finding of Iwasaki *et al.* (2003). They found the increase in LFP with a moderate exercise training program (three to four times/week) from the baseline to three- and six-month time points (Iwasaki *et al.* 2003). On the other hand, in some randomised longitudinal training studies, a significant effect of aerobic training on daytime HRV in 24-hour recording has been found to be due to an increase in a vagal modulation of cardiac activity (Stähle *et al.* 1999, Tulppo *et al.* 2003). Cold adaptation may mimic exercise training effects, as both stresses acutely increase the level of stress hormones and sympathetic activity (Huttunen *et al.* 2001, McArdle *et al.* 1996, Leppäluoto *et al.* 2008). The increase in LFP may either reflect an increase in cardiac sympathetic modulation (Pagani *et al.* 1986), or an increase the cardiac parasympathetic modulation, only (Hedman *et al.* 1992, Uusitalo *et al.* 1996) or increase both of them (Akselrod *et al.* 1985). It seems that the LFP of the RRI is dependent on both cardiac sympathetic and vagal activities (Pomeranz *et al.* 1985, Goldstein *et al.* 1994). The predominance of the sympathetic activity during stressful conditions may occur, while at rest the HRV may be mainly vagally modulated (Uusitalo *et al.* 1996). In the present study, the measurements were performed during supine rest, which allows us to conclude that the LFP could be mainly dependent on vagal activity. However, the physiological mechanism of the vagal regulation of HFP and the LFP of the RRI could be different, because HFP did not change significantly and even tended to increase.

6.3.3 Neuromuscular performance

To our knowledge, the adaptation of neuromuscular performance has not been mentioned in literature, though humans can physiologically adapt to the cold. The results of the present study however showed that the single WBC decreased flight time in the drop-jump exercise, but after three months of repeated WBC, this change had almost vanished. After repeated WBC, the activity of the agonist muscle increased more and the activity of the antagonist increased less, indicating reduced co-contraction (Bawa *et al.* 1987). As the subjects did not train the drop-

jump exercise during the three months' period, these results allowed us to argue that neuromuscular adaptation had taken place due to the repeated WBC.

In the present study, the averaged EMG-activity of the agonist muscle increased more after repeated WBC. The EMG-activity of the antagonist muscle was less during the shortening phase, indicating diminished co-contraction. These changes in muscle activity may explain, at least partially, the smaller reduction in flight time that was observed in the drop-jump test after repeated WBC. There may be a great number of mechanisms inducing the observed changes in the drop-jump. One potential candidate could be the adaptation of the muscle spindle. Oksa *et al.* (2000) have reported that after acute cooling, the decreased spindle activity was seen as a decreased aEMG activity of the agonist muscle and an increased aEMG activity of the antagonist muscle during the shortening phase of the stretch-shortening cycle. If the muscle spindle is able to adapt to the cold, it should be viewed as a reduced co-contraction (Bawa *et al.* 1987) or breaking effect (Oksa *et al.* 1995, 1997), which in fact was the case in this study.

It is known that temperature is an important modulator of the neuromuscular function (Davies & Young 1983, De Ruyter *et al.* 1999). However, to our knowledge, no research has been conducted regarding the effect of repetitive WBC on neuromuscular functions, but there are studies using milder and different types of exposures. Geurts *et al.* (2005) investigated the effects of cold acclimation on the neuromuscular function of the hand. In their study, the subjects immersed one hand in 8 °C water for 30 minutes, 5 days a week for three weeks, which means 15 immersions. Likewise Geurts *et al.* (2006) examined the effects of repeated cold exposure (2 weeks, daily) with an elevated core temperature from exercise (cycling) on the neuromuscular function of the hand. In their local cold exposure studies (Geurts *et al.* 2005, 2006), no adaptation in neuromuscular function was found. Geurts *et al.* (2005) speculated that a larger change in the temperature of the hand may be needed to obtain significant changes in neuromuscular function after repeated cold exposures. It should be noted that the subjects had 36 exposures in our study. Therefore, we may assume that considerably more than 15, or exposures of different kinds, are needed so that changes/adaptation in the neuromuscular function may be observed.

Dietz *et al.* (1981) and Komi (1983) have reported that during the pre-activity phase, an anticipatory effect (increased EMG activity) occurs before the stretch phase of the stretch-shortening cycle. It has been reported that after cooling, increased EMG-activity enhances the utilisation of the elastic components of the working muscles (Asmussen *et al.* 1976). In the present study, during the pre-

activity phase after repeated WBC, the averaged EMG-activity of the agonist muscle increased more than after a single WBC, suggesting that the utilisation of the elastic components could be enhanced after repeated WBC. If so, this increase in muscle activity during the pre-activity phase may also explain the smaller reduction in flight time after repeated WBC and support the notion of adaptation in neuromuscular performance. Since pre-activity is mainly regulated via higher levels of the central nervous system and not on the level of muscle spindle, we may speculate that the “pre-activation model” may also be modulated because of repeated WBC.

In the wrist flexion test after repeated WBC, the averaged EMG-activity of the agonist muscle tended to increase more (ns) than after a single WBC, whereas the EMG-activity of the antagonist tended to increase less (ns) than after a single WBC, suggesting a reduced level of co-contraction after repeated WBC. Although there were changes in the EMG-activity that might be able to reduce performance, the level of maximal force was unaffected. This may be due to a different type (isometric) of exercise. In human studies, the maximal isometric force level has been found to be relatively stable within the muscle temperature range of 27 to 40 °C (Clarke & Royce 1962). The muscle temperature is not known after WBC, but the skin temperature of the forearm decreased rapidly to 5.2 °C. The temperature however recovered rapidly after exiting the therapy-room (-110 °C). It is thus assumed that only superficial parts of the muscles are cooled and due to a strong vasoconstriction and short exposure duration, the “critical” muscle temperature of 27 °C for the reduction of maximal isometric force was not reached.

The cooling of the working muscles has also been related to the increased TPT (Ranatunga *et al.* 1987) and RT (Wiles & Edwards 1982). The reasons for these changes have been explained by slowed ATP hydrolysis (Ferretti 1992), slowed Ca^{2+} release and uptake from the sarcoplasmic reticulum (Kössler *et al.* 1987), and decreased Ca^{2+} sensitivity of actomyosin. In parallel with the unchanged maximal force level, TPT and RT either made no change, which may refer that the change in muscle temperature was not sufficient to induce changes in these parameters either.

7 Conclusions

The main findings and conclusions of the present study are as follows:

1. WBC (-110°C , 2 minutes) involves no risk of frostbite, if the persons stand rather still. The lowest local skin temperatures were recorded on the forearm, 5.2°C , and on the calf, 5.3°C . After WBC, all skin temperatures recovered rapidly, indicating that the temperature dependent analgetic effects of WBC only occur during a limited period after the exposure. Thus, if WBC is used for therapeutic means, the exercises should be done immediately after the WBC.
2. Repeated exposures to WBC in healthy women were mostly well tolerated and comfortable. This may be due to the short exposure times without significant core cooling with concomitant autonomic effector responses and/or psychological factors. The results indicate that during repeated severe whole-body cold stress of short durations, thermal sensations and comfort become habituated during the first exposures.
3. WBC increased the average levels of blood pressure temporarily, but the variation of the individual responses was vast. The magnitude of the increase is however supposed to be safe for healthy persons. Neither significant gender differences nor adaptation in blood pressures were found during or after repeated WBC.
4. The observed acute increase in the high frequency power (HFP) of RR-intervals induced by WBC indicates an increase in cardiac parasympathetic modulation. After three months of repeated WBC, the increase in the parasympathetic tone was attenuated, which may be interpreted as an adaptation of the autonomic function. The repeated WBC exposures related to an increase in resting the low frequency power (LFP) of RR-intervals during the three months resembles the response induced by exercise training. Although we have no information on the maximal heart rate response during WBC, we are inclined to suggest that the WBC is safe and even beneficial for the autonomic functions of healthy people.
5. Neuromuscular adaptation may take place, especially in dynamic performance, after three months of repeated exposure (3 times a week) to WBC. A single WBC decreased flight time in drop-jump exercise. However, after repeated WBC, these changes almost disappeared. This adaptation was confirmed by the change of the activity of the agonist muscle, which

increased more, and by the change of the activity of the antagonist muscle, which increased less or did not change after repeated WBC. This indicates reduced co-contraction and neuromuscular adaptation. If the same type of adaptation of neuromuscular functions occurs in patients, it might reduce their pain and stiffness and allow them to perform the therapeutic exercises more effectively after repeated exposures to WBC. To confirm this hypothesis, further studies are required.

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Appendix 1

Table. A1 Summary of the studies examining the effects of WBC.

| Subjects | Cold exposure | Effects of cold | Study |
|---|-----------------------------|---|--|
| Healthy subjects | -110 °C, 2 min | increased level of norepinephrine | Leppäluoto <i>et al.</i> (2008) |
| Sportsmen | -110 °C, 4 min | aural and skin temperatures decreased | Savalli <i>et al.</i> (2006) |
| Healthy subjects | -110 °C, 2 min | minor bronchoconstriction | Smolander <i>et al.</i> (2006) |
| Healthy women | -110 °C, 2 min | changes were mild in antioxidant capacity | Dugue <i>et al.</i> (2005) |
| Male subjects | -110 °C, 2 min 30 s | heart rate and blood lactate decreased, parasympathetic tone increased during exercise (compared without WBC) | Joch <i>et al.</i> (2005) |
| Students | -110 °C, 2 min | improvement in sprint performance | Fricke <i>et al.</i> (2000) |
| Healthy subjects | -110 °C, 1, 2 and 3 min | increased muscle strength and performance | Fricke <i>et al.</i> (1999) |
| Healthy subjects and patients with rheumatoid arthritis | -120 °C, 1 to 3 min | improved transitory bronchodilatory effect | Engel <i>et al.</i> (1989) |
| Patients with rheumatoid diseases and healthy subjects | -110 °C, 30 s to 1 min 30 s | heart rate and blood pressure increased temporarily | Taghawinejad <i>et al.</i> (1989b) |
| Patients with rheumatoid diseases | -105 °C, 2 min 30 s | pain decreased | Metzger <i>et al.</i> (2000) |
| Patients with rheumatoid diseases | -100 °C, 1 min 30 s | heart rate and blood pressure increased temporarily, oral temperature tended to decrease | Taghawinejad <i>et al.</i> (1989a) |
| Patients with rheumatoid arthritis | -60 °C, -110 °C, 2 min | pain decreased and disease activity decreased | Hirvonen <i>et al.</i> (2006) |
| Patients with rheumatoid arthritis | -110 °C, 2 to 3 min | increased pain threshold, a feeling of better general health, decreased duration of morning stiffness, decreased disease activity | Ksiezopolska-Pietrzak <i>et al.</i> (2000) |

| Subjects | Cold exposure | Effects of cold | Study |
|---|---------------------------------------|--|-----------------------------------|
| Patients with rheumatoid arthritis | -110 °C to -160 °C, 2 min | ACTH, cortisol and beta-endorphins increased, no arrhythmias or ischemic changes | Zagrobelyny <i>et al.</i> (1992) |
| Patients with rheumatoid arthritis | -110 °C, 30 s to 3 min | pain decreased, improvement of general well being and joint function, heart rate and blood pressure tended to increase temporarily | Fricke (1989) |
| Patients with joint disease | -110 °C, 3min | pain relieved | Fakhari <i>et al.</i> (2000) |
| Patients with systemic joint disease | -110 °C, 0 to 3 min | pain relieved, improvement of general well being and joint function | Birwe <i>et al.</i> (1989) |
| Patients with ankylosing spondylarthritis | -110 °C, exposure time not reported | inflammation, swelling and pain decreased | Wichman & Fricke (1997) |
| Patients with fibromyalgia | -67 °C, 3 min | pain decreased, general well being improved, thermal tolerance increased | Gutenbrunner <i>et al.</i> (1999) |
| Patients with fibromyalgia | -150 °C, 3 to 4 min | pain decreased | Samborski <i>et al.</i> (1992) |
| Patients with low back pain | -120 °C, 2 to 3 min | inflammation tended to decrease | Cholewka <i>et al.</i> (2006) |
| Sportsmen after orthopaedic surgical intervention in rehabilitation | -110 °C, 2 to 4 min | pain decreased, improvement of recovery in muscles | Savalli <i>et al.</i> (2005) |
| Patients with affective and anxiety disorders | -110 to -160°C, 2 to 3 min | reduced anxiety and depressive symptoms, increased life satisfaction | Rymaszewska <i>et al.</i> (2007) |
| Patients with depressive symptoms | -150 °C, 2 min 40 s | depressive symptoms decreased | Rymaszewska <i>et al.</i> (2003) |
| Asthmatic patients | -175 °C, , exposure time not reported | lung function improved | Yamauchi (1988) |

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