Cold intolerance

From thermoregulation to nerve innervation

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Koude intolerantie

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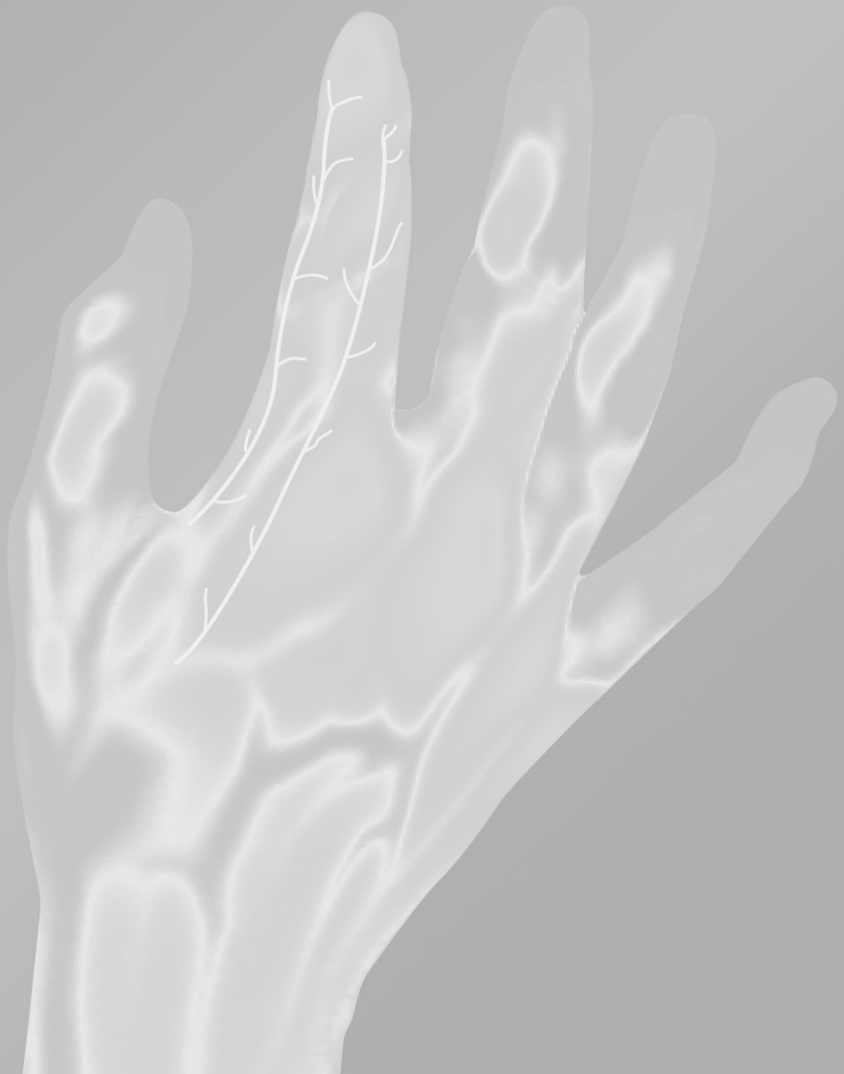
General introduction



Clinical Case

Bram Beukers is a 42-year-old experienced butcher. He works in the family butchery since he was 16 years of age. He feels comfortable with the anatomy of his products and with handling his equipment. It is one week before Christmas and Bram is working overtime. He already skipped family dinner -again- to prepare the next day deliveries. On a Tuesday evening in this busy week before Christmas he is working on the last piece of lamb. In a split second of inattention he chops of his little finger.

In deliberation with the hand surgeon, the butcher chooses not to try to replant his little finger. He will not have the time or the motivation for the extensive and long-lasting rehabilitation, since he needs to work in this busy period. The stump/defect is closed following the regular protocol, while giving special care to the prevention of neuromas.

Eighteen months after the trauma during a beautiful summer day he is preparing a lot of orders for the outside grill. While he is preparing meat in the refrigeration room, he is forced to leave because of an unbearable pain of his stump. He has felt this before and it painfully reminds him of his injury and the limitations he has to cope with during his work. From then onwards, he experiences he can only work for two minutes in the temperatures of the cooling cell.

Shortly after this incident, Bram Beukers decides to visit the outpatient clinic. He has to prepare this visit carefully. Even though the weather is beautiful, he won't be able to ride his bike without a glove on his injured hand. The wind, combined with an inevitable chill factor, results in unpleasant sensations.

He is worried that because of his complaints, he will have to sell the butchery that is run by his family since 1846. In the multidisciplinary consultation of the hand-surgeon, anaesthesiologist, rehabilitation physician, occupational health physician and the psychologist, it is concluded that unfortunately for him the only solution for the complaints is to avoid cold environments.

The impact of cold intolerance

Trauma to the hand is frequently seen in people working with machinery, during occasional handyman work in and around the house. In the Netherlands (a population of 16.7 million) 50.000 fractures of hand and fingers, 790 amputations of fingers and 200 nerve injuries are to be count every year.[1]

The largest population of hand and wrist injuries comprises of working class male between 20-64 years of age, like Bram Beukers. The absolute number of patients with complex soft tissue injuries such as a traumatic amputation or a nerve injury is relatively low, but at the level of the individual patient, these injuries cause high health-care costs and a major loss of production.[2] In 2007, hand and wrist injuries in the Netherlands annually account for 740 million U.S. dollar and rank first as most expensive injury types. In comparison, hip fractures cost 532 million U.S. dollar.[2] Of the 740 million U.S. dollar costs of hand and wrist injuries, 411 million dollar (56%) is related to productivity costs. Per case these productivity costs have an average of 1580 U.S. dollar.[2]

One of the most bothersome long-term effects of a nerve injury is cold intolerance. Cold intolerance is an abnormal sensitivity to a cold environment or cold temperatures, and is defined as abnormal pain after exposure to mild or severe cold, with or without symptoms such as discoloration, numbness, weakness, or stiffness of the hand and fingers.[3-5] It has been reported that cold intolerance seriously influences a patients daily life by being the most bothersome, prolonged and disabling symptom, affecting both work and leisure activities.[5-9] Most likely, the cold intolerance develops within the first months after injury and the symptoms of cold intolerance often do not diminish over time.[8-14] However, as in the story of Bram Beukers, it can have a later onset as well.

Development of cold intolerance is seen in patients after ulnar (56%) or median (70%) nerve injury,[14] a traumatic amputation 51%,[15] a flexor tendon injury (66%),[16] radial for arm harvesting (31%).[17] At the start of this research project, the prevalence of cold intolerance in patients with fractures to the hand was still unknown.

Chronic pain

About 20% of the adult Europeans suffer from chronic pain of moderate to severe intensity.[18] Neuropathic pain affects 6-8% of the general population and has a great impact on the patient's quality of life and disability.[19,20] Loss of hand function caused by pain will be invalidating for activities of daily life for both younger and older patient. Hands are the primary tool for interacting with our environment and, crucial for receiving information about our surroundings.[21]

As cold intolerance does not diminish over time, it can be seen as a chronic pain syndrome.[22] Possibly, cold intolerance is part of a (chronic) neuropathic pain syndrome. Often patients with chronic pain experience the inability of doctors to treat their symptoms satisfactory. For

both patient and doctor this can be very frustrating and might eventually lead to a feeling of distrust in the medical abilities of the doctor. Inevitably, patients with chronic pain are tagged as “difficult”. At this point, we have passed a decade dedicated to pain control and research,[23] but unfortunately the public and professional understanding and knowledge of pain and pain management are often still unsatisfactory for patient and professional.[23,24]

The high prevalence of cold intolerance in patients and the reduced quality of live as direct result, urge both clinicians and researchers to collaborate. Cold intolerance is still not completely understood and therefore sufficient treatment cannot be given. So, even with the best possible intention, the hand surgeon cannot promise the patient a cold intolerance free outcome after surgery.

History and definition of cold intolerance

Research performed on the painful effect of cold on the human body has been a topic of interest for almost a century. Observations of the influence of cold upon the skin have been described in 1930 by Lewis.[25,26] It is plausible that is was the severity of complaints of cold intolerance that urged researchers such as Sir Lewis to get involved (Figure 1).

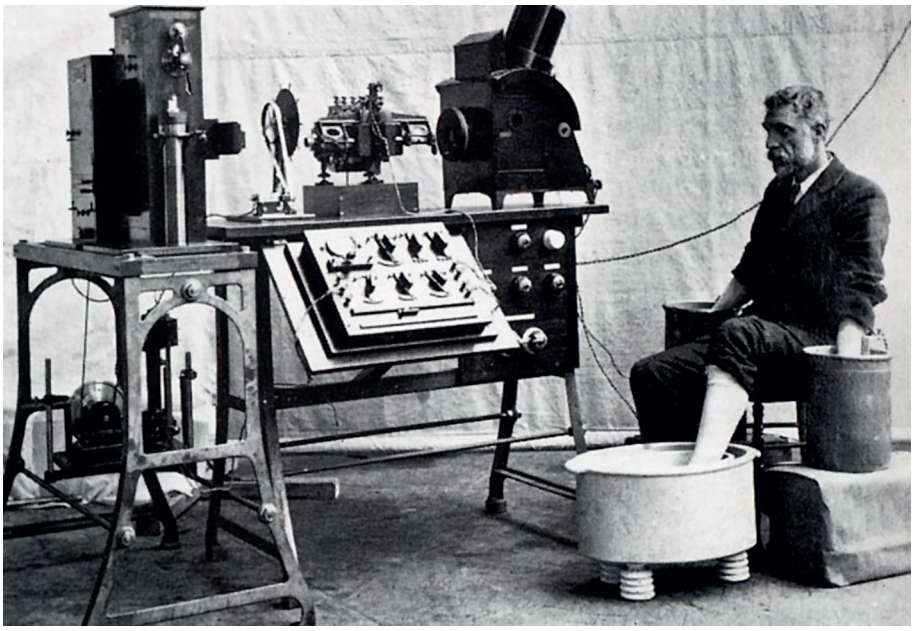


Figure 1 | Sir Lewis performing fundamental research on the heart, the pulse and blood pressure. Mastering the technology of the electrocardiogram in 1912.

Posttraumatic cold intolerance has several names and descriptions. The following terms are commonly used: cold hypersensitivity,[27] cold sensitivity,[10,11,13,28-30] cold allodynia,[31,32] Abnormal Cold Intolerance (ABCI)[22] and Trauma Induced Cold Associated Symptoms (TICAS).[3] Throughout this thesis the term cold intolerance will be used. This is a commonly accepted term for posttraumatic cold intolerance[4,5,8] and actually describes the most predominant complaint of the patient, namely pain and discomfort when exposed to cold, at a temperature which healthy people can easily withstand. Cold intolerance is most commonly defined as abnormal pain after exposure to mild or severe cold, with or without discoloration, numbness, weakness, or stiffness of the hand and fingers. While using this definition, it should be stressed that patients without a trauma of the hand with similar complaints and signs such as discoloration as seen in Raynaud's phenomenon for example are not suffering from cold intolerance as described in this thesis.
Cold intolerance appears after several types of traumas. The best described and most bothersome injuries to the hand that cause cold intolerance are:
Nerve injury[14]
Replantation of a digit[7,30,33]
Amputation of a digit[34]
Arterial repair (with and without additional nerve injury)[22]
Flexor tendon injury[16]
Hand and arm vibration syndrome[15]
Cold injury, Freezing and non freezing[89-90]

Several variables are influencing the development of cold intolerance. Predictive variables are the level of nerve injury, the level of vascular injury, presence of peripheral nerve injury, presence of bone injury, crush injury, poor somatosensory recovery, early post-operative pain, and when a patient smokes.[10-12,14,28,35-41]

Development of questionnaires

To determine the presence and severance of cold intolerance, several questionnaires have been developed. The most commonly used is the Cold Intolerance Symptom Severity (CISS) questionnaire. In addition to the CISS questionnaire, VAS scores using single or multiple questions and modification of the cold sensitivity severity scale (CSSS) are used.[5,9,10,17,42,43] The CISS questionnaire is a validated questionnaire that measures the presence and severity of cold intolerance and contains six questions about the complaints of cold intolerance. The CISS score ranges from a minimum of 0 to a maximum of 100, where a maximal score indicates most severe complaints. The first question is about the type of symptoms and is not included in the final score. The following five questions include the frequency and time of occurrence, change in behavior to ease or prevent symptoms, severity of symptoms during special actions, and severity of activities affected by the symptoms.

The first version of a validated cold intolerance questionnaire Cold Sensitivity Severity Scale (CSSS) was made by McCabe et al.[13] Six years later, Irwin et al. developed the Cold Intolerance Symptom Severity (CISS) questionnaire,[12] which was modified by Ruijs et al.[44] Several cut-off values have been determined for this questionnaire. Sweden has a cut-off value of 50, in The Netherlands a cut-off value of 30 was found.[44,45] To diagnose cold intolerance, throughout this thesis the CISS questionnaire will be used with the adjusted cut-off value of 30 for the Dutch normative population.

Clinical Problem

Part of this thesis concerns cold intolerance in patients after a hand fracture. A reason why cold intolerance is such a bothersome complaint, is because in a part of the patient group, the complaints of cold intolerance do not diminish over time.[6,8,9,22] One study described complaints of cold intolerance more than a decade after the trauma.[22] A classic presentation of cold intolerance is when the symptoms develop during the first four months after trauma, gradually increasing in intensity to one year after the trauma.[6,11] The prevalence of cold intolerance in patients with a hand fracture is unknown. The cold intolerance problems of patients who visit the outpatient clinic or primary care after a hand fracture might have been ignored or not taken seriously, because cold intolerance after hand fracture has not been investigated or described in the literature. It has been suggested that there is an increased chance to develop cold intolerance if a bone is injured.[11] However, the patient group of cold intolerance after a hand fracture without additional damage to surrounding tissue such as nerve and vascular trauma has not been described. By describing the prevalence and severity of complaints of this group, awareness of the doctor may increase and the complaints of patients will be recognized. Secondly, the patient group that suffers from a hand fracture and experiences complaints of discomfort to cold is of substantial interest. The patients have had a trauma to the hand after which cold intolerance has evolved. The presence of cold intolerance and no major nerve and/or vascular lesions could be an interesting patient group. In the patient group with cold intolerance and no major nerve and/or vascular lesions we would be able to test the first hypothesis; is a disordered vascular function the origin of cold intolerance? However before we test this patient group the prevalence and severity of this patient group should be investigated to outline the impact of this clinical problem.

The pathophysiology of cold intolerance is unfortunately unclear. Until now, two possibilities are feasible: 1) A disordered vascular function leading to an impaired thermoregulation of the extremities and the skin, and/or 2) A neurological dysfunction leading to a changed neurological response to cold stimuli that causes the complaints of intolerance to cold. In the next part of this introduction these possible hypotheses will be further explained.

Vascular origin

When skin temperature differs locally, underlying mechanisms that influence the skin temperature such as vascular dysfunction can be investigated. As a direct result of vascularisation of the digits, skin temperature can vary when either vasodilatation or constriction takes place. In other words, evaluating skin temperature can be a tool for recognizing vascular dysfunction.

Measuring temperature is not an experiment of the last decades, it has been described in 170 AD by Claudius Galenus. The first thermometer using mercury and a scale was developed in the early 18th century by Ole Christensen Romer and is considered the origin of the thermometer used in many modern days in clinical and non-clinical settings. These two tools (i.e. the mercury and scale) are the basic essentials to measure skin temperature and make research possible to evaluate changes in temperature. A common used technique is to measure temperature in the rectum, ear or under the tongue, changes in temperature can give an insight in the underlying mechanism, such as the activity of the metabolism which can indicate a fever after infection.[46]

The skin, the largest organ of the body, serves as the body thermoregulator, controlling blood flow within a few millimeters of surface.[47] Differences in skin temperature between the injured and the uninjured hand are suggestive of a disturbance in thermoregulation. The skin temperature in digits and palms correlates with blood flow, changes in blood flow and therefore skin temperature can be monitored.[48,49] Nowadays, changes in skin temperature can accurately be assessed in several manners using simple thermometers, thermocouples or more expensive and sensitive methods such as continuously infrared recording. A thermocouple is a frequently used tool to assess this thermoregulation and measures reactions to cold exposure, in humans and rodents.[50-53] Skin temperature changes measured with an infrared recording after immersion into cold water has proven to be a useful tool to assess thermoregulation this could be was (?) conformed and used by Ruijs et al.[52,53]

Cold intolerance has been related to low skin temperature, discolored fingers and a poor vascular function. A poor vascular function is suggested to be a key factor in the development of cold intolerance.[41] The fact that in the smoking population the incidence of cold intolerance is higher made the influence of the vascular function on cold intolerance unavoidable.[12]

In this thesis, two possible techniques will be described to assess thermoregulation function: 1) performing a (short) cold stress test and measuring variables such as the rewarming pattern afterwards[53,54] and 2) cooling for a longer period followed by measuring the cold induced vasodilatation (CIVD) reaction.

Rewarming after a cold stress test

Results of vascular dysfunction can be expressed by an abnormal rewarming pattern after cold stress. Differences in blood flow of the skin can be substantial. Under normothermic conditions, the blood flow of the skin is approximately 5% of the cardiac output. However, the absolute amount of blood in the skin can vary from nearly zero during vasoconstriction in a cold stress test to 60% of the cardiac output during a heat stress (which results in maximal vasodilatation).[55] The use of thermography in evaluating vascular function in the hands of a patient with cold intolerance is based on the presence of asymmetries between the involved limb and the healthy contralateral limb.[47] Based on the study of Uemsta et al. performed in 1988, we assumed a link between asymmetry in skin temperature and the underlying mechanisms that influence the skin temperature. In line with this, Ruijs et al. demonstrated that sensory recovery was strongly related to the presence of active rewarming patterns in patients with peripheral nerve injury, suggesting sensory recovery is an important aspect of the pathophysiology of cold intolerance.[53]

Cold induced vasodilatation

The human thermoregulation reacts to changes in internal (core) temperature and skin temperature.[46] When the skin temperature drops during cooling, direct vasoconstriction of the skin body vessels occurs. This is to prevent that the core temperature will decrease as well.[46] A CIVD reaction is a mechanism to protect the hands, feet, chin and nose against frostbite.[50,56] The previously mentioned Lewis et al. described a so called Hunting reaction in 1930.[26] This reaction describes a difference of 60% in distribution of blood over the body surface during cold and heat stress[55] and a significant change of the skin temperature. A consequence of the vasoconstriction of microvasculature and the high surface area-to-volume ratio is a rapid and exponential decrease of the skin temperature of the fingers to a level approaching that of the ambient (cold) environment.[57] Due to a reduced nutritional blood flow tissue damage and even necrosis may occur, this is also seen in non-freezing cold injuries such as frostbite.[58] To protect fingers from frostbite, a temporary increase in blood flow can occur where skin temperature can rewarm by even 10 degrees Celsius. These cyclic changes of skin temperature may appear repeatedly, which in fact is considered to be a CIVD reaction.

In healthy humans, a CIVD reaction generally occurs after a minimum cooling time of 5 minutes in an environment to a maximum of 15°C.[59] However, changes in CIVD response in individuals who live in cold environments appear to be neither guaranteed nor predictable.[60] The presence and nature of a CIVD reaction depends on a large number of variables. For example, a higher body core temperature as well as the intake of food leads to a stronger and faster CIVD reaction.[50,61-63] Changes in the CIVD reaction have been described in patients with cold intolerance after a traumatic peripheral nerve injury.[51,64-66] It has been suggested that there is a relation between CIVD and the treatment of posttraumatic cold intolerance.[67] To assess the thermoregulation and consequently the vascular function in cold intolerant patients measuring the CIVD reaction can be an effective tool.

Neurological origin

To understand neurological dysfunction, knowledge of the physiology of injuries of the peripheral nervous system is required. An injury of a peripheral nerve provokes a cascade of reactions that may lead to a variety of symptoms. These reactions serve to limit damage and to create an environment that supports nerve regeneration.[68]

A peripheral nerve is a cordlike structure filled with cables of axons and provides a common pathway for the electrochemical nerve impulses that are transmitted along each of the axons to peripheral organs. In the nervous system, the analogous structures are known as tracts. Within a nerve, a layer of connective tissue surrounds each axon.[68] There are two types of somatosensibility that can be described: vital sensibility and gnostic sensibility. Vital sensibility refers to the sensation of pain, temperature and touch, while gnostic sensibility refers to the sensation of movement, position, vibration and discrimination. With the skin one is able to feel several sensations of touch, vibration, mechanical and thermal (pain) stimuli. These stimuli and sensations are registered by different fibers such as the A beta β-, A delta δ- and C fibers.

Damage to nerves may be caused by physical injury, entrapment (e.g. carpal tunnel syndrome), autoimmune diseases like Guillain-Barre syndrome, and infections such as neuritis, diabetic neuropathy or failure of the blood vessels surrounding the nerve. Nerve damage is usually accompanied by numbness, muscle weakness or paralysis and sometimes pain. The pain can occur when a nerve is damaged. Signaling is altered for all parts of the area from which the nerve receives input, not just the site of the damage.

Besides cold intolerance as a complication of nerve injury, a neuroma is also considered a serious complication. A neuroma is the sprouting of neural fibers and connective tissue. In 3-5% a neuroma is symptomatic and a painful hypersensitivity to normal light tactile stimuli will be present.[69,70,88] Throughout this thesis we will not include patients with a neuroma and will not discuss this subject. A third common known complication of a nerve injury is neuropathic pain. Neuropathic pain may be associated with abnormal sensations and pain produced by normally non-painful stimuli (allodynia). Neuropathic pain may result from disorders of the peripheral nervous system. Because neuropathic pain is sometimes a “vague” clinical description and not a “hard” diagnosis, it could be that the neurological origin of cold intolerance has not been recognized as an important standalone subgroup within the neuropathic pain patient group.

With the knowledge of the possible consequence of a nerve injury, the influence of a neurological dysfunction on cold intolerance has not been investigated to its fullest extent. Two possible techniques will be described to assess neurological dysfunction that is possible related to cold intolerance.

Quantitative Sensory Testing

Quantitative sensory testing (QST) is an noninvasive measurement tool designed to determine detection thresholds for sensations of touch, vibration, mechanical and thermal (pain) stimuli.[71] It gives quantified information on detection of the previous mentioned thresholds. QST can assess the full somatosensory range in one single diagnostic tool. QST can be an effective tool to evaluate somatosensory changes in different pathologies, and is regarded a useful diagnostic instrument in pain conditions such as neuropathic, arthritic, myofascial and fibromyalgic pain.[72-78] The German Research Network on Neuropathic Pain designed and validated a standardized set of tests comprising all somatosensory submodalities mediated by different primary afferents (C-, Aδ-, Aβ-), including the assessment of the deeper layers.[79] As the different fibers can be disordered in pain syndromes such as cold intolerance, it could be of interest to detect thermal pain thresholds, and investigate somatosensory changes in patients with cold intolerance. A relationship in the QST outcome and the corresponding changes in A- and C- fibers activity will provide an insight in the underlying pathophysiology of cold intolerance.

Conditioned Pain Modulation

With the conditioned pain modulation (CPM) effect the function of the central descending inhibitory system can be assessed.[80,81] This central descending inhibitory system is of interest because it can inhibit or facilitate transmission of noxious information. Recent studies have shown that this system is involved in the pathophysiology of chronic pain[81,82] and that pain modulation patterns are reduced in various patient groups with idiopathic acute and chronic pain syndromes. How one reacts to pain is different between people and interpersonal variability in pain experience is most likely based on differences in pain modulation. Descending pathways in pain modulation are of major importance because they can either enlarge or weaken transmission of noxious (painful) information.[82,83] Over 30 years ago Le Bars et al. described a phenomenon of pain inhibiting pain in rats. This so called diffuse noxious inhibitory control effect was later called a conditioned pain modulation effect.[84] Variability in how pain inhibits pain in individual patients can be evaluated by quantifying the conditioned pain modulation CPM effect.[80] In general, the objective is to inhibit ongoing pain in remote areas when a new pain is introduced. An explanation why some patients develop more cold intolerance then others could relate to the interpersonal variability in pain modulation.

Objective, research questions and outline of the thesis

The general aim of this thesis was to increase our understanding of the pathophysiology of cold intolerance. Why do a large number of patients with a trauma to the hands develop cold intolerance? By answering this question and investigating the pathophysiology of cold intolerance step by step, customized therapy might be developed and sufficient pain relief could be achieved. To pursue this goal, the general aim can be divided into “major” questions with several sub-questions:

– How large is the clinical problem?
– Is there evidence for a vascular origin that can explain the complaints of cold intolerance?
– Is there evidence for a neurological origin that can explain the complaints of cold intolerance?

Clinical problem

In a posttraumatic hand, additional bone fractures increase the chance to develop cold intolerance.[11] However, the prevalence of cold intolerance in patients with a hand fracture has not been investigated yet. Because patients with a hand fracture do not have major vascular or nerve injury, the patient group could be of interest to further understand the mechanism of cold intolerance.

Chapter 2 We performed a retrospective study to measure the prevalence and severity of cold intolerance in patients who had suffered a fracture to the hand. The widely accepted CISS questionnaire was used to measure the degree of cold intolerance in this patient group.

Part I: Does a vascular origin lie at the root of cold intolerance?

Chapter 3 To investigate the vascular dysfunction hypothesis in hand fracture patients, we evaluated rewarming patterns in patients with cold intolerance. We performed a cold stress test to the hands; rewarming patterns were measured by way of infrared videothermography recordings. Patients with a hand fracture with and without cold intolerance were compared to healthy controls to study the relation between the severity of cold intolerance and rewarming patterns after cold stress testing.

Chapter 4 The relation between dysfunctional thermoregulation and cold intolerance was further investigated. Because of the complexity of hand trauma with multifactor tissue damage, micro-lesions to vessels and nerve endings, an animal model was chosen to explore the underlying mechanism of cold intolerance. Rats underwent different types of peripheral nerve injury. Examining the rewarming patterns and comparing this data to the severity of cold intolerance could assess the influence of thermoregulation on cold intolerance.

Changes in a Cold Induced Vasodilatation (CIVD) reaction have been described in patients with cold intolerance after a traumatic peripheral nerve injury. It has been suggested that there is a relation between CIVD and the treatment of posttraumatic cold intolerance. It is unknown if a CIVD reaction during cold stress is modified in rats with peripheral nerve injuries and whether the presence or absence of a CIVD reaction is related to cold hypersensitivity. As in cold stress testing, evaluation of the CIVD reaction may be a tool to assess the quality of the thermoregulatory system in rats after peripheral nerve injuries.

Chapter 5 The aim was to determine the role of the sympathetic system in initiating a Cold induced vasodilatating (CIVD) response. Therefore, rats underwent different types of peripheral nerve injury, the hind limbs were cooled and skin temperature was recorded to evaluate the presence of CIVD reactions. The results could be of interest to examine the role of the peripheral nerve and the sympathic system in the initiation of the mechanism of a CIVD reaction in the hind limb of a rat.

Chapter 6 A recently published article on the use of the term cold-induced vasodilatation urged us to comment on the used definition. The authors interpreted the data when the cooling had ended. Unfortunately a CIVD reaction is only seen during continuously cooling. In addition there was no cyclic oscillation described. Therefore most likely an active rewarming pattern was seen as described in chapter 3 and 4 of this thesis instead of a CIVD reaction.

Part II: Does a neurological origin lie at the root of cold intolerance?

Chapter 7 We used quantitative sensory testing to investigate if a neurological dysfunction is a predominant mechanism of cold intolerance. The quantitative sensory testing is ideal to interpret in a noninvasive manner the behavior of the different types of small nerve fibers (Aβ-, Aδ- and C) abnormalities in patients with cold intolerance. The study can assess and quantify sensory nerve function. In addition the possibility of the quantitative sensory testing device as a diagnostic tool for cold intolerance was evaluated.
An explanation why some patients develop more cold intolerance then others could relate to the interpersonal variability in pain modulation.
Chapter 8 We used the phenomenon of 'pain inhibits pain' to measure a conditioned pain modulation (CPM) effect. This CPM effect is useful to understand idiopathic pain syndromes. The CPM effect was measured in cold intolerance patients and compared to healthy controls. A reduced CPM effect could indicate an altered function of the central descending inhibitory system. This could be of interest for the use of Selective Serotonin and Noradrenalin Reuptake Inhibitors (SSNRI). Secondly, the CPM effect can be a predictive for the development of chronic pain such as cold intolerance.
Chapter 9 The main results from the previous chapters and their implications are discussed. Conclusions are made, research questions are answered, the main objective evaluated and possibilities for future research are shared.

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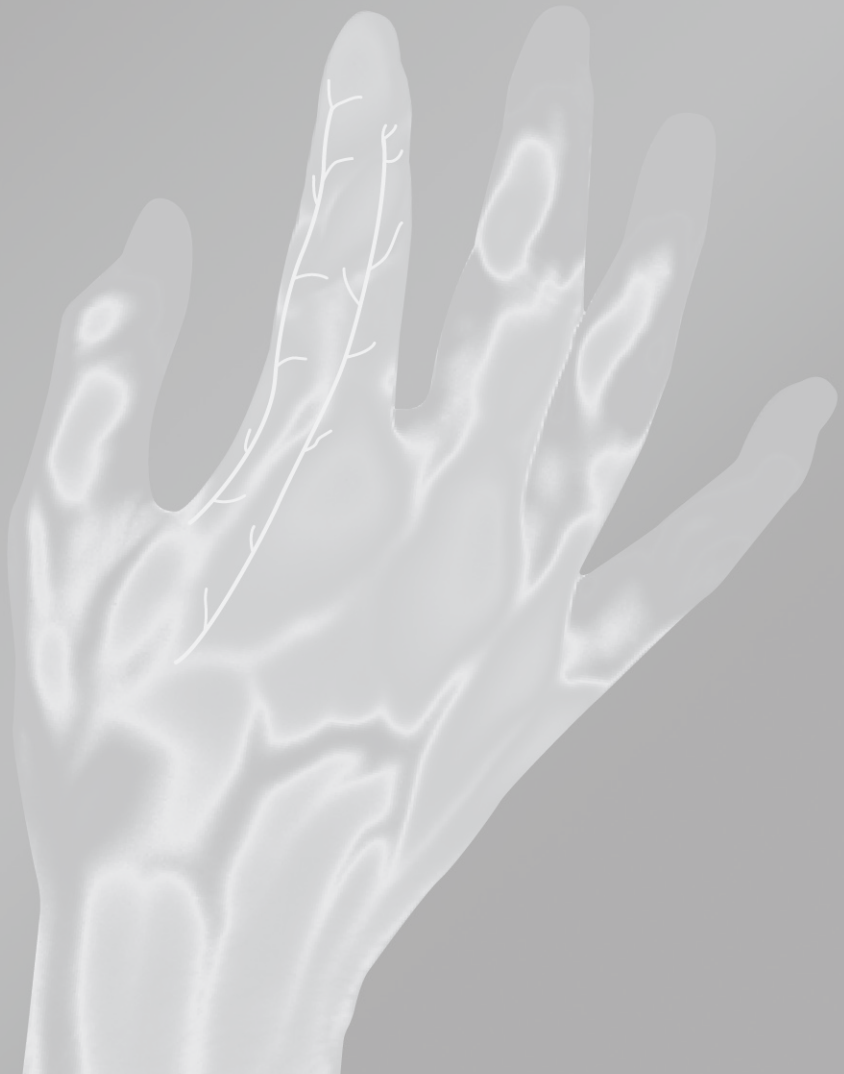
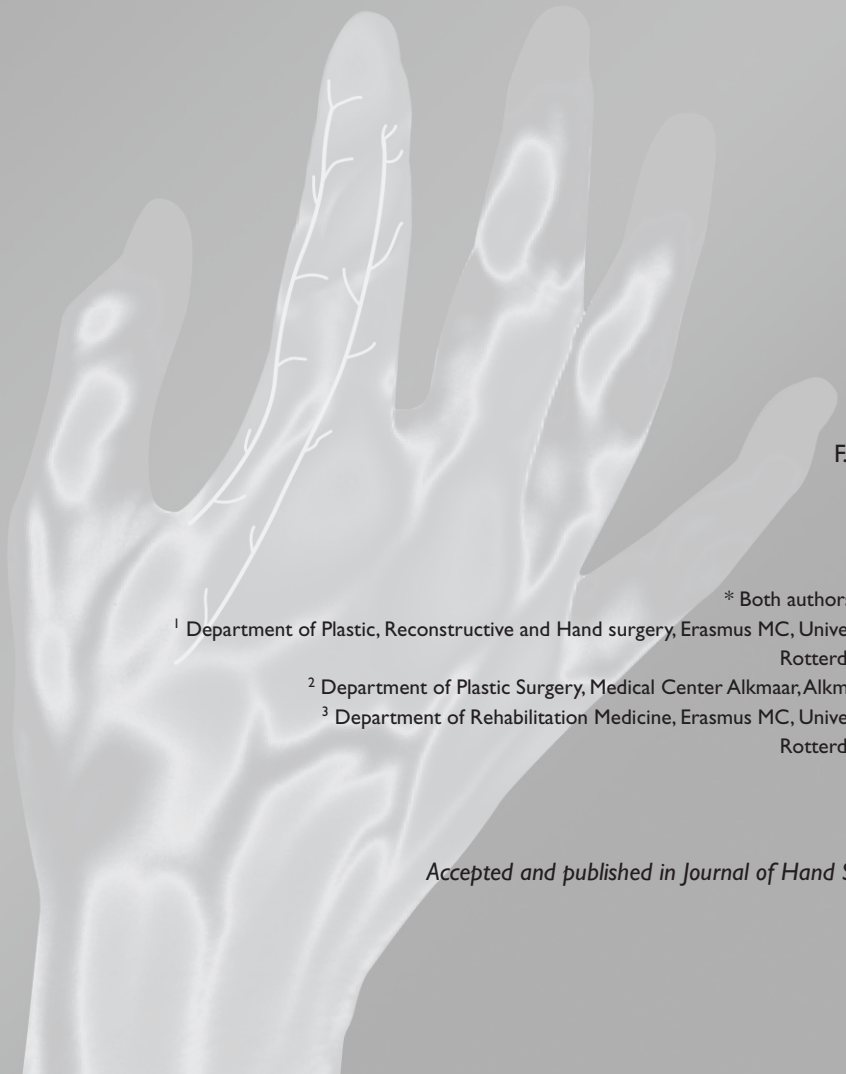
Clinical problem



Chapter 2

Prevalence and severity of cold intolerance in patients with a hand fracture



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Abstract

Introduction

Cold intolerance is a well-known phenomenon that develops in the first months after hand injury and generally does not decrease over time. A high prevalence of cold intolerance after nerve injury and replantation is described in literature. In this study, we evaluate the prevalence and severity of cold intolerance in patients with a hand fracture.

Methods

To evaluate the prevalence and severity of cold intolerance, 129 patients treated for a hand fracture completed the Cold Intolerance Symptom Severity (CISS) questionnaire. This questionnaire assesses the severity of the cold intolerance using 6 questions. The CISS test score ranges between a minimum of 0 and a maximum of 100. The cut-off point for the diagnosis cold intolerance is ≥ 30. Patients with nerve and/or vascular injuries were excluded. The response rate was 59%. The mean CISS score of the patients was 23. Thirty-eight percent (95 confidence interval: 24-53%) was diagnosed with pathological cold intolerance.

Conclusion

Cold intolerance is increasingly accepted as a serious problem for patients with trauma in their extremities. We found that 38% of our patients with hand fractures had pathological cold intolerance

Introduction

Cold intolerance after fracture of the hand is not well documented. Cold intolerance is defined as abnormal pain after exposure to mild or severe cold, with or without discoloration, numbness, weakness, or stiffness of the hand and fingers.[3,7,13,16,18] Symptoms of cold intolerance develop in the first months after injury and generally do not decrease over time.[4,5,10,20,23,25,27] While current treatment includes the use of pharmacological agents, operations of the autonomic nervous system and biofeedback techniques, behavioral change such as a change of occupation is still considered the most effective strategy.

Although cold intolerance is a frequent sequel of upper limb trauma, the prevalence is particularly high after nerve injury. It has been reported that in the majority of peripheral nerve-injured patients, cold intolerance is the most bothersome, prolonged and disabling symptom, affecting both work and leisure activities.[1,8,18,23,25] Ruijs et al. (2007) studied the prevalence of cold intolerance in patients with ulnar or median nerve injuries and found that 56% of the patients with a single nerve injury and 70% with a combined nerve injury suffered pathological cold intolerance according to the CISS questionnaire. In addition, cold intolerance has been documented in other hand disorders such as Raynaud's disease,[12,19,21,22] upper extremity trauma,[15] digital replantations[1,2,8,9,11,17,24,25,28] and after raising a radial forearm flap.[29,30]

In most studies, cold intolerance has been evaluated using single 'yes' or 'no' questions.[3-5,16,18] More recent questionnaires such as the Cold Intolerance Severity Scale,[20] Blond McIndoe[10] and the Cold Intolerance Symptom Severity (CISS) questionnaire[10,26,27] contain multiple questions on the impact of cold intolerance in the patients daily life and calculate a sum score, expressing the severity of cold intolerance in more detail than a single yes or no question. These questionnaires do not appear to have been used to study cold intolerance after hand fractures. Therefore, we used the CISS questionnaire to examine the prevalence and severity of cold intolerance in patients with a hand fracture.

Methods

One hundred and twenty-nine patients treated for one or more hand fractures between 2005 and 2006 were asked to participate in this study. Patients had between one and seven fractures of the metacarpals or phalanges. All patients received treatment at the Erasmus MC in Rotterdam, The Netherlands. Exclusion criteria were a nerve or vascular injury, vascular and systemic diseases (such as Raynaud's disease, diabetes, and rheumatoid arthritis), age lower than 18 years, use of vasodilatating medicines, previous hand fractures, nerve lesions, and previous hand surgery. All these criteria were evaluated using a questionnaire with, for their greater part, closed questions and an open question on additional diseases to exclude systemic and vascular diseases. The fractures types were categorized by their anatomic description. The Medical Ethics Committee of the Erasmus MC approved this study and all participants signed informed consent.

Patients completed the Cold Intolerance Symptom Severity (CISS) questionnaire which was developed by Irwin et al. (1997) and was modified by Ruijs et al. (Table 1).[26,27] The CISS test score ranges between a minimum of 0 and a maximum of 100. The cut-off point for the diagnosis cold intolerance is ≥ 30, based on the 95% confidence interval in normal subjects.[26] We also recorded age, profession, use of medication, hand dominance, side of injury, number of fractures, type of fractures, number of hospital visits, and costs of treatment.

Patients were contacted by a letter that included the CISS questionnaire, a form with the additional questions and consent form. If there was no response, the patient was contacted by telephone and the questionnaire was scored by telephone.

Data analysis

Descriptive values such as mean, median and standard deviation were calculated with the SPSS 16.0. Independent t-tests were used to test for significant differences between the responders and non-responders. One way analysis of variance (ANOVA) was used to test for effects of fracture type, fracture location and type of work on the CISS score. Pearson correlation was used to test for relations between the CISS score and a number of variables.

Table 1 | CISS Questionnaire.

Question	Score*
1. Which of the following symptoms of cold intolerance do you experience in your injured limb on exposure to cold?	
Pain, numbness, stiffness, weakness, aching, skin colour change (white/bluish white/blue)	Not scored
2. How often do you experience these symptoms? (Please tick)	
Continuously / all the time	10
Several times a day	8
Once a day	6
Once a week	4
Once a month or less	2
Never†	0
3. When you develop cold induced symptoms, on your return to a warm environment are the symptoms relieved (please tick):	
Not applicable†	0
Within a few minutes	2
Within 30 minutes	6
After more than 30 minutes	10
4. What do you do to ease or prevent your symptoms occurring? (please tick)	
Take no special action	0
Keep hand in pocket	2
Wear gloves in cold weather	4
Wear gloves all the time	6
Avoid cold weather / stay indoors	8
Other (please specify)	10
5. How much does cold bother your injured hand in the following situations (please score 0-10)	
Holding a glass of ice water‡	10
Holding a frozen package from the freezer‡	10
Washing in cold water‡	10
When you get out of a hot bath/shower with air room temperature‡	10
During cold wintry weather	10
6. Please state how each of the following activities have been affected as a consequence of cold induced symptoms in your injured hand and score each (please score 0-4)	
Domestic chores	4
Hobbies and interests	4
Dressing and undressing	4
Tying your shoe laces	4
Your job	4

*In question one, patients are asked to score their symptoms as follows: "Please give each symptom a score between 0 and 10, where 0=no symptoms at all and 10=the most severe symptoms you can possibly imagine." However, the scores given in this question do not count towards the final CISS test score.

†These are two questions developed by Ruijs et al. to improve the scoring

‡These are the questions of the McCabe cold sensitivity test.

Results

The characteristics of responders and non-responders are presented in Table 2. Seventy-six (58.9%) of the 129 patients completed our questionnaire. The average age of responders at the time of injury was 39 years (range 15-75); 50 were men. Statistical differences between the responders and non-responders were found for age and gender (Table 2) but not for the other patient or fracture characteristics.

The main cause of the injury was falling (62%), followed by punching (13%), accidents with a machine (10%), entrapment (8%) and other (e.g. sports and unknown, 7%). The metacarpals were injured in 42% of cases. The mean follow-up time of the responders, defined as the number of days between the injury and completion of the questionnaire, was 669 days (median follow-up time was 674 days).

Mean CISS score for all patients was 27 (range 0-75). Twenty-nine (38%; 95% confidence interval 24-52%) of the patients had a CISS score larger than 30, indicating pathological cold intolerance. In Figure 1, the distribution of CISS scores can be seen, showing a large group of patients with a low CISS score (< 10), while the other scores between 10 and 80 are more or less equally distributed. Figure 2 shows the CISS scores plotted against age, indicating no relation between the age of the patient and the CISS score.

No correlation was found between fracture type or location and the CISS score. In addition, no correlation was found between time since injury and CISS score, and no significant differences were found between the CISS scores and white collar workers, blue collar workers, and unemployed. However, significant correlations were found between CISS score and the number of fractures, the number of hospital visits and the number of rehabilitation visits (see Table 3), indicating that more severe or complex injuries lead to higher CISS scores.

Table 2 | Characteristics of responders and non-responders.

Characteristic	Responders	Non-responders	P-value
Age, Mean (SD)	39 (SD 17)	32 (SD 13)	0.003
Male : female	50 : 26	40 : 13	0.016
Duration since injury (in days), (Median)	674	732	0.346
Fracture Geometry			
Transversal	20	21	0.874
Comminuted	18	9	
Avulsion	12	10	
Oblique	11	6	
Spiral	8	6	
Longitudinal	1	0	
Unknown	6	1	
Fracture Location			
Metacarpal	32	25	0.910
Distal phalanx	20	14	
Proximal phalanx	14	8	
Middle phalanx	5	5	
Unknown	5	1	
Dominant hand affected			
Yes : no : unknown	35 : 35 : 6	26 : 21 : 6	0.685

P-values indicate the differences between the responders and the non-responders.

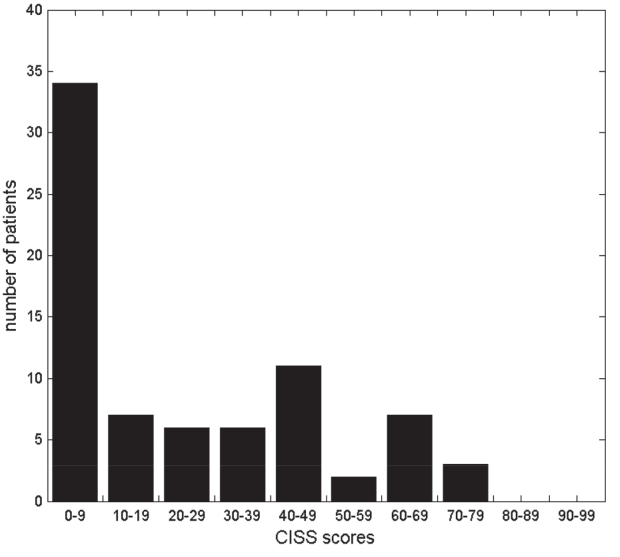

Figure 1 | Histogram indicating the number of patients with scores in the different CISS-score ranges.

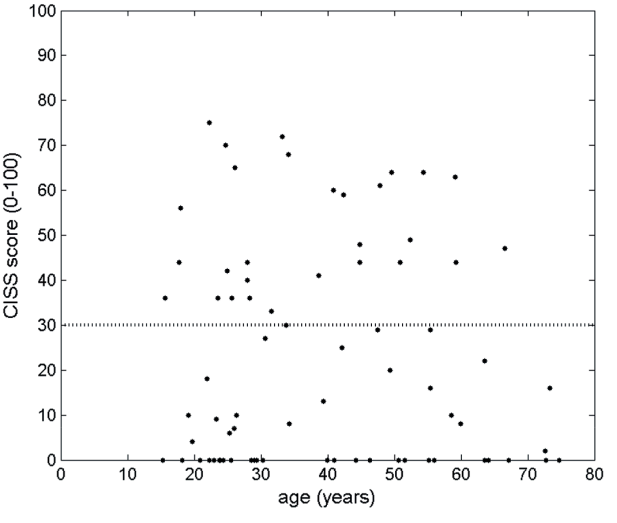

Figure 2 | Scatter plot of age versus CISS score for all patients. The dotted line indicates the cut-off value for pathological cold intolerance.

Table 3 | Pearson correlations between CISS score and variables indicating the severity of the fracture (number of fractures in the same hand, number of visits and absence of work) and CISS scores for the different fracture types, fracture locations and work type.

Variables	Correlation with CISS	P-value
Number of fractures	0.237	0.039
Number of hospital visits	0.250	0.036
Absence at work due rehabilitation	0.201	0.175
Number of rehabilitation visits	0.263	0.027
	CISS mean (SD)	
Fracture type		
Avulsion (N=12)	32.7 (29.6)	
Comminuted (18)	22.8 (25.1)	
Longitudinal (1)	0	0.706
Oblique (11)	19.6 (25.4)	
Spiral (8)	16.1 (17.0)	
Transverse (20)	24.8 (23.0)	
Unknown (6)	19.5 (24.2)	
Fracture location		
Metacarpal (32)	20.5 (22.6)	
Proximal phalanx (14)	26.4 (22.1)	0.699
Middle phalanx (5)	6.6 (14.8)	
Distal phalanx (20)	26.0 (28.2)	
Unknown (5)	19.0 (27.0)	
Work type		
Blue collar (24)	27.3 (26.7)	
White collar (18)	24.2 (23.0)	0.309
Unemployed (26)	16.8 (23. 4)	

The P-values indicate the significance of the correlation coefficients or the significance of the overall effects of fracture type, fracture location and work type on the CISS score.

Discussion

Cold intolerance is increasingly accepted as a serious problem for patients with trauma in their extremities. The prevalence and severity of cold intolerance is unknown. We found that 38% of our patients with hand fractures had pathological cold intolerance.

There are a number of limitations of this study. Firstly, it should be noted that the CISS questionnaire consists of questions that describe only the symptoms of cold intolerance (see Table 1). Since the etiology of cold intolerance is unknown, it is not clear whether the score relates to disease severity or progression. However, we believe that the questionnaire indicates the severity of the complaints of cold intolerance (the higher the score, the more severe the complaints). A second limitation is the low response rate of 59%. A low response rate is commonly seen in these populations.[6] In the unlikely case that none of the non-responders suffered cold intolerance, our study would indicate a minimum rate of 22%. It should also be noted that the follow-up since injury is relatively long. Although most studies related to other injuries in the upper limb indicate that cold intolerance develops in the first few months after trauma and generally does not decrease significantly over time.[4,5,10,20,23,25,27]

It should be noted that we do not have data on the presence of cold intolerance before injury. However, the cut-off value of 30 for the CISS questionnaire is based on an evaluation of the range of values found in healthy subjects (95% confidence interval). Therefore, on statistical grounds, it could be expected that approximately 5% of the subjects had a CISS score larger than 30 before the trauma.

The prevalence in our study on patients with hand fractures (38%) is lower than those found after single nerve injury (56%) and combined nerve injury (70%) (Ruijs et al. 2007). It is also lower than the 83% prevalence in replantation patients.[14] These higher rates may be explained by the more extensive nerve injury in those pathologies as well as the vascular damage in replantation cases. However, at present, the relation between cold intolerance and nerve damage or vascular damage remains unclear.

The pathophysiology and predictors of cold intolerance remain controversial. Although the symptoms have been associated with reduction of blood flow in response to cold, the reason for the decreased flow is unclear. For example, the assumption that cold intolerance after finger replantation is caused by macrovascular problems or capillary microcirculatory failure seems incorrect based on the results of Klein-Weigel et al., (2007) who demonstrated that diminished skin vessel density reduced thermal modulation capacities in the fingertips of cold-sensitive replanted digits.

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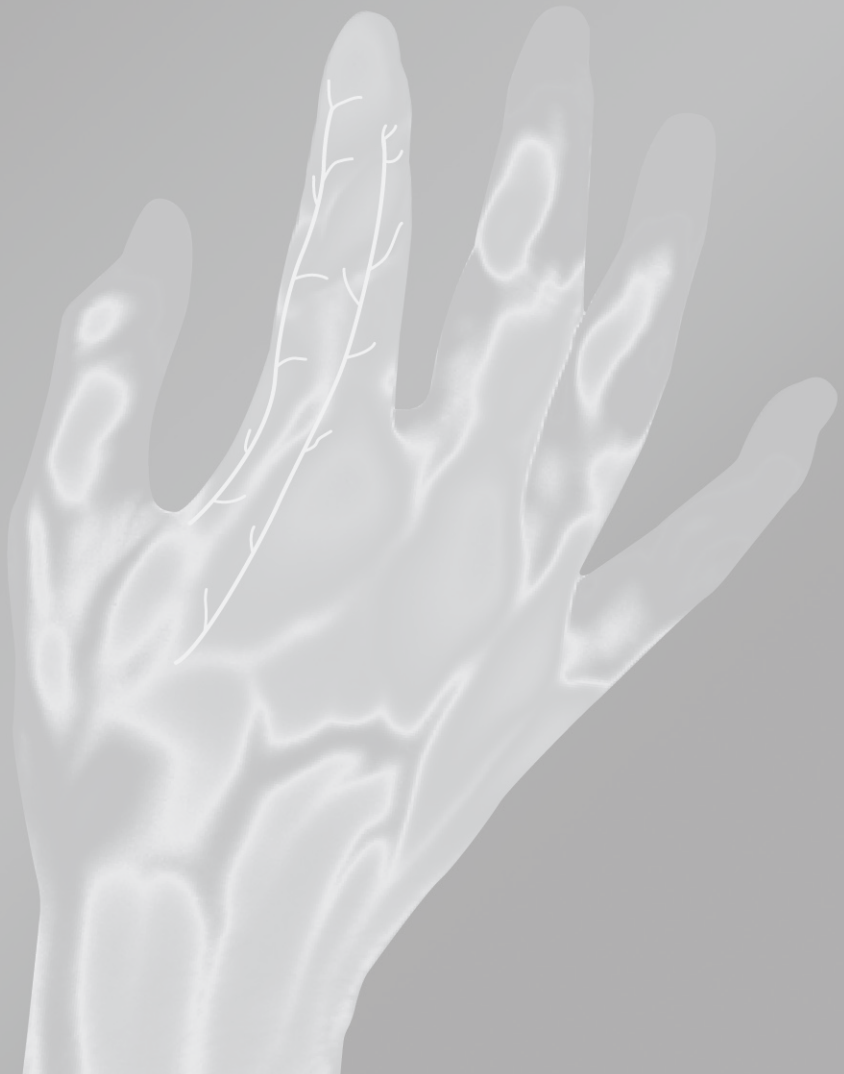
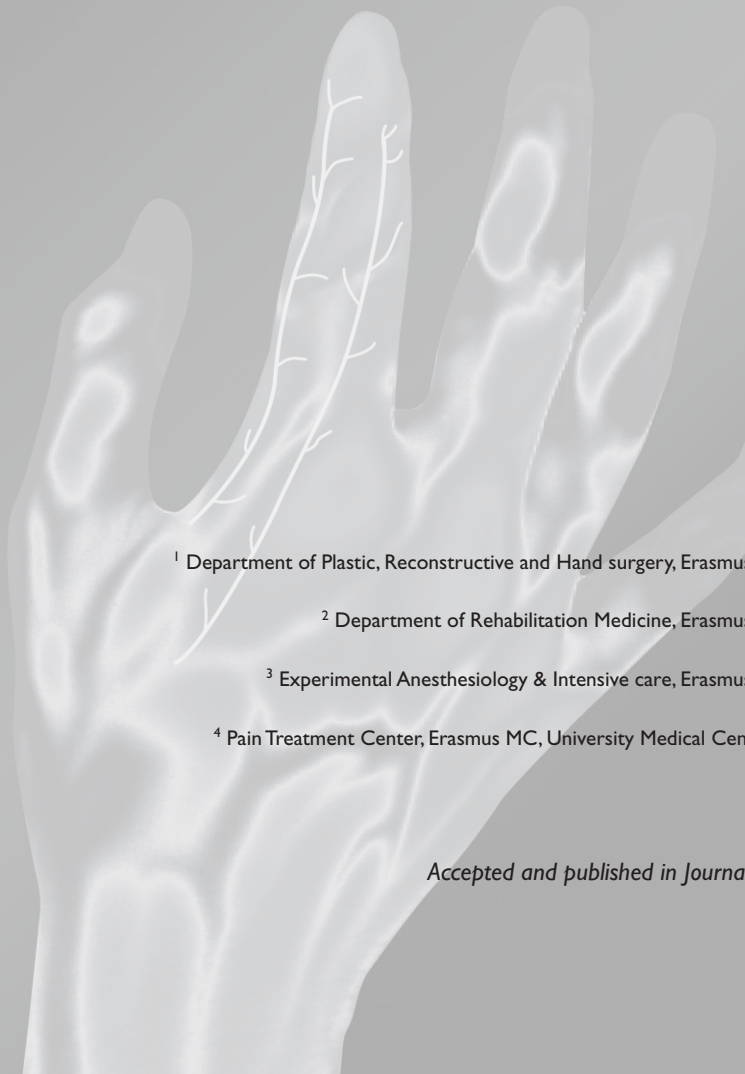
Part I



Chapter 3

Re-warming patterns in hand fracture patients with and without cold intolerance



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Abstract

Introduction

It is often assumed that cold intolerance is associated with abnormalities in the skin temperature due to changes in the blood flow of the hands. In this prognostic study, we determined whether patients with and without cold intolerance after a hand fracture or healthy controls have a diminished re-warming after a cold stimulus.

Methods

The severity of cold intolerance was evaluated using the Cold Intolerance Symptom Severity (CISS) questionnaire. To determine if abnormal re-warming plays a major role in the underlying pathophysiology of cold intolerance, a cold-stress test was applied 30 months after the patients recovered from a hand fracture. Temperature during the re-warming phase was measured using videothermography.

Results

Thirteen control subjects and 18 patients participated. Control subjects did not report any symptoms of cold intolerance (CISS score 0) and no loss of sensibility was measured. Mean CISS score of all patients was 27.8; nine patients scored above the cut-off value for normal cold intolerance.

No significant differences were found in the re-warming patterns between 1) the affected and non-affected hand of the patients, 2) the dominant and non-dominant hand of the control subjects, and 3) the patients and controls.

Conclusions

The results of this study revealed no relation between the severity of cold intolerance and re-warming patterns after cold stress testing. This may suggest that temperature regulation of the hands in this patient group may not be responsible for the symptoms of cold intolerance.

Introduction

Cold intolerance is a disabling symptom that is defined as abnormal pain following exposure to mild cold that often occurs after trauma.[4,10,16,18,21,26,31,33] In a study on patients with a hand fracture, the prevalence of cold intolerance was found to be 38%. In addition, cold intolerance is also commonly reported by patients with Raynaud's disease,[12,22,24,25] upper extremity trauma, digital replantations,[1,2,6,7,11,15,20,28,29,35] the hand and arm vibration syndrome and after a radial forearm flap.[36,37] In many studies, the presence of cold intolerance is determined using a single yes/no question. Alternatively, the severity of cold intolerance can be determined in more detail using the Cold Intolerance Symptom Severity (CISS) questionnaire, developed by Irwin et al.[10] and adjusted by Ruijs et al.[32]

The pathophysiology of cold intolerance is still unclear.[4] Nevertheless, it is assumed that cold intolerance is related to vascular dysfunction.[11] In a healthy person, after extensive cooling of the extremity, the thermoregulatory system increases the blood flow to the extremity to counteract the decrease in hand temperature and prevent pain and/or frostbite.[17] One of the substances involved in this thermoregulatory process is Nitric Oxide (NO), having a direct effect on the smooth muscle activity.[13] In this process sympathetic fibers play a key role, providing control of adrenergic neurotransmitters by peripheral nerves and vasculature. As described by Ruch et al., the vascular flow can be separated in nutritional flow and thermoregulatory flow, from which only thermoregulatory flow determines the digital temperatures.[30] Thermoregulation of the skin in part relies on input from temperature sensors and nerves located in the peripheral location. There are also alternative hypotheses on the pathophysiology of cold intolerance. For example, experimental studies are performed on Transit Receptor Potential (TRP) channels, which are poly model receptors that respond to temperature, pain and pressure. Recent studies suggest that an up-regulation of TRP channels after peripheral nerve trauma may also explain cold intolerance.[3,14] When vascular dysfunction exists, abnormal re-warming will be one of the results. One way to elucidate abnormal re-warming is to perform a cold-stress test.[19] In this test, both hands are cooled in water and the re-warming pattern is recorded. Measuring temperature during re-warming after a cold stress test in cold intolerant patients has not been extensively studied. In cold intolerant patients with measureable peripheral nerve damage, a diminished thermoregulation was observed.[34] Until now, it is still unclear if, and to what extent, cold intolerance is indeed related to abnormal re-warming and not, for example, to neurological changes or interaction between both.[5,6]

The aim of this study was to investigate the correlation between cold intolerance and abnormal re-warming after a cold stress test. In order to elucidate this relation we analyzed re-warming patterns for possible thermoregulatory dysfunction in control subjects and in hand fracture patients with and without cold intolerance. Additionally, we wanted to study the correlation between the score of the CISS questionnaire and the re-warming pattern.

Methods

Subjects

We invited patients form an earlier study on the incidence and severity of cold intolerance after a hand fracture to participate.[27] All subjects completed the cold intolerance symptom severity (CISS) questionnaire,[10,23,32] which has a minimum score of 0 and a maximum score of 100. Based on the range of normative values, a patient with a score of 30 or higher has abnormal cold tolerance.[32] Thirteen control subjects (five men and eight women) participated in this study. Average age was 38 year (range from 22 to 58 years), one subject was left hand dominant, twelve right hand dominant (see Table 1). Three subjects smoked 2-10 cigarettes a day (average 6/day), but were asked to refrain from smoking starting the evening before testing. In addition, eighteen hand fracture patients (nine women and nine men) participated in this study. The mean time after fracture was 135 weeks with a range of nineteen weeks. All patients had one or more fractures in their metacarpal and/or phalangeal bone(s). Average age at injury was 45 years (range from 21 to 75 years) and 17 patients were right hand dominant. Five subjects smoked between 3-40 cigarettes a day, these patients were asked to refrain from smoking starting the evening before testing. This study was approved by the Medical Ethical Committee of our hospital and all patients and control subjects gave their written informed consent (MEC 2008-088). No external funding was used to fulfill this study.

Measurements

During the patient's and control subjects acclimatization for 15 minutes in a room with a mean temperature of 23°C (range: 22.5 to 23.5) and a relative humidity of 50% (range: 45 to 55), the CISS question list was completed. Hereafter the possible loss of sensibility in the patients hands was measured using Semmes Weinstein monofilaments (North Coast Medical Inc, Morgan Hill, CA) and outcome was classified according to Imai et al.[9] On each hand, 5 filaments ranging from 2.83 till 6.65 gram were used. Filaments were placed for 1 to 2 seconds on the fingers and we asked the patient to indicate without looking which finger was touched.

Cooling and temperature recording

For cooling, both hands were immersed up to the ulnar styloids for 90seconds in a water bath with a constant temperature of 13°C. Traynor and McDermid showed that immersion of hands in 13 degrees C water for 90 seconds is sufficient to initiate active rewarming.[38]

After removing the hands from the water, the examiner carefully and quickly blotted the hands dry using a cotton towel. Temperature measurements of the affected and unaffected hand were made with the hands placed on a clean towel. The delay between the removal of the hands from the water and the start of the thermographic recording was less than 10 seconds.

Skin temperature from the dorsal side of both hands was registered with a computer-assisted videothermography (ThermaCAM SC2000, Flir Systems, Berchem, Belgium) which calibrated automatically. The measurement protocol was previously used by Ruijs et al.[34] The thermal sensitivity was 0.05°C at 30°C, the spectral range 7.5 to 13.0 µm, and the built-in digital video has 320x240 pixels (total 76.800 pixels). Data were obtained through a high-speed (1Hz) data acquisition system (ThermaCAM Researcher 2001 HS, Berchem, Belgium) connected to a desktop-PC. The distance between the camera and the hands was 70cm; the pixel size of the temperature recordings was 0.8x0.8mm. Temperature was measured until both hands had re-warmed to a stable temperature of 34°C with a minimum duration of ten minutes and a maximum of thirty minutes.

Statistical methods

Temperature data were exported to text files using FLIR Thermacam Researcher Pro (version 2001-HS). For each digit, temperature was measured in the middle of the nail bed placing a circle with an average diameter of 8 mm (see Figure 1). Figure 2 shows a thermographic image of an example of normal active re-warming after applying the cold stress test. The active re-warming starts distal in the fingertips and proceeds in proximal direction. Data was plotted in a time-temperature curve for further analysis of the re-warming (Figures 3 and 4).

The temperature readings of each digit, stored in a text file, were imported to Matlab® (version 7.1) to be analyzed. In Matlab® the calibration points of the camera were removed and a second order low pass Butterworth filter with a cut-off frequency of 0.015Hz was applied. An algorithm was developed in Matlab® to automatically determine the start and stop events of the active re-warming in each curve (Figures 3 and 4). We defined the active re-warming as the time between the maximum increase and decrease of the second temperature derivative.[31] Since the automatically selected points were not always accurate because of, for example, irregularities due to calibrations of the camera or movement of the fingers, all selected events were visually verified and, when needed, manually corrected.

To quantify the re-warming, the duration of re-warming (between the start and stop events) and the area under the curve (Q) between these events was calculated (Figures 3 and 4). This Q-value was divided by time to calculate the average re-warming per second (Q/s), indicating the re-warming velocity of the digit.

Statistical analysis

Statistical analysis was performed using SPSS 16.0. The Q and Q/s value of the affected hands were compared with the opposite digits, and the Q-value between patients and control subjects where compared.

The Pearson correlation was used to describe the relation between the re-warming time, indication of energy added (Q), re-warming velocity (Q/s) of the digits and the CISS score.

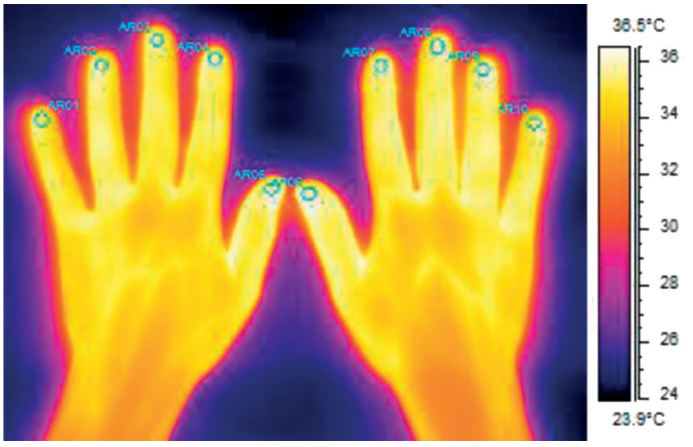

Figure 1 | Videothermographic image of both hands.

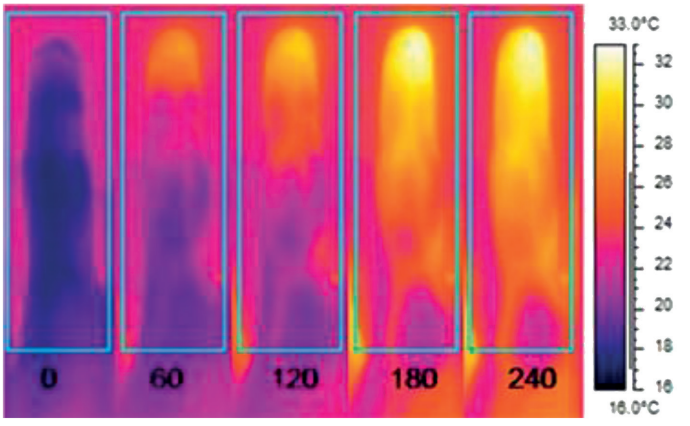

Figure 2 | Consecutive thermographs during the active re-warming phase of a control subject. Active re-warming starts in the fingertips and proceeds in proximally.

Results

Mean CISS score of the control subjects was 0, indicating that all of the control subjects had normal cold tolerance. The mean CISS score of the patients was 27.8 (range: 0-75). Nine of the 18 patients had abnormal cold intolerance based on the CISS Score.

The Semmes Weinstein test did not show abnormal sensitivity in any of the control subjects. Three of the eighteen patients had a decreased sensibility in the fractured finger as assessed by using the Semmes Weinstein test (Table 1).

Cooling the hands in water of 13°C was not experienced as unbearable pain. Figure 3 and 4 show typical examples of a normal and an abnormal re-warming curve. The normal active re-warming of the control subject in Figure 3 starts approximately 50-80 seconds after removing the hands from the cold water. This active re-warming continues until the hands reach an average temperature of 34 degrees Celsius. Figure 4 illustrates an abnormal re-warming pattern in a patient with a hand fracture and with a high level of cold intolerance (CISS=61), affected hand (left) and unaffected contralateral (right) are plotted. The re-warming curve illustrates a noteworthy reduction in the rate of active re-warming in the affected hand compared to the non affected hand.

Although re-warming was abnormal in a number of hand fracture patients, we did not find systematic and meaningful differences between groups, when comparing the duration of re-warming, Q and Q/s. Additionally, no differences between the affected and unaffected hand of the patients, between the dominant and non dominant hand of the controls, and between the affected hand of patients and the hands of the control subjects were found for the determined variables. Consequently, no significant correlations between the variables describing the re-warming patterns (duration, Q and Q/s) for the patients and the CISS scores were found. We also compared only the patients with cold intolerance to the healthy subjects and again found no significantly different re-warming between both groups (for all determined variables $p \geq 0.48$).

Table 1 | Characteristics of study population.

Characteristic	**Control group N=13 (Sd)**	**Patient Group N=18 (Sd)**
Gender (M: F)	8: 5	9: 9
Age (years)	38 (22-58)	45 (21-75)
BMI (kg/m²)	25.0 (21-28)	24.0 (18-35)
CISS-Score	0	27.8 (0-75)
Smokers (cigarettes a day)	3 (2-10)	6 (2-40)
Fracture Geometry	**(%)**	**N (%)**
Transverse		7 (39)
Spiral		4 (22)
Avulsion		2 (11)
Comminuted		2 (11)
Oblique		2 (11)
Longitudinal		1 (6)
Semmes Weinstein		
A (Normal) (2.83)	13 (100)	15 (83)
B (Residual texture) (3.61)		1 (6)
C (Residual protective sensation) (4.31)		2 (11)
D (Loss off protective sensation) (4.56)		0
E (Residual deep pressure) (6.65)		0

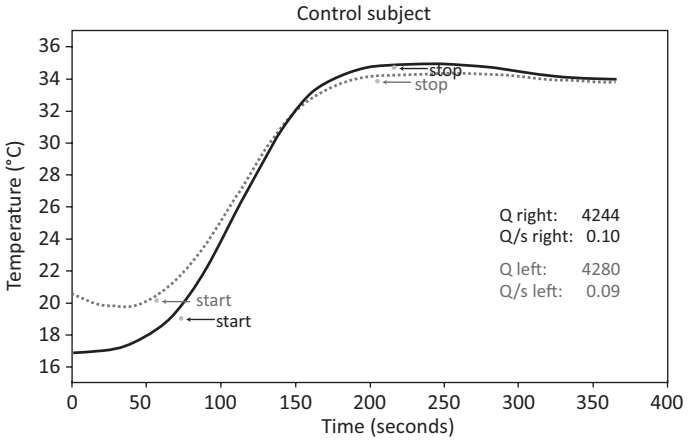

Figure 3 | Re-warming curve single digit of a control subject. Q value as indication of efficiency of heating in a digit, Q/s average re-warming per second. The x-axis indicates the time in seconds, the y-axis the temperature in degrees Celsius.

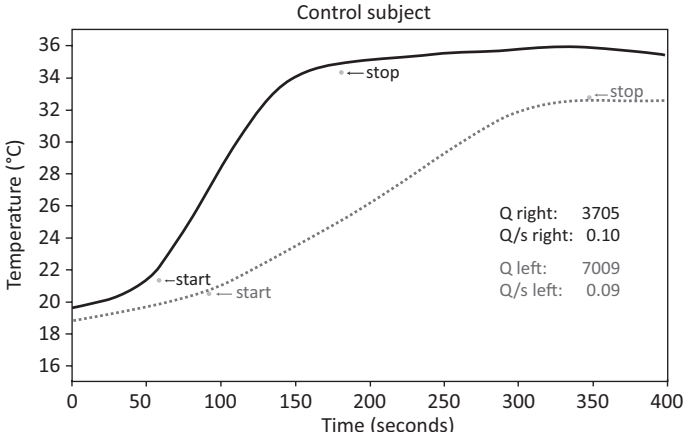

Figure 4 | Re-warming curve of a single digit in a patient with a fracture in the left hand and no injury in the right hand. Q value as indication of efficiency of heating in a digit, Q/s average re-warming per second. The x-axis indicates the time in seconds, the y-axis the temperature in degrees Celsius.

Discussion

The aim of this study was to determine if abnormal re-warming after a cold stress test could be related to cold intolerance. In this study, we found no difference in re-warming patterns between the fractured hand and the non-affected hand of patients with and without cold intolerance or between patients and controls. Furthermore, no correlations for CISS score and the re-warming variables (duration, Q and Q/s) were found, indicating that the severity of cold intolerance in hand fracture patients is not related to the re-warming pattern. These findings indicate that thermoregulatory dysfunction may not be cause of the experienced cold intolerance in these patients.

Disturbed thermoregulation as a result of pathology within the extremities has been reported in a number of studies. For example, the study of Jayanetti et al. revealed abnormal re-warming curves in children with Raynaud's phenomenon.[12] In addition, Suominen et al. reported that a radial forearm flap affects the thermoregulatory system of the donor hand resulting in abnormal re-warming.[36] Greenstein et al. reported that subjects with Raynaud's phenomenon have an impaired thermoregulation which he suggests to explain the cold sensitivity in this patient group.[8] In this study we found abnormal re-warming in both healthy subjects and patients. Secondly, we found no relation between re-warming patterns after cold stress testing in patients with cold intolerance recovering from a hand fracture and subjective cold intolerance. This finding may seem in contrast with the findings from Ruijs et al.,[34] who reported abnormal re-warming in peripheral nerve injury patients with cold intolerance. However, an important difference between both studies was that Ruijs et al. studied nerve injury patients after trauma whereas in the present study patients with nerve injuries were excluded, although damage to the more distal and smaller nerve branches as a result of the trauma cannot be excluded. Our finding of an absent relation between re-warming after cold stress testing and subjective scoring cold intolerance is in line with a study by Traynor and MacDermid on healthy subjects.[38] In addition, the study of Suominen et al., also concluded that abnormal re-warming in patients with a radial forearm flap did not correlate with subjective cold intolerance.[36]

The lack of relation between re-warming and cold intolerance may have important implications for understanding cold intolerance, since it may indicate that disrupted re-warming may not be responsible for the symptoms of cold intolerance. It also put the findings of Ruijs et al. of a relation between abnormal re-warming and subjective cold intolerance in nerve injury patients in a different perspective, indicating that these factors may both be symptoms of nerve injury but without cold intolerance being the direct result of disrupted re-warming. In addition, it can be concluded that direct vascular pathophysiology in, for example Raynaud phenomenon, could cause cold intolerance.

Although this study indicates that cold intolerance is not related to abnormal re-warming and that other mechanisms have to be present, quantifying re-warming may still be important since patients may have more complaints if they have cold intolerance in combination with disrupted re-warming. Future studies are needed to investigate these relations in more detail.

The present study has a number of limitations. A first limitation is that we found our algorithm to determine the start and stop of the active re-warming not working successfully in a number of recordings. Therefore, in the cases where the algorithm failed, we manually selected start and stop of the active re-warming. Although two researchers independently selected both points, we especially encountered difficulties when defining these events in the situation of abnormal re-warming. A second limitation was that the number of patients in this study was relatively small (N=18), leading to a relatively small statistical power. However, in addition to finding no significant differences, we also found no trends of differences between patients and controls and between involved and uninvolved hand, as well as no trends of significance in the correlation between CISS scores and re-warming data, indicating that clinical relevant effects are not present. A third limitation could be the absence of a second cold stress test evaluation to increase the reliability of the results. On the other hand, Traynor and MacDermid concluded that the results between two cold stress test evaluations in the same group were comparable.[38] Another study of Ruijs et al. with a almost identical measurement design concluded that the test retest reliability for the Q value, maximum slope (Q/s) and the cold stress testing was good.[34] Hence, an additional evaluation in both patient and control groups seems unnecessary.

This study revealed that there is no direct relation between cold intolerance in patients with a hand fracture and abnormal re-warming after cold stress testing. As a result, alternative hypotheses are needed to understand cold intolerance, such as mechanisms based on changes in the properties of the temperature-sensory system or other neurological mechanisms. Future studies should include a longitudinal follow-up on a larger and similar patient group starting earlier after injury, this may provide more detailed information about the developments and the courses of the cold intolerance.

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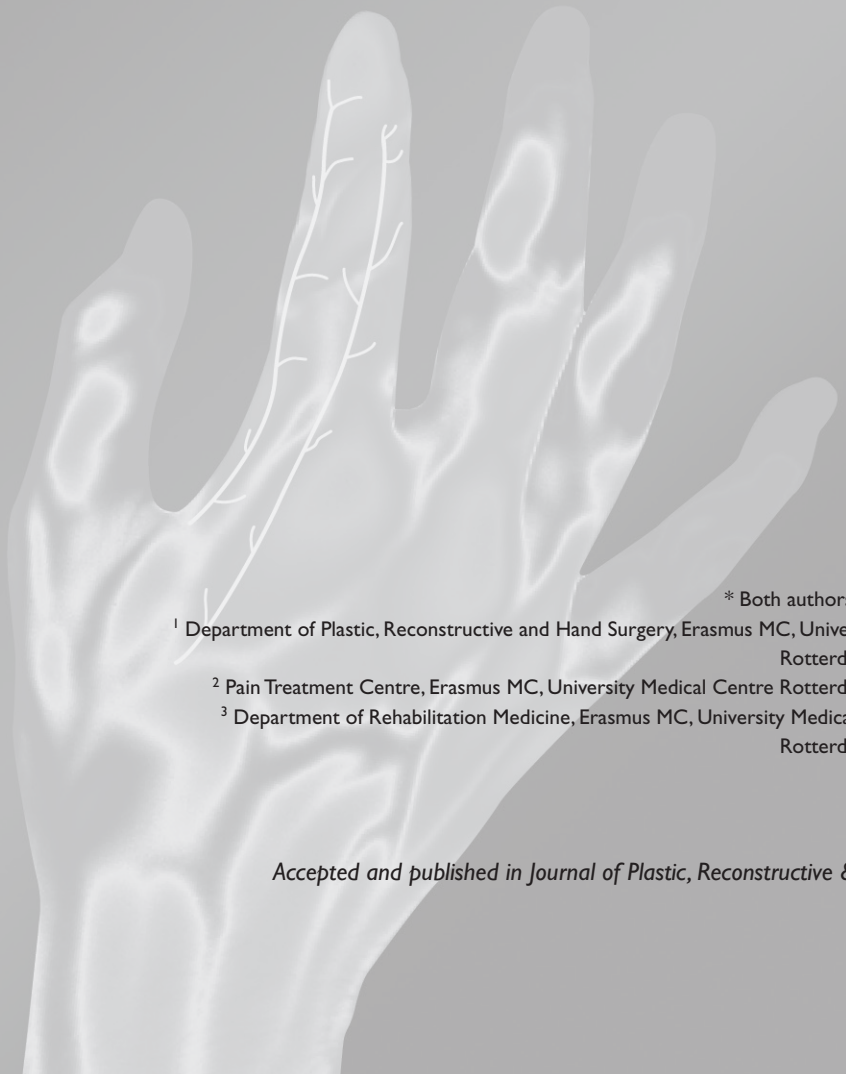
Chapter 4

Thermoregulation in peripheral nerve injury induced cold intolerant rats



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Abstract

Cold intolerance is defined as pain after exposure to non-painful cold. It is suggested that cold intolerance may be related to dysfunctional thermoregulation in upper extremity nerve injury patients. Purpose of this study was to examine if the rewarming of a rat hind paw is altered in different peripheral nerve injury models and if these patterns are related to severity of cold intolerance. In the Spared Nerve Injury (SNI) and Complete Sciatic Lesion (CSL) model the rewarming patterns after cold stress exposure were investigated preoperatively and at 3, 6 and 9 weeks post-operatively with a device to induce cooling of the hind paws. Thermocouples were attached on the dorsal side of the hind paw to monitor rewarming patterns. The Von Frey test and cold plate test indicated a significantly lower paw-withdrawal threshold and latency in the SNI compared to the Sham model. The CSL group, however, had only significantly lower paw-withdrawal latency on the cold plate test compared to the Sham group. While we found no significantly different rewarming patterns in the SNI and CSL group compared to Sham group, we did find a tendency in temperature increase in the CSL group 3 weeks post operatively. Overall, our findings indicate that rewarming patterns are not altered after peripheral nerve injury in these rat models despite the fact that these animals did develop cold intolerance. This suggests that disturbed thermoregulation may not be the prime mechanism for Cold Intolerance and that other, most likely, neurological mechanisms may play a more important role. There is no direct correlation between Cold Intolerance and rewarming patterns in different peripheral nerve injury rat models. This is an important finding for future developing treatments for this common problem, since treatment focusing on vaso-regulation may not help diminish symptoms of Cold Intolerant patients.

Introduction

Cold intolerance (CI) is defined as abnormal pain after exposure to mild to severe cold, with or without discoloration or stiffness of the hand and fingers.[1-5] CI is a frequent sequel of upper extremity injury, especially when neurovascular structures are involved.[5-9] Its reported incidence varies, probably due to variability in testing method, between 56% and 83% in humans.[1,4,10] CI has been found to be the most disabling symptom following peripheral nerve injury, and it does not diminish over time.[1,3,5,10,11] CI has been reported in a wide range of pathologies, such as patients with Raynaud's disease,[12-15] upper extremity fractures,[15] digital replantation,[6,7,16-19] and after raising radial forearm flaps. Unfortunately, because the pathophysiology of CI is still unclear, there is no treatment available, other than symptomatic relief.

CI may be related to dysfunctional thermoregulation in peripheral nerve injury patients. [16,20] In healthy people, after cooling of an extremity, the thermoregulatory system increases blood-flow to counteract decrease in temperature and to prevent pain and frostbite.[21] After a peripheral nerve injury, thermoregulation may be impaired, as was shown by Ruijs et al.[20] However, other studies do not support this theory, because low blood flow does not correlate with subjective symptoms of CI.[7,16,18] This was both found in patients with digital replantation or revascularization and in patients followed by raising a radial forearm flap for reconstruction purposes.[7,22-25] The diversity and complexity of the pathogenesis of CI could be a reason for the inconsistent results. Co-morbidity, such as vascular damage, could be a major factor. Gender, age and different definitions of CI could also be attributed for different outcomes.

An unresolved issue remains how different types of nerve injury influence thermoregulation. In clinical studies, because of the complexity of upper extremity traumas, regeneration and re-innervation of damaged and undamaged nerves is difficult to determine. An animal study with a nerve injury model that systematically inflicts CI would be a suitable set-up to answer these questions. Well-documented nerve injury rat models that are known to be cold hypersensitive are the Spared Nerve Injury[26] (SNI) and the Complete Sciatic Lesion (CSL)[27] models. Advantages of these models are the reproducibility and circumscriptive nerve injury application and the subsequent well-characterized CI behaviour. However, there are no studies that investigate the relation between peripheral nerve injuries causing CI in a rat model and thermoregulation.

To determine the function of thermoregulation, the re-warming pattern after cold stress exposure was used as a measure, a frequently performed and validated method to evaluate thermoregulation.[20,28] Our hypothesis was that thermoregulation of rat hind paw is altered in peripheral nerve injury and that these patterns are related to the severity of CI.

Methods

The Animal Ethic Experiment Commission approved all experimental procedures. Twenty-four male Wistar rats, weight 300-350g, were divided into three groups. The first group (N=8) was operated on the left hind limb using the SNI model.[26] The second group (N=8) received the sham model,[26] while the third group (N=8) had the CSL operation. Two of the eight SNI rats, however, were excluded from further analysis because of auto mutilation. All measurements were at the same location under similar conditions.

Surgery

Under isoflurane (2%) anaesthesia the skin on the left lateral surface of the thigh was incised and a division was made through the biceps femoris muscle exposing the sciatic nerve and its three main branches: the sural, common peroneal, and tibial nerves. A transection and ligation with 5.0 silk of the tibial and common peroneal nerves was performed leaving the sural nerve intact in the SNI model (Figure 1A). Great care was taken to prevent contacting or stretching the intact sural nerve. CSL involved a ligation of the sciatic nerve, 1 cm above the three main branches of the sciatic nerve (Figure 1A). Sham controls involved exposure of the sciatic nerve and its branches without nerve damage.

Measurements

Measurements were performed one day preoperatively and at three, six and nine weeks postoperatively. Von Frey test and Cold plate test were taken prior to the measurements. Before each experiment the rats were habituated to the experimenter, the experiment room and the cages where the behavioural tests were performed for 5 days. Thereafter, the rats where habituated for 30 minutes prior to each experiment to reduce stress.

Cold plate test

During the cold plate test, rats were placed in a clear covered chamber black-blinded Plexiglas cage (50 cm × 50 cm × 50 cm) on an aluminium plate with a surface temperature of 5°C. We measured the paw withdrawal latency, defined as the latency of the first operated paw lift after rat placement on the cold plate. An experimenter who was blinded for the test groups manually measured the latency of the operated hind paw.

Von Frey test

During the Von Frey test, withdrawal threshold to touch was measured with a set of Von Frey hairs ranging from 0.6 to 300 g in a set of 14 filaments steps. The rat was placed in a chamber with a mesh metal floor, covered by a Plexiglas dome of 20x30 cm and 10 cm high. The dome enabled the rat to walk freely but not to rear up on its hind limbs. Hence, the experimenter was

able to reach the plantar surfaces of the operated paw from below, unobserved by the rat. Each Von Frey hair was applied for 2 s at 5 s intervals. The Von Frey test was considered positive when at least 3 of 5 applications evoked responses.

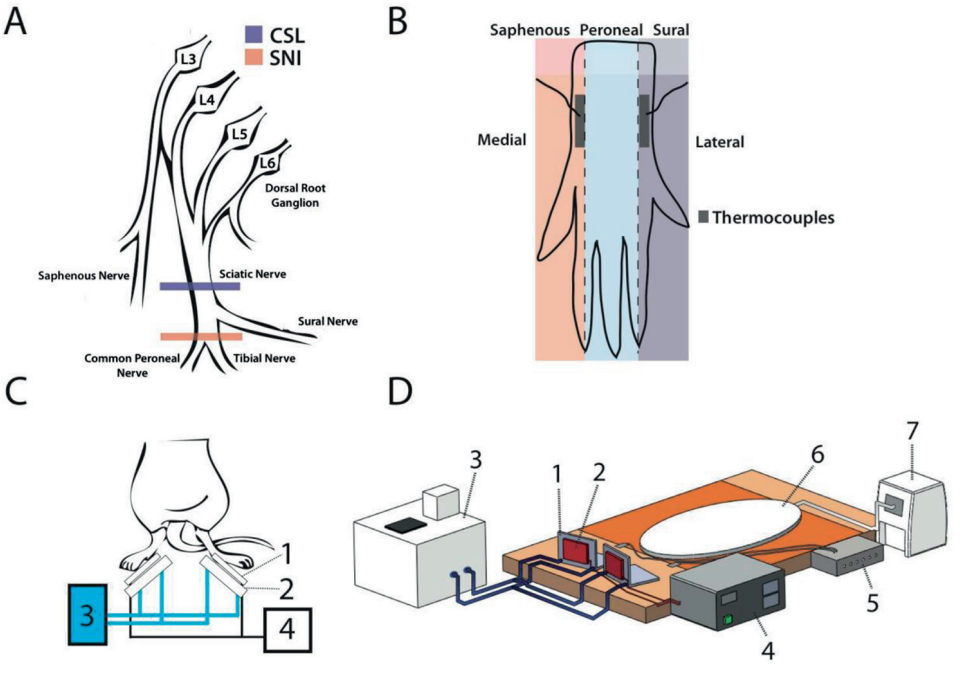

Figure 1 | Illustration of the experiment. **A**: The complete Sciatic Lesion model (CSL) comprises a lesion of the sciatic nerve while the Spared Nerve Injury model (SNI) comprises a lesion of the tibial and common peroneal nerve. **B**: Dorsal sides of a rat hind paw with its nerve innervations areas. Thermocouples are attached on the medial (saphenous) and lateral (sural) part of the paw. **C**: Illustration of the cooling set-up. **1**: Peltier elements, 2: Water coolers, **3**: Thermostatic bath, **4**: Power supply. Under anesthesia the rat is positioned on the dorsal side with the hind paws placed on the Peltier elements for cooling. **D**: Illustration of the complete cooling set-up. **1**: Peltier elements, **2**: Water coolers, **3**: Thermostatic bath, **4**: Power supply, **5**: USB-based 8-channel thermocouple input module, **6**: Heating mattress, **7**: Heating mattress regulator.

Rewarming after cold exposure

Our department developed a set-up to induce cold stress exposure bilaterally in the hind paws of a rat and to measure skin surface temperature at the same time.[29]

The anaesthetized rats were placed in a dorsal position, with the hind paws in prone position. The ventral sides of the hind paws were placed onto two vertically positioned Peltier cooling elements using surgical tape (Figure 1C). A Peltier cooler is a solid-state active heat pump that transfers heat from one side of the device to the other to generate cooling. The dorsal side of the paws was left exposed for thermocouple attachment to measure the temperature.

The thermocouple probes were placed on lateral and medial side of both limbs (Figure 1B). A rectal probe was used to monitor the core temperature and one thermocouple measured the ambient temperature. A sample rate of 2 measurements every second was used by an USB-based 8-channel thermocouple input module (Measurement Computing); Calibration of the device and the thermocouples was performed using Instacal (v5.82, Measurement Computing). The data storage was provided using a custom developed program in Labview (v7.1, National Instruments). Because both hind limbs were cooled, body core temperatures had a tendency to decrease, therefore a heating mattress (39.5°C) was used to maintain body core temperature. Both Peltier elements were set at a temperature of three degrees Celsius. Respiratory rate was monitored as an indicator of depth of anaesthesia throughout the experiment.

The measurement had three phases. A pre-phase (five minutes) to measure the temperature without cooling and also to measure the standardized baseline temperature of an anesthetized rat. Secondly, we cooled the rats for 20 minutes. In the third phase (also 20 minutes) we measured the rewarming pattern of the rat, after the hind limbs were detached from the Peltier elements. The total measurement took 45 minutes for each rat.

Quantification of the rewarming pattern

Measured temperatures were stored in an in-house developed Labview program; the raw data were exported to Matlab. We used two methods to quantify the rewarming patterns. First, the increase in temperature of the rewarming curve was determined by calculating the difference between the start of the rewarming curve and the highest temperature during the first 325 seconds of the rewarming phase (Figure 3A). In addition, the first derivative of the time-temperature curve was calculated. From this first derivative, we determine the maximum rate of temperature change (°C/min) (Figure 3B) during the first 325 seconds.

Statistical analysis

Values are means ± SE. The software package SPSS was used for all statistical analysis. Effects were considered significant if $P \leq 0.05$. We used a two way ANOVA with one repeated-measures factor time (preoperatively and three, six and nine weeks postoperatively), and one between-subject factor group (SNI, CSL and Sham). To test differences between medial and lateral side of

the hind paw we also used the Two-way ANOVA. To test the normality of the data of each week we used a Shapiro-Wilk test. In case of a significant main effect, a Bonferroni-corrected post-hoc test was performed. For the Von Frey test we used a non-parametric Kruskal-Wallis test with the same factors (time and group) and a Dunn's post-hoc test.

Results

Cold Plate and Von Frey Test

The sham rats displayed no paw withdrawal responses (PWL) when placed on a temperature controlled surface of 5°C up until 140 seconds. Paw withdrawal latency in SNI rats was significantly reduced as compared to the sham operated rats at all postoperatively time-points ($P < 0,001$). CSL rats showed a significant reduced PWL only at 6 and 9 weeks compared to Sham rats ($P < 0,001$). There was no significant difference between the CSL and SNI group at 6 and 9 weeks postoperatively (Figure 2A).

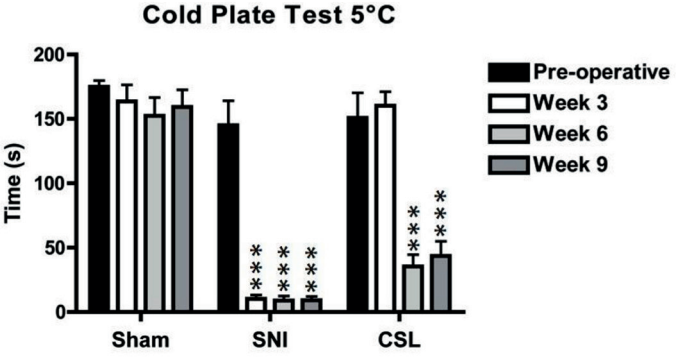

Figure 2A | Cold Plate Test and Von Frey Test. **A**: The paw lift latency in seconds during the different measurement times in all three groups. Paw lift was significantly faster in the SNI group at all postoperative times (3W: 10.2±3. 6W: 8.9±3.0 9W: 9.0±3.4) as compared to the sham group (175.0±4.9). For the CSL group there was no significant difference at 3 weeks postoperatively as compared to the sham group in paw withdrawal latency. Only at 6 (35.4±10.8) and 9 weeks (43.6±9.1) postoperatively there was a significantly lower paw lift withdrawal latency in the CSL group as compared to the sham group. * $P < 0,05$, ** $P < 0,01$, *** $P < 0,001$.

The SNI group had a significantly lower withdrawal response of the paw during the Von Frey test at all investigated postoperatively times as compared to Sham ($P < 0,01$). The CSL group did not have a significantly lower withdrawal response compared to the Sham group. However there was no significant difference between the CSL and SNI group at 6 and 9 weeks (Figure 2B).

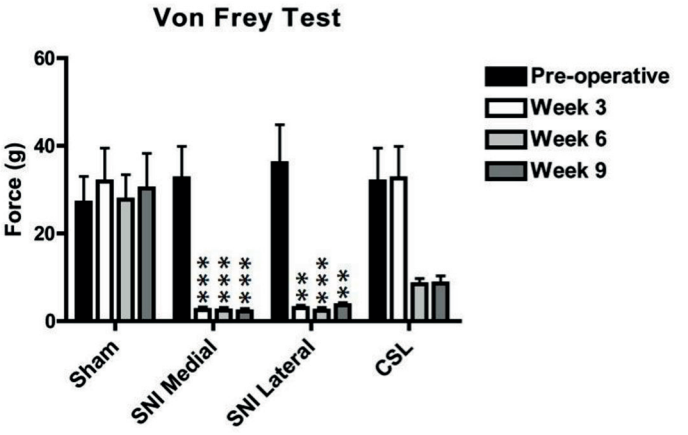

Figure 2B | Amount of force of the Von Frey filaments needed to obtain a mechanical reflex withdrawal response. Force was reduced for the SNI group at 3 (3.0±0.7), 6 (2.4±0.7) and 9 (3.6±0.6) weeks postoperatively compared to the Sham group (27.0±6.0) while the CSL group had no significant lower withdrawal (3W; 32.6±7.3, 6W; 8.4±1.3, 9W; 7.6±1.4) response compared to the Sham group. However there is no significant difference between the CSL and SNI group at 6 and 9 weeks postoperatively. * P < 0,05, ** P < 0,01, *** P < 0,001.

Ambient Temperature and Core Temperature

Mean ambient temperature during the cold exposure experiment was 25.1°C, and minimum surrounding temperature did not drop below 22.8°C. The core temperature of the rat was stable; 35.9°C, SD 1.4°C. Minimum temperature dropped below 35°C in 24 measurements. All surrounding and rectal temperatures are displayed in Table 1.

Rewarming Pattern

Figure 3 shows videothermographic images of the cold stress exposure during cooling with the 3°C Peltier plates and during rewarming of the hind paws. Figure 4 shows a typical example of the rewarming pattern of the lateral side after 20 minutes cold stress exposure measured preoperatively in a Sham rat.

We found no significant difference in increase in temperature for the SNI group compared to the Sham group at 3, 6 and 9 weeks post-operatively (P=0.337) (Figure 5). For the CSL group there is a higher tendency in increase in temperature 3 weeks postoperatively compared to the Sham group. At week 6 and 9 this stabilizes to pre-operative levels. There was no difference between the medial and lateral side in Δ temperature in all groups.

Between the different groups, post-surgery, we found no significant difference in rate of rewarming (P=0.780) (Figure 6). In addition, there was also no significant difference between the medial and lateral side of the operated hind paws in the different groups postoperatively.

Table 1 | Ambient and rectal temperatures during the experiments.

	Mean	Std. Deviation
Mean rectal	35,87	1,43
Minimal rectal	35,07	1,95
Maximum rectal	36,88	,90
Mean surrounding	25,10	,83
Minimal surounding	24,63	,73
Maximal surounding	25,74	,89

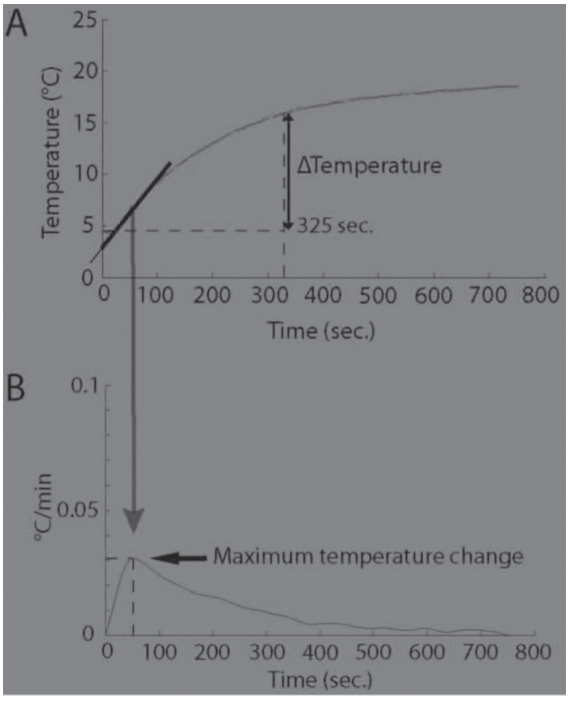

Figure 3 | Typical example of the temperature during the rewarming phase and illustration of the outcome measures. A: Increase in temperature is determined by calculating the difference between the lowest and highest temperature of the rewarming curve (Δ Temperature) during the first 325 seconds in the rewarming phase. B: Maximum rate of temperature increase was calculated by taking the first derivative of the time temperature curve. The highest point in the first derivative graph (°C/min.) is the maximum change in temperature during the rewarming phase.

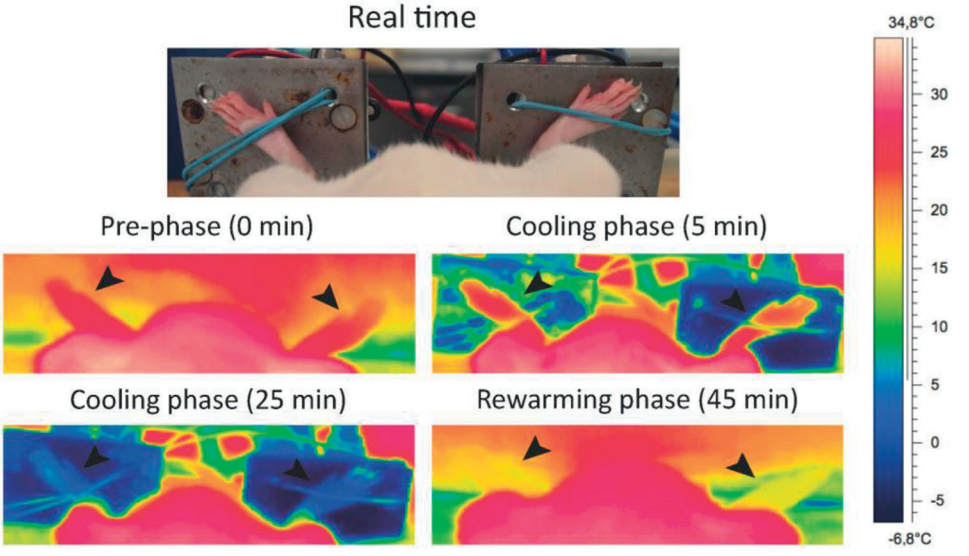

Figure 4 | Thermographic imaging of the experiment illustration the body of the rat, both hind paws and the Peltier element. **Pre-phase** (5 min.): Rat is dorsally positioned under anesthesia to measure baseline temperatures of the hind paws. **Cooling phase** (20 min.): Both hind paws are attached to the Peltier elements. The color indicates that both Peltier elements and hind paws get equally cooled. **Rewarming phase** (20 min.): Hind paws are detached from the Peltier elements and the elements are displaced, therefore not being able to affect the environmental temperature.

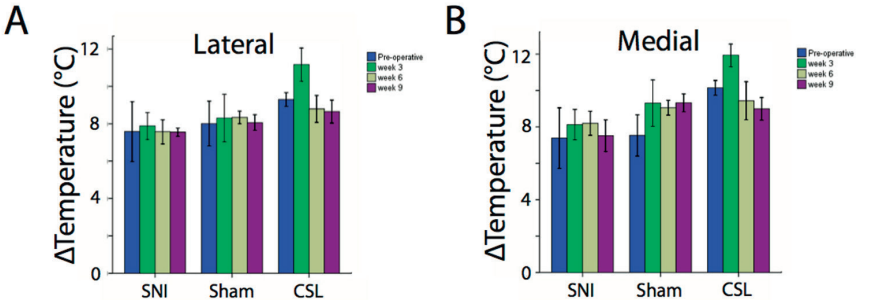

Figure 5 | Increase in Temperature: Graphs show the increase in temperature in the rewarming phase. There is no significant difference in the SNI group post-operatively compared to the Sham group. In the CSL group there is a tendency in higher increase in temperature on week 3, compared to the Sham and SNI group. There is no difference in increase of temperature between the measured medial and lateral side. On week 3 with the ANOVA test there was no significant difference between the CSL and Sham group on week 3.

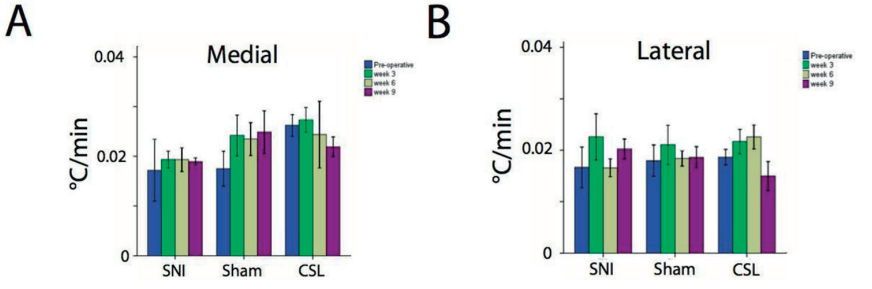

Figure 6 | Maximum Change in Temperature. We found no significant difference in maximum change in temperature between the different groups post-operatively and no difference in acceleration speed between the medial and lateral side.

Discussion

The purpose of this study was to examine if the rewarming pattern of a rat hind paw is altered in peripheral nerve injury models and if these patterns are related to severity of CI. We used the SNI and the CSL models, which, based on Cold Plate Test, both developed cold hypersensitivity. However, only the SNI group displayed a significantly lower PWL. While no significantly different rewarming was found in the SNI and CSL group compared to the Sham group, we did find a tendency in temperature increase in the CSL group 3 weeks postoperatively. However, overall our findings indicate that rewarming patterns are not altered after peripheral nerve injury in these rat models despite the fact that these animals did develop CI.

Our findings seem in contrast with Ruijs et al.,[20] who reported abnormal rewarming in peripheral nerve injury patients with cold intolerance. However, an important difference between both studies was that Ruijs et al. studied nerve injury patients after trauma, where possible vascular damage cannot be excluded. This study used a Sham model as a negative control and to exclude vascular damage. Our findings of an absent relation between rewarming pattern after cold stress exposure in CI rats is in line with other clinical studies, although in these studies the extent of nerve and vascular damage is not known.[7,16,18,23-25,30] Nevertheless, Smits et al. showed that in patients with finger fractures there was no relation between CI complaints and abnormal rewarming pattern after cold stress exposure.[31]

It is known that Transient Receptor Potential channels (TRP) register ambient temperature, and that deregulation of the properties of these channels could possibly contribute to CI in patients with peripheral nerve injury.[32-34] Despite this, our study indicates that peripheral thermoregulation can still be intact after a peripheral nerve injury in a rat model, possibly suggesting that other receptors could interact with blood vessels and react with different ambient temperatures.

Another explanation for the discrepancy between Ruijs et al. and this study could be the different ratio between extremity size and body size in humans compared to paw size and body size in rats. Extremities of humans are relatively large and distant from the body. Therefore, disruption of thermoregulation could be more difficult for the body to cope with than in rats, where the paws are relatively small and close to the body.

The finding of intact thermoregulation after denervation of the sympathetic system has been reported in a previous study. Kalincik et al. showed that rats with a complete T11 spinal cord transection did not have a change in tail surface temperature and arterial flow during 20 minutes cold exposure of 6-9°C.[35] In addition, they found a full tail vasoconstriction in the spinal cord transection group during cold exposure.

Although the above-mentioned study did not find substantial alterations in thermoregulation after nerve transection, Laird et al. showed in a T4 spinal cord transection rat model an increase in tail temperature at room temperature (23°C).[36] In our study, the CSL model had a higher temperature increase in week 3, but this stabilizes at 6 and 9 weeks postoperatively.

The temperature increase in week 3 and stabilization in week 6 and 9 could be explained by a combination of two mechanisms. First, absent sympathetic activity that leads to a loss of vasoconstrictor tone in cutaneous blood vessels and thus increased skin blood flow may cause a higher temperature increase of the hind paw during the rewarming phase. However, there was no significant difference between the medial and lateral side of the CSL model. Higher temperature increase in the larger denervated lateral and centre area could lead to a higher temperature increase in the smaller innervated medial area. There could be still strong direct vasoconstrictive response by the peripheral vasculature to cold; an in vivo study showed that cutaneous circulation is locally regulated by directly cooling the skin.[37]

An alternative explanation for the normal thermoregulation could be the hyperactivity of adrenoceptores of blood vessels to circulating catecholamines. It is known that cold exposure results in an increase in levels of plasma catecholamines, thereby still controlling the denervated vasculature in cold environment.[38-40] A possible explanation for the normal rewarming pattern in the SNI model is that undamaged nerves innervated the measured areas. Sprouting of nerve fibres from the medial area to the centre and lateral area in the CSL group starts at 4 weeks, whereby at 3 weeks the fibres are to far from the denervated blood vessels in the centre and lateral area to exert action on, therefore stabilizing the rewarming pattern at week 6 and 9.[41]

Skin temperature correlates highly with skin blood flow and skin sympathetic nerve activity. Therefore, changes in central or peripheral mechanisms of blood flow regulation are, in part, represented by changes in skin temperature, measured by the thermocouples.[20,42]

Ruch et al.[43] described that skin blood flow can be divided into nutritional flow and thermoregulatory flow. Thermoregulatory blood flow, which is regulated by arteriovenous anastomoses (AVA), accounts for 90% of skin blood flow in humans and therefore skin temperature has a good correlation with skin blood flow.[44] The percentage of thermoregulatory blood flow in rats is unknown. However, because rats are living in homeostasis with their environment, the amount of thermoregulatory blood flow is likely to be similar as in humans. Therefore, we believe that also in rats, AVA's determine the thermoregulatory flow and are related to the hind paw temperature. However, due to shunting of the AVA's, the relation between thermoregulatory flow and skin temperature could be altered. Therefore, future studies may focus on directly measuring blood flow in these CI induced rat models.

A limitation of our study was the utilization of anesthetized rats instead of awake rats, which may affect our results. However it is not possible to measure these CI rats while awake, since they are cold hypersensitive and will avoid contact with cold surfaces. Another limitation was that the sham-treated animals showed differences in withdrawal response over time during the Von Frey test, although not significant. Presently, the Von Frey test is the only test to measure mechanical sensitivity in rodents. Variability in the sham-treated test could be explained by the non-continues values, where the last three hairs of the test are 15, 26 and 60 grams. In summary, this study revealed that rewarming patterns are not altered after a peripheral nerve

injury in these rat models and that these patterns do not relate to CI. This indicates that CI is not influenced by disturbed thermoregulatory vascular responses. Our study suggests that other aetiologies such as changes in properties of sensory system or other neurological mechanisms or abnormal shunting with nutritional deprivation may play an important role in development and maintaining of CI.

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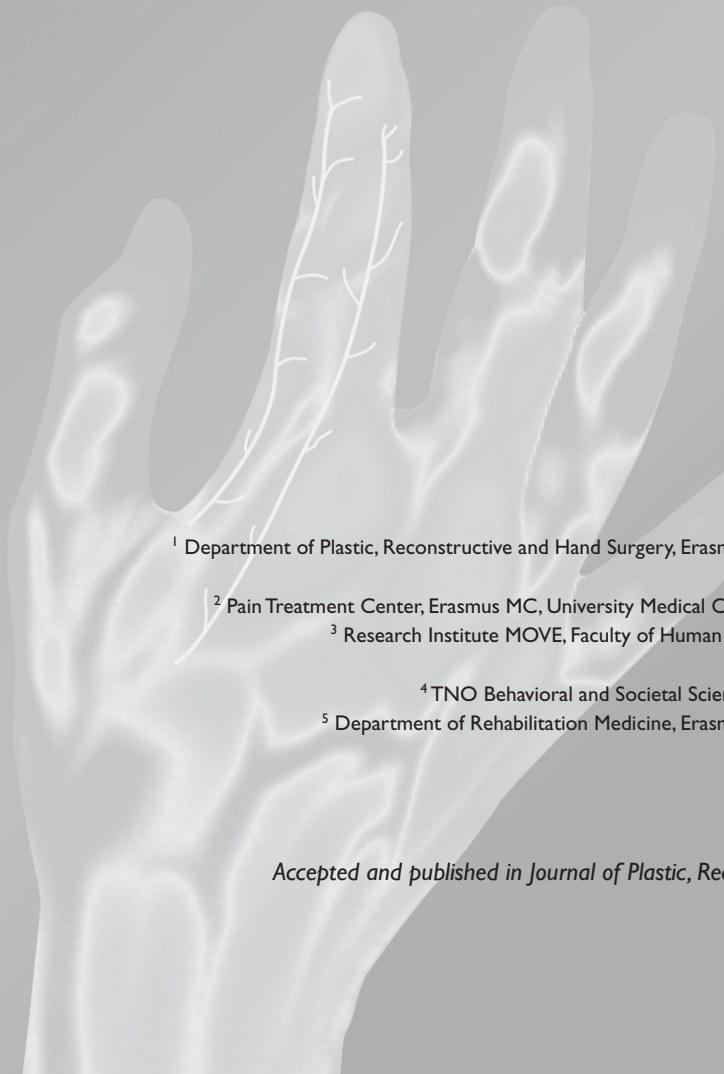
Chapter 5

Cold-induced vasodilatation in cold-intolerant rats after nerve injury



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Abstract

Purpose

Cold Induced Vasodilatation (CIVD) is a cyclic regulation of blood flow during prolonged cooling of protruding body parts. It is generally considered to be a protective mechanism against local cold injuries and cold intolerance after peripheral nerve injury. The aim of this study was to determine the role of the sympathetic system in initiating a CIVD response.

Methods

Eight rats were operated according to the Spared Nerve Injury (SNI) model, eight underwent a Complete Sciatic Lesion (CSL) and six underwent a sham operation. Prior to operation, three, six and nine weeks postoperatively, both hind limbs were cooled and skin temperature was recorded to evaluate the presence of CIVD reactions. Cold intolerance was determined using the cold plate test and mechanical hypersensitivity measured using the Von Frey test.

Results

No significant difference in CIVD was found comparing the lateral operated hind limb for time (preoperatively and three, six and nine weeks postoperatively; P=0.397) and for group (SNI, CSL and Sham; P=0.695). SNI and CSL rats developed cold intolerance and mechanical hypersensitivity.

Conclusion

Our data show that the underlying mechanisms that initiate a CIVD reaction are not affected by damage to a peripheral nerve that includes the sympathetic fibers. We conclude that the sympathetic system does not play a major role in the initiation of CIVD in the hind limb of a rat.

Clinical Relevance

No substantial changes in the CIVD reaction after peripheral nerve injury imply that the origin of cold intolerance after a traumatic nerve injury is initiated by local factors and has a more neurological cause. This is an important finding for future developing treatments for this common problem, since treatment focusing on vaso-regulation may not help diminish symptoms of cold intolerant patients.

Introduction

Abnormal pain after exposure to cold is one of the most common long-term complaints of patients after a nerve injury. The incidence of cold intolerance in humans with a nerve injury is estimated to be 56% to 83%.[1-4] Unfortunately, the only remedy described for cold intolerance is to instruct this patient group to avoid cold stimulations.[5] An important reason why treatment is unavailable may be that the pathophysiology of cold intolerance is still unclear, in particular the mechanism of Cold Induced Vasodilatation (CIVD).

CIVD is a cyclic regulation of blood flow during prolonged cooling of protruding body parts, such as the hands, feet, chin and nose.[6,7] Lewis et al. first described CIVD in 1930,[8] and it is generally seen as a protective mechanism for local cold injuries.[9,10] In healthy humans, CIVD generally occurs after a minimum cooling time of 5 minutes in an environment of maximally 15°C.[11] However, changes in CIVD response in individuals who live in cold environments appear to be neither guaranteed nor predictable.[12] The presence and nature of a CIVD reaction depends on a large number of variables. For example, a higher body core temperature as well as the intake of food leads to a stronger and faster CIVD reaction.[6,13-15] Changes in the CIVD reaction have been described in patients with cold intolerance after a traumatic peripheral nerve injury.[16-19] It has been suggested that there is a relation between CIVD and the treatment of posttraumatic cold intolerance.[19]

It is undisputed that the sympathetic nervous system plays a role in the magnitude of CIVD, since the magnitude of CIVD is strongly dependent on central body temperature.[14,20] However, it is still debated if the sympathetic system is initiating the CIVD response or if peripheral triggers are responsible. Flouris et al. (2008) exposed ten appropriately dressed adults to -20°C and observed oscillatory changes in finger blood flow that were related to body core temperature.[21] They concluded that these changes, which they called CIVD, appeared to be the 'eventuality of the thermoregulatory function based on the suppression and activation of the sympathetic vasoconstrictor system'. Daanen (2009), however, argued that these changes should not be named CIVD, since the finger skin temperatures ranged from 7.2 to 33.5°C and the observed fluctuations during the experiment did not resemble the typical cyclic CIVD reaction.[22] Later, Flouris and Cheung (2009) concluded from changes in heat balance during exposure of hands to cold air that "CIVD is a centrally originating phenomenon caused by sympathetic vasoconstrictor withdrawal".[23] It was challenged that these observations showed that finger blood flow and body heat content were unrelated.[24] Since eliminating the sympathetic drive is hard to accomplish in humans without side effects, we decided to investigate the role of the sympathetic system in rat paws in a systematic way.

Although the locations where CIVD is observed differ between rats (mainly tail and paws) and humans (mainly fingers and toes), the mechanism and control seems to be comparable, the Arterio-Venous Anastomoses (AVA's) that are under sympathetic control are the anatomical

structures where CIVD is observed.[24-27] In this study, we evaluated the CIVD reaction in rats with different types of nerve injury because this allows us to standardize the nerve lesion and to exclude additional factors caused by the trauma such as vascular damage. To do so, a spared nerve injury model (SNI), and complete sciatic lesion (CSL) model[28,29] was used. The SNI and CSL models are well-documented nerve injury models that are known to be cold intolerant.[29,30] As in cold stress testing, evaluation of the CIVD reaction may be a tool to assess the quality of the thermoregulatory system in rats after peripheral nerve injuries. It is presently unknown if the timing and amplitude of the CIVD reaction during cold stress is modified in rats with peripheral nerve injuries and whether the presence or absence of a CIVD reaction is related to cold intolerance.

The aim of this study was to determine the role of the sympathetic system in initiating a CIVD response. To do so, prior to operation as well as three, six and nine weeks postoperatively, both hind limbs were cooled and skin temperature was recorded to evaluate the presence and absence of CIVD reactions. The rats were measured if they experienced presence of cold intolerance and mechanical hypersensitivity.

Material and methods

A recently developed and validated set-up[31] was used to measure the CIVD response bilaterally. All animal experimental procedures were approved by the Animal Experiment Commission of the Erasmus MC and were performed in accordance with the National Institutes of Health guidelines on animal care.

Twenty-two male Wistar rats, (weights 350-500g) were randomly divided into three groups. In one group (N=8) the left hind limb was operated according to the SNI model of Decosterd and Woolf,[29] in the second group (N=8) the left hind limb of the rats underwent a Complete Sciatic Lesion (CSL)[30] and in the third group (N=6) the left hind limb was operated according to the sham model[29] to compare and verify these models. All Wistar rats underwent the same general treatment and were not significantly different for gender, weight, habitat and nutrition.

Surgery

Under isoflurane (2%) anesthesia, the skin on the left lateral surface of the thigh was incised and a deviation was made through the septum of the biceps femoris muscle exposing the sciatic nerve and its three main branches: the sural, common peroneal and tibial nerves. The SNI procedure comprised an axotomy and ligation of the tibial and common peroneal nerves leaving the sural nerve intact. The common peroneal and the tibial nerves were tightly ligated with 8.0 ethilon and sectioned distal to the ligation, removing 1.0 mm of the distal nerve stump. Great care was taken to avoid any contact with, or stretching of the intact sural nerve. Septum and skin were

closed in two layers. CSL compromised a ligation of the sciatic nerve, 1cm above the three main branches of the sciatic nerve (Figure 1A). Sham controls involved exposure of the sciatic nerve and its branches without any lesion.

Cold plate test

To measure the presence of cold intolerance we evaluated the limb withdrawal time. The rats were placed in a clear covered chamber black-blinded Plexiglas cage (50 cm × 50 cm × 50 cm) on an aluminum plate with a surface temperature of 5°C. We measured the limb withdrawal latency, which defines the latency of the rat first limb lift after the rat has been placed on the cold plate.[32] An experimenter who was blinded for the test groups manually measured the latency. Measurement of the limb withdrawal latency was performed preoperatively and three, six and nine weeks postoperatively.

Von Frey test

To measure the presence of mechanical hypersensitivity we used the Von Frey test. Withdrawal threshold to touch was measured with a set of Von Frey hairs ranging from 0.25 to 60.0g. The rat was placed in a chamber with a mesh metal floor, covered by a Plexiglas dome of 20x30 cm and 10 cm high. The dome enabled the rat to walk freely but not to rear up on its hind limbs. Hence, the experimenter was able to reach the plantar surface of the limbs from beneath, unobserved by the rat. Each Von Frey hair was applied for 2s at 5s intervals. When at least 3 of the 5 applied applications evoked responses, the Von Frey test was considered positive. At threshold, rats responded by a quick limb flick. An experimenter who was blind for the different test groups measured this manually. Measurement of the Von Frey test was performed preoperatively and three, six and nine weeks postoperatively.

Evoking CIVD reactions

The rats were placed in a supine position (Figure 1C). We measured temperature on seven different points on the lower extremities as well as rectally. The ventral sides of the hind limbs were placed onto two vertically positioned Peltier cooling elements (Figure 1 C') using surgical tape. A Peltier cooler is a solid-state active heat pump that transfers heat from one side of the device to the other to generate cooling. The dorsal sides of the limbs were left exposed for thermocouple attachment and to allow thermographic recording.

The thermocouple probes were placed on the marked areas on both limbs (Figure 1B). One thermocouple was attached to a rectal probe to monitor the core temperature and one thermocouple measured the ambient temperature. Thermocouple registration with a sample rate of 2 measurements every second was done by an USB-based 8-channel thermocouple input module (Measurement Computing). Calibration of the device and the thermocouples was performed using Instacal (v5.82, Measurement Computing). The data storage was provided using an in house developed program in Labview (v7.1, National Instruments) and data were

exported to Matlab (version 7.8 R2009a). Peltier elements were controlled at a temperature of 3°C, resulting in the cooling of both limbs of the rat. In order to prevent a drop in rectal temperature in an anesthetized rat due to hind limb cooling, a heating mattress (39.5°C) was used to maintain body core temperature during the pre phase and the cooling phase. Respiratory rate was monitored as indicator of depth of anesthesia throughout the experiment.

The measurement was divided into two phases. We started with a pre-phase of five minutes to measure the temperature of the anesthetized rat at rest without cooling. Thereafter we cooled the rats for twenty minutes to evoke a CIVD reaction. Based on the literature of work in rat tails by Thomas et al. and Gardner et al. and in humans by Daanen[6,25,28] we chose a cooling period of 20 minutes to investigate the role of the sympathetic system in initiating a CIVD response.

Temperature measurements (CIVD reactions) were performed pre-operatively and on three, six and nine week's post-operative. All measurements took place at the same location under similar conditions. All rats were cared for each day for the duration of the entire experiment. Rats stayed in the experiment room until the anesthesia had worn out. The rats were provided with water and food ad libitum. All rats were habituated for at least seven days before any measurements were executed. The weight of the rat was recorded on every day of the measurement.

The measurement period started 375 seconds after the start of cooling and lasted for 850 seconds. As a first step, we fitted the data using a low-pass Gaussian filter with a standard deviation of 44 seconds to model the overall change in temperature and to remove higher frequency noise (see Figure 2). CIVD was defined as an increase in temperature of the filtered signal of at least 0.4°C. When CIVD was present, we scored the number of CIVD reactions in each rat as well as the increase in temperature of each CIVD. The amount of CIVD waves was counted; the height of the CIVD reaction was scored from 1 to 4. The height was scored from 1-4 based on temperature increase in tenths of °C. We plotted the raw and the filtered data and cut and enlarged the CIVD reaction. Three investigators scored the blinded plots. When the investigators disagreed in scoring they discussed the situation and together they defined the score. When all CIVD's were identified, we scored the number of CIVD reactions in each rat and calculated the time of onset of the CIVD after cooling was initiated.

Statistical analysis

Values are means ± SEM. The software package SPSS was used for all statistical analysis. Effects were considered significant if $P \leq 0.05$. We used a two way ANOVA with one repeated-measures factor for time (pre operative and three, six and nine weeks post operative) and one between-subject factor for group (SNI, CSL and Sham) to evaluate changes of time and differences between groups. To test the normality of the data of each week we used a Shapiro-Wilk test.

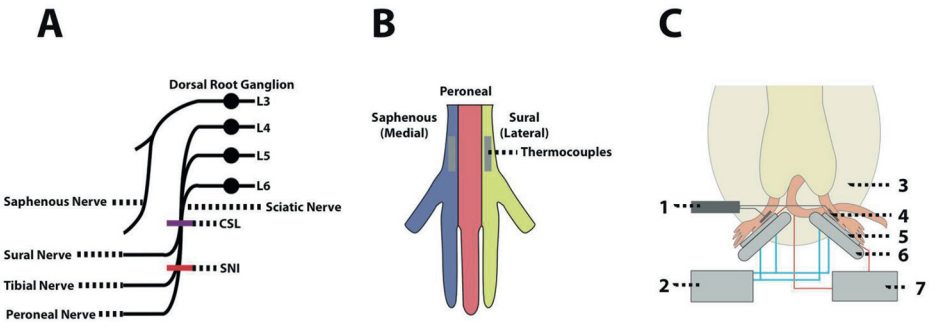

Figure 1 | 1A: Operation methods of the Spared Nerve Injury (SNI) and the Complete Sciatic Lesion (CSL). The SNI procedure a ligation of the tibial and common peroneal nerves leaving the sural nerve intact. CSL compromised a ligation of the sciatic nerve, 1 cm above the three main branches of the sciatic nerve. Sham controls involved exposure of the sciatic nerve and its branches without any lesion. **1B**: Positioning of the thermocouples on the hind limbs of the rat. Innervated areas of the nerves are colored on the hind limbs. **1C**: Illustration of the cooling setup and positioning of the rat. **1**: USB-based 8-channel thermocouple input module, **2**: Thermostatic bath, **3**: Heating mattress, **4**: Thermocouples, **5**: Peltier elements, **6**: Water coolers, **7**: Power supply.

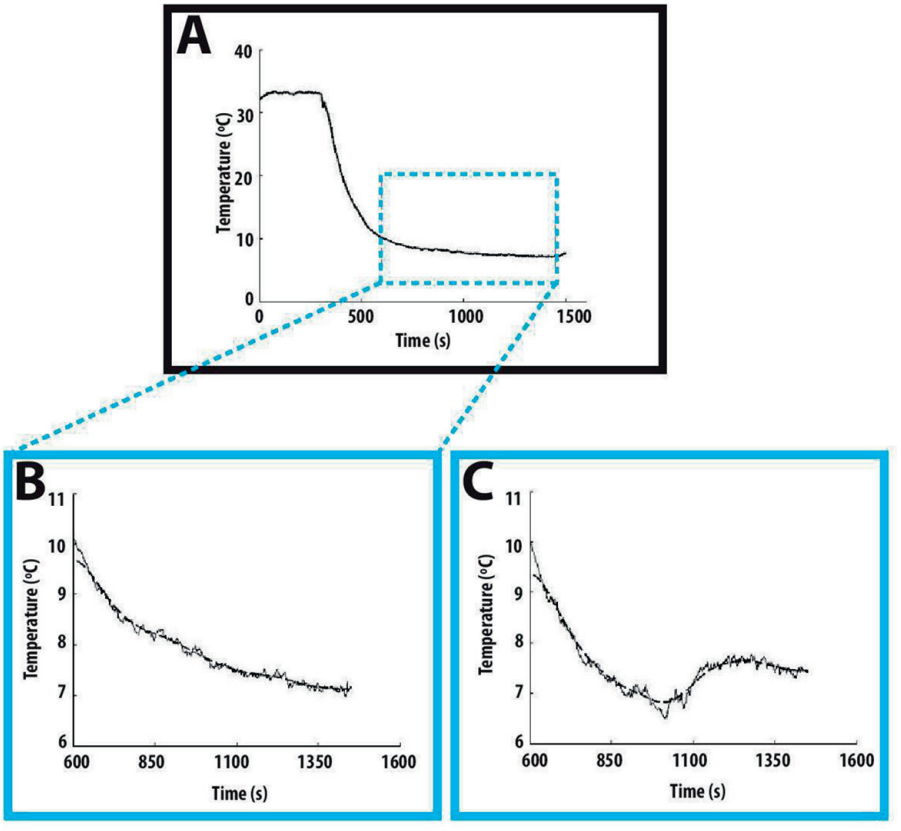

Figure 2 | Typical example of temperature during the cooling phase, illustration of the Cold Induced Vasodilatation, the so-called Hunting reaction, in one of the rats. **A**: Illustration of the total pre- and cooling phase. The analysis period started 375 seconds after the start of cooling and lasted for 850 seconds. The selected cooling phase in Figure 1B and 1C are raw and filtered data. **B**: A typical example of a cooling phase without CIVD reaction. **C**: A typical example of a CIVD reaction during the cooling phase. A CIVD was identified when the temperature of the filtered signal increased at least 0.4°C during the cooling phase.

Results

Ambient temperature during the experiment averaged 25.1°C. The minimum ambient temperature was 22.8°C (SD 0.73°C). The core temperature of the rats was stabile at 35.9°C (SD 1.4°C). Mean temperatures of the operated paw and rectal temperatures in °C are displayed in Table 1, showing the variation in temperature during cooling. One rat passed away after the measurements of the third week. The rat was part of the SNI group, reducing the number of rats in this group to 7.

Cold plate test

For the reference test for cold intolerance, we found that the sham group showed no paw withdrawal response when exposed to the cold plate surface of 5°C for 140 seconds. In contrast, the SNI-group showed a significant reduced paw-withdrawal latency at all postoperative time points ($P < 0.001$). The CSL-group had a significant reduced paw-withdrawal latency only at 6 and 9 weeks as compared to the sham group ($P < 0.001$). There was no significant difference between the SNI and CSL rat at 6 and 9 weeks in paw withdrawal response due to exposure to a 5°C cold plate (Table 2). These results show that the SNI and CSL rats developed cold hypersensitivity due to nerve injury.

Von Frey test

The results of the Von Frey test display that the SNI group has a significant mechanical hypersensitivity due to nerve injury (Table 3). For the reference for mechanical hypersensitivity, we found that the amount of force of the Von Frey filaments needed to obtain a mechanical withdrawal reflex was lower in the SNI group at all postoperatively (3, 6 and 9 weeks) time-points as compared to the Sham group. The CSL displayed a lower mechanical withdrawal threshold as compared to the Sham group at 6 and 9 weeks postoperatively; however, this difference was not statistically significant. No significant differences were found between the mechanical withdrawal thresholds of the SNI and CSL rats.

CIVD reactions

The investigators differed in 5.9% of the manual CIVD scores of the temperature curves but rapidly came to consensus. No significant effect of time (preoperatively and three, six and nine weeks postoperatively; $P=0.397$) and group (SNI, CSL and Sham; $P=0.695$) were found for the number of CIVD's in the lateral operated hind limb (Figure 3).

Table 1 | Displayed are the mean operated paw and rectal temperatures during cooling in °C at the different time points.

Location	Time	Mean Temperature °C (SD)
Operated paw	*Pre-operative*	27.4 (3.1)
	3 weeks	26.7 (3.2)
	6 weeks	27.2 (0.6)
	9 weeks	25.9 (2.3)
Rectal	*Pre-operative*	36.5 (1.1)
	3 weeks	36.0 (1.2)
	6 weeks	34.8 (1.9)
	9 weeks	35.9 (1.1)

Table 2 | Cold plate test for cold hypersensitivity. The paw lift latency is shown in seconds with standard deviation between parenthesis during the different measurement times in all three groups. After 3 weeks SNI rats developed a significant cold hypersensitivity as compared to the sham group and the CSL rats. The latter group also developed these symptoms after 6 weeks.

Cold Plate Test (s)	3 Weeks	6 Weeks	9 Weeks
CSL	160.3 (28.5)	35.4 (24.2)	43.6 (29.9)
SNI	10.2 (7.8)	8.9 (9.0)	9.0 (8.3)
Sham	163.5 (33.9)	152.4 (37.8)	159.2 (35.5)

Table 3 | Von Frey Test for mechanical hypersensitivity. The amount of force (g) is shown of the Von Frey filaments needed to obtain a mechanical reflex withdrawal response with standard deviation between parentheses. After 3 weeks SNI rats developed a significant mechanical hypersensitivity as compared to the sham group and the CSL rats. The latter group also developed these symptoms after 6 weeks.

Von Frey Test (g)	3 Weeks	6 Weeks	9 Weeks
CSL	43.6 (20.7)	8.4 (3.5)	8.6 (4.7)
SNI	3.0 (1.7)	2.4 (1.9)	3.6 (1.5)
Sham	31.9 (20.2)	39.0 (20.0)	30.3 (21.1)

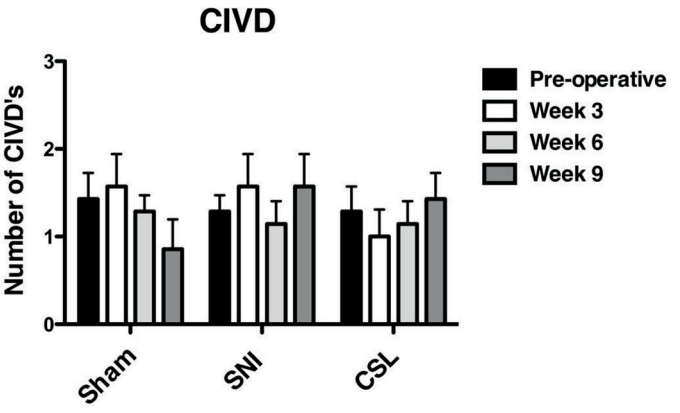

Figure 3 | The amount of CIVD reactions displayed. No significant differences were found between the groups. The X-axis shows the three operated groups: A sham operated group, a spared nerve injury model (SNI) operated group and a complete sciatic lesion (CSL) operated group. The Y-axis shows the number of CIVD reactions during the cooling phase.

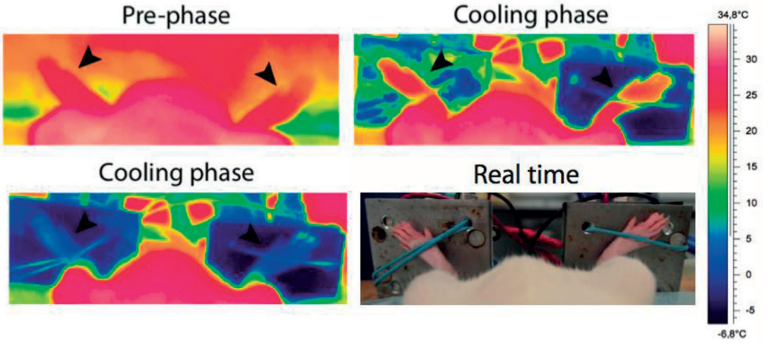

Figure 4 | Thermographic imaging and a real time photo of the body of the rat during the experiment, both hind limbs and the Peltier element. **Pre-phase**: Rat is dorsally positioned under anesthesia to measure baseline temperatures of the hind limbs. **Cooling phase** (20 min.): Both hind limbs are attached to the Peltier elements. The color indicates that both Peltier elements and hind limbs get equally cooled.

Discussion

The aim of this study was to determine the role of the sympathetic system of initiating a CIVD response. The results of the cold plate test and the von Frey test clearly demonstrated that the SNI and CSI rats developed cold intolerance and mechanical hypersensitivity. The nerve lesions of the tibial nerve resulted in cold and mechanical hypersensitivity at 3, 6 and 9 weeks after the lesion. The sciatic nerve lesion resulted in cold and mechanical hypersensitivity at 6 and 9 weeks after the operation, but not after 3 weeks.

In this study no significant differences in CIVD reactions were observed between CSL, SNI and the sham operated rats. The sympathetic nerves are part of the nerves that were cut during the operation. Therefore, we can conclude that the sympathetic system initiating the CIVD response has no effect on the CIVD reaction and that peripheral triggers are more likely responsible for initiating a CIVD reaction.

A possible explanation for the absence of changes in patterns of the CIVD reactions could be that vascular control can function without peripheral nerve innervation and that the smooth muscles in the vessel wall have no effect on any type of denervation of the peripheral nerve. It could be possible that more local factors such as norepinephrine have more influence[33] than denervation of the hind paw by a nerve lesion. Active cutaneous vasodilatation occurs via cholinergic nerve co-transmission and has been shown to include potential roles for nitric oxide, vasoactive intestinal peptide, prostaglandins, and substance P. It has been proven both interesting and challenging that not one substance has been identified as the sole mediator of active cutaneous vasodilatation.[34] It could be that noradrenaline plays an important role, as Stephens et al. (2001) identified in young men. Stephens et al. (2001) also showed that complete postsynaptic blockade of norepinephrine-mediated vasoconstriction did not completely inhibit the reflex vasoconstrictor response to 15 min of progressive decrease in skin temperature, although presynaptic inhibition with bretylium abolished response.[34,35]

In the study of Eide (1976) tail temperature increased after an initial temperature decrease produced by immersion in ice water, followed by several CIVD cycles after varying time periods.[6] In contrast, the CIVD response was completely absent in the study of Thomas et al. (1994) in cold injured rat-tails. CIVD remained absent at least several weeks after exposure to the injury condition, implicating the loss of CIVD.[25,37] For future studies on the onset of a CIVD, we suggest cooling the rat's paw for at least 20 minutes between 0-5°C, and measure with a time interval of maximally 1 second. When the experimental setting would allow this, cooling for longer periods (e.g, 40 minutes) would yield multiple CIVD reactions in a single recording.

Our findings may seem in contrast with a recently published study of Wen Hu et al. 2012[38] that showed a significant difference in CIVD reaction of the hindpaw following an autograft repair after a sciatic nerve injury when compared to a control group. However, the differences may be explained by the fact that we measured during cooling and Wen Hu et al. measured after

cooling. Wen Hu et al. initially cool the hind paws in smashed ice for 5 minutes, immediately dry the hind paws with a towel and then measure the blood perfusion by means of a laser Doppler when the cooling has already stopped. This approach in not in line with the common definition of a CIVD reaction as a cyclic vasodilatation response during cooling.[39] Wen Hu et al. show that after the cooling phase, a vasodilatory response occurs in the first minutes which disappear after about 10 minutes; after this period no cyclic vasodilatation or vasoconstriction occur. Therefore we believe that the measured blood perfusion changes are not CIVD's but are active rewarming patterns, which, due to vasodilatation,[40] occur after a period of cold exposure and have been shown to be disturbed in patients with peripheral nerve injury.[41] Other explanations between the discrepancies between our and their study may be the usage of different strains of rats (Wistar vs Sprague-Dawley) and/or different models of nerve manipulation. In the current study the nerve injury leads to distinct neuropathic pain behavior, whereas in the study conducted by Wen Hu et al. the nerve injury and reconstruction does not result in any pain behavior. This implies that peripheral nerves are altered differently in the two models, which in turn may alter peripheral thermoregulation and can lead in different results.

Generating cold intolerance using the operation models for SNI and CSL has proven to be successful, as demonstrated with the cold plate test (cold intolerance) and the Von Frey test (mechanical hypersensitivity). In addition, the sham operation apparently has no consequences for the sensitivity to temperature or mechanical pain, and SNI and CSL are therefore appropriate models to evoke cold intolerance. It has been described that the innervation differs between the different operation models. In the SNI model, the medial and lateral sides of the foot sole are still innervated, these are stimulated during the behavioral tests.[29,42,43] In the CSL model the foot sole is de-innervated after a sciatic nerve lesion. It is known that after approximately 3 weeks after nerve injury sprouting of non-injured fibers starts.[27,44] Therefore in the CSL model the foot sole probably starts to re-innervate after 3 weeks, therefore the hypersensitivity to mechanical and cold stimuli is evident only after 6 weeks.

We continued to monitor the rat when it was removed from the Peltier elements and occasionally the rectal probe shifted and was exposed to surrounding temperature, this explains why the minimum rectal temperatures sometimes dropped below the 35°C. Reevaluating the data, we found that when the probe was placed back into the rectum, core temperature had not decreased lower than 35°C. Core and surrounding temperature were monitored very strictly during the entire experiment as previous work described the importance of a stable environment and core temperature.[6]

The observed cyclic changes after cooling the rat hind limbs are strongly indicative of the phenomenon of CIVD, and appear qualitatively similar to CIVD reactions reported in rats tail and in human's, and display considerable pattern variability as also observed in humans and in rat tails.[6,8,25] Moreover, these cyclic patterns appear comparable to patterns of CIVD reported in an in vitro preparation of isolated segments of rat tail arteries exposed to cold.[28] The currently

employed study design[31] may add to the existing methods. CIVD research is hampered by the absence of a uniform quantification of CIVD responses. Therefore, we would like to stress that studies should try to develop a schematic algorithm to define a CIVD reaction which takes different variables such as core temperature, cooling temperature, subject and surrounding temperature into account.

Greenfield et al. demonstrated in 1951 that intact peripheral nerves are not essential to the vasodilatation phase of the CIVD in humans.[45] Daanen and Ducharme et al. demonstrated in humans that the axon reflexes do not occur in a cold-exposed hand and thus are unlikely to explain the CIVD phenomenon.[46] Based on previous work and the results of our study we conclude that rats and possibly humans that underwent a peripheral nerve injury still experience the so-called hunting reaction. Humans and rats will still experience an aching in the hands and paws respectively which corresponds to cutaneous vasoconstriction, and periods of warmness and tingling may follow corresponding to vasodilatation and renewed blood flow.[8,28] We suggest that the underlying mechanisms that initiate a CIVD reaction are not affected by damage to a peripheral nerve. We conclude that the sympathetic system, which initiates the CIVD response, has no effect on the CIVD reaction and local triggers are more likely responsible for the CIVD reaction. No substantial changes in the CIVD reaction after peripheral nerve injury imply that the origin of cold intolerance after a traumatic nerve injury is initiated by local factors. Further research on the pathophysiology of cold intolerance should concentrate on local biochemical changes and their effects on small nerve fiber endings and micorovascularisation.

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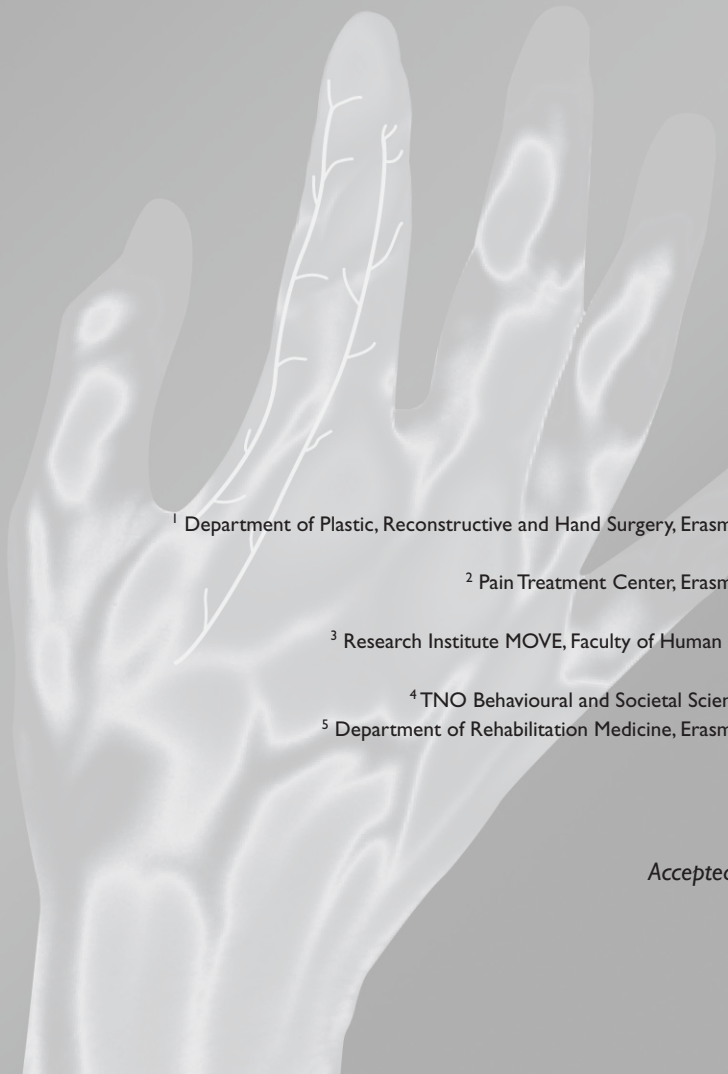
Chapter 6

Comments to the term "cold-induced vasodilatation" in "laser doppler perfusion imaging of skin territory to reflect autonomic functional recovery following sciatic nerve autografting repair in rats"



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Dear Editor,

In this letter we would like to challenge the correctness of the term cold-induced vasodilatation in a recent paper in this journal. In microsurgery 32:136-143, 2012, the group of Hu et al. contributed to the debate on the influence of nerve injury and nerve repair on the cold induced vasodilatation (CIVD) reaction.[1] The study reported a significant decrease in the CIVD reaction of the hind paw following a nerve transection of the sciatic nerve as compared to a sham-treated group (same operation procedure, without nerve manipulation). In addition, they measured the CIVD reaction after an autograft repair of the sciatic nerve and found no significant differences with the sham-treated group. Therefore, the authors concluded that the CIVD reaction is controlled by the nervous system, more specifically by the autonomic nervous system. In this reply, we question if Hu et al. use the definition of a CIVD in an accurate manner. It is possible that the CIVD reaction defined and measured by Hu et al. was not a CIVD reaction but an active rewarming pattern.

A CIVD is currently defined as a cyclic oscillation in blood flow that occurs in extremities upon cold exposure.[2-4] The study of Hu examined the CIVD reaction by placing the hind paws in smashed ice for 5 minutes, thereafter immediately drying them with a towel and measuring blood perfusion by means of a laser Doppler. However, measuring the CIVD when the cooling phase has ended is not in line with the current definition which states that a CIVD reaction presents upon cold exposure. Since there is no active cooling, we believe that it is better characterized as rewarming.

A second argument why the pattern reported by Hu et al. is not a CIVD, is the course of blood perfusion. The study shows that after the cooling phase, a vasodilatory response occurs in the first minutes, which stabilizes after ±10 minutes. Afterwards, neither vasodilation nor vasoconstriction occurs for up to 20 minutes. Since a CIVD is a clear cyclic oscillation in blood flow induced by subsequent vasoconstrictions and vasodilatation, we believe that the findings by Hu et al. are not CIVD reactions but are active rewarming patterns. In a recent study,[5] we demonstrated that active rewarming due to vasodilatation occur after a period of cold exposure and that this active rewarming can be disturbed in patients with peripheral nerve injury,[6] which is in line with the results of Hu et al.

There are methods that permit simultaneous cooling and measuring blood perfusion or skin temperature, which would allow for measuring CIVD in animal experiments. However there is still no clear definition of a CIVD reaction in humans and animals, which makes comparing studies very difficult. In order to avoid confusion, we propose to limit the term CIVD to vasodilation during local cold exposure and not after cold exposure,7-9 which is a commonly respected definition.[4]

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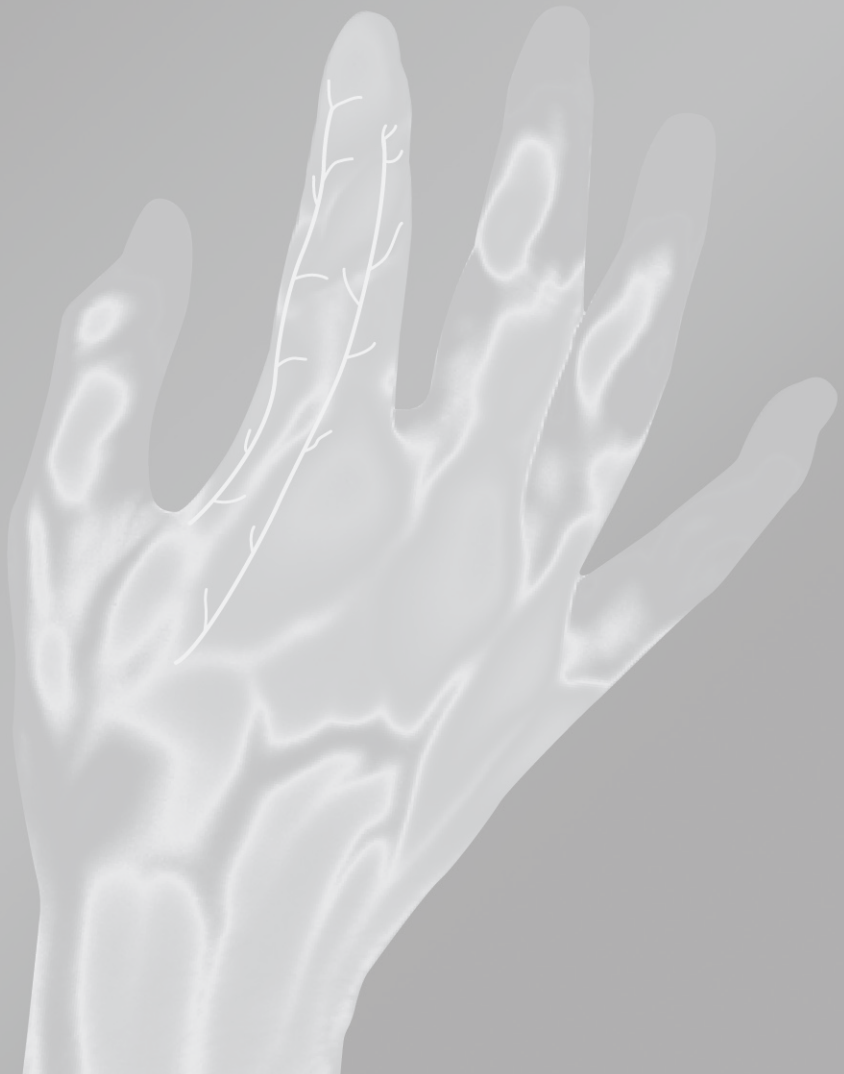
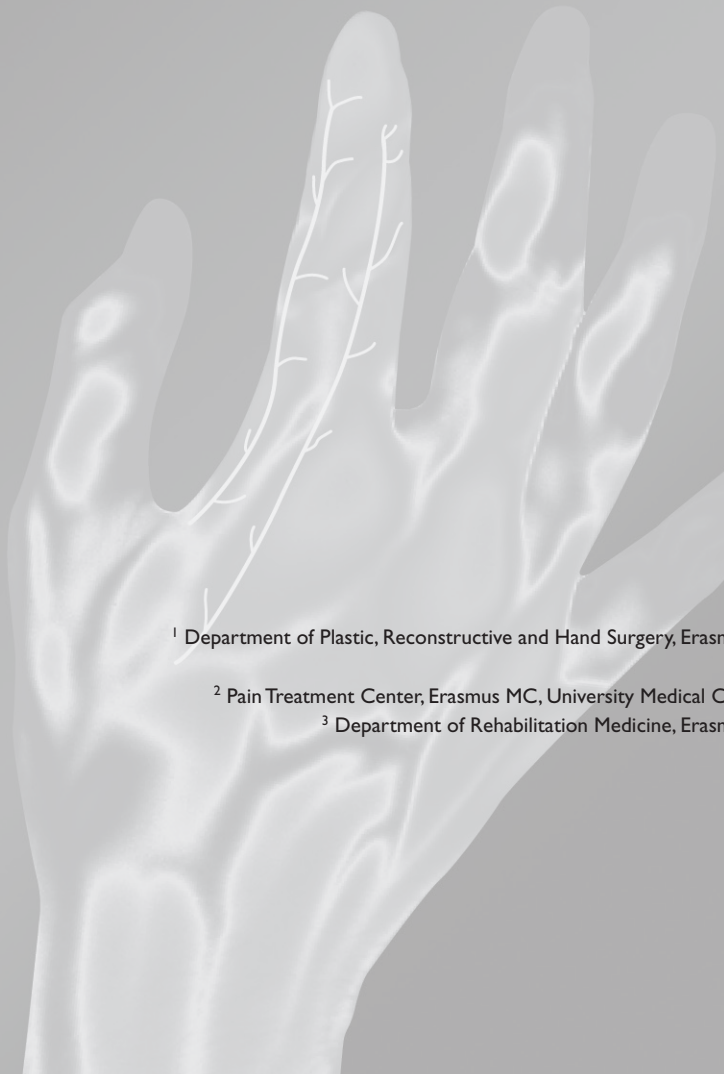
Part II



Chapter 7

Quantitative Sensory abnormalities in patients with posttraumatic cold intolerance



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Submitted

Abstract

Background

Cold intolerance remains a condition of unknown cause with varying symptoms. Quantitative sensory testing is a valid and quantitative technique that directly interprets the behavior of the different types of small nerve fibers. Changes in the nerve endings in patients with cold intolerance may clarify the cause of cold intolerance.

Method

Fifthteen healthy controls and 32 patients with cold intolerance participated in the study. Eleven patients had a median or ulnar nerve injury, fifteen patients an amputation of one or more fingers and six patients a hand fracture. Cold intolerance was determined by symptom severity of cold intolerance (CISS) questionnaire. All patients were subjected to quantitative sensory testing using a Medoc TSA-II Neurosensory analyzer. The subjects were measured at the dermatome where they experienced the most complaints.

Results

The values of all patient groups were significantly different from the control group for the cold detection threshold, warm detection threshold, thermal sensory discrimination, mechanical detection threshold and vibration detection threshold. Patients with cold intolerance had a higher threshold for the detection of both hot and cold temperature changes compared to the control group. Upon cooling, patients reported pain at higher temperatures than the control group.

Conclusions

Based on the literature the outcome of this study indicates that these changes in peripheral sensory channels of A-delta and C fibers may be disrupted in patients with cold intolerance.

Introduction

Cold intolerance defined as abnormal pain, with or without discoloration, numbness, weakness or stiffness of the hand and fingers after exposure to mild to severe cold.[1-5] Cold intolerance is a frequent sequel of upper extremity trauma, the incidence is particularly high following upper extremity nerve injury or amputation of one or more digits (56%-83%), and is also present in patients with a fracture of the hand (38%).[6-14] Affecting work and leisure activities, cold intolerance is a bothersome and disabling symptom.[8,15-17]

The standard tool to assess cold intolerance is the Cold Intolerance Symptom Severity (CISS) questionnaire, which was developed by Irwin et al. and adjusted by Ruijs et al.[6,18] Unfortunately, this questionnaire only illustrates the patient's experience of the possible painful symptoms and is not a sensitive diagnostic tool to identify the unknown underlying pathophysiology of cold intolerance. Recent literature investigating the pathophysiology of pain in neuroma patients describes that abnormal connections between A- and C-fibers can result in thermal hyperalgesia.[19,20] Therefore, abnormal sensitivity and spontaneous activity of injured axons can be caused by sensitized C-nociceptors.[4,19-24]

Quantitative sensory testing (QST) is an acknowledged method designed to determine detection thresholds for cutaneous sensations of touch, vibration, mechanical and thermal (pain) stimuli. QST can be an effective tool to evaluate somatosensory changes in different pathologies,[25] and is regarded a useful diagnostic instrument in pain conditions such as neuropathic, arthritic, myofascial and fibromyalgic pain.[26-32] The German Research Network on Neuropathic Pain (DFNS) designed and validated a standardized set of tests comprising all somatosensory submodalities mediated by different primary afferents (C-, Aδ-, Aβ-), including the assessment of the deeper layers.[33]

In this study, we applied quantitative sensory testing to three different subgroups of patients with cold intolerance diagnosed using the CISS score: 1) patients with a peripheral nerve lesion, 2) patients with an amputation to one or more digits and 3) patients with a hand fracture. The aim of this study is to investigate with QST patterns of somatosensory changes in patients with cold intolerance in different subgroups. Hereby, we will be able to quantify the changes in QST outcome and evaluate if patients with cold intolerance are characterized by specific sensory changes.

Methods

Subjects

The following inclusion criteria had to be met for the inclusion of patients: clinically diagnosed cold intolerance, a nerve lesion to the median and/or ulnar nerve, an amputation of one or more digits, a fracture in the hand, the patient had to be older than 18 years old, and the trauma had to be at least 10 months ago. Patients were excluded if they were pregnant or unable to participate because of insufficient knowledge of Dutch language. Patients with a hand fracture were excluded when they had additional nerve lesions, to enable comparisons of the subgroups. Medication, smoking and patient characteristics were registered.

All subjects completed the cold intolerance symptom severity (CISS) questionnaire,[18,34] which has a minimum score of 0 and a maximum score of 100.[18] Based on the range of Dutch normative values, a patient with a score of 30 or higher is defined as having abnormal cold tolerance.[18,34] The patient group of 32 patients was divided in to 3 subgroups based on the underlying etiology: 1) nerve lesion, 2) amputation of one or more digits, or 3) a fracture of the hand. We measured a control group of 15 healthy volunteers to compare the results of our patient groups and also used the reference values of Magerl et al.[35] This study was approved by the Medical Ethical Committee of our hospital (MEC 2009-363) and all patients provided written informed consent.

Assessments

All patients and control subjects underwent the QST protocol as developed by the German Research Network on Neuropathic Pain (DFNS) and completed the CISS questionnaire to measure the severity of cold intolerance. The DFNS was developed a standardized QST battery that consists of 13 parameters.[33,36]

Thermal testing comprises detection thresholds and pain thresholds for cold or warmth stimuli (C- and A-delta fiber mediated): Cold detection threshold (CDT), warmth detection threshold (WDT), cold pain threshold (CPT) and heat pain threshold (HPT). In addition, we evaluated thermal sensory limen (TSL) (different limen for alternating warm and cold stimuli) and the presence of paradoxical heat sensations (PHS) (report of heat sensations during innocuous cold stimuli). Thermal tests were conducted using the TSA 2001 (Medoc Inc.) with a thermode contact area of 3.0×3.0 cm. Mechanical stimulus detection comprises of mechanical detection thresholds (MDT) using von Frey-filaments (rounded tip 0.5mm diameter) between 0.25 and 512 mN for A-beta fiber function, and mechanical pain threshold (MPT) using pinprick stimuli (8, 16, 32, 64, 128, 256 and 512 mN) for A-delta fiber mediated hyper- or hypoalgesia.

Pain stimulus-response testing was tested using 1) mechanical pain sensitivity (MPS) using pinprick stimuli, 2) dynamic mechanical allodynia (ALL) using pinprick stimuli to assess A-delta mediated sensitivity, and A-beta fiber mediated pain sensitivity using 3) stroking light touch (cotton wisp; cotton wool tip; brush).

The wind-up phenomenon (increasing pain with repeated stimulation) was tested using the wind-up ratio (WUR), comparing the numerical ratings of a single pinprick stimulus with a series of 10 repetitive pinprick stimuli of the same force, repeated for 5 series, to calculate the WUR as a ratio. In addition, the vibration detection threshold (VDT) using a 64 Hz tuning fork was used to assess A-beta fiber function. Pressure pain threshold (PPT) was tested using an algometer to test deep pain sensitivity, most probably mediated by muscle C- and A-delta fibers.
The assessments were always performed in the same order by the same nurse practitioner in the same room with monitored surrounding temperatures of 23°C (±1.0). Patients acclimatized to the room temperature for 30 minutes. Following advice by the DFNS, the nurse practitioner was trained by a member of the German Research Network on Neuropathic pain to ensure conformity in the data. Results were reported, calculated and stored following Rolke et al.[36] Each test was carried out on the location of the dermatome were the patients experienced the most complaints of cold intolerance.

Statistical analysis

All data were analyzed for distribution properties. If the data of a test subgroup was not distributed normally, we transformed the data logarithmic. To compare patients QST data profile with each other independent of different units of measurements, a z-transformation was performed.[36] QST variables were compared between groups using an independent sample t-test.

Results

Patient characteristics

Fifteen healthy control subjects and 32 patients with cold intolerance participated in the study. Eleven patients had a median or ulnar nerve injury or combined nerve injury, fifteen patients had an amputation to one or more digits and six patients had a hand fracture (Table 1). All patients were measured between December 2009 and December 2010. Each group consisted of more men than woman except in the group with hand fractures. Patients had a mean CISS score of 48 (SD 15.7) and a mean age of 44 (SD 15) years. The description of the subgroups is presented in Table 1.

QST results in patients subgroups

Quantitative sensory testing outcomes of control subjects and patient subgroups are displayed in Table 2. Mean and standard deviation of each subgroup are displayed. We found that the CDT, TSL, CPT, HPT and VDT were normally distributed; the remaining test parameters were logarithmic transformation, as advised by Rolke et al.[36] For each subgroup, the frequencies of quantitative sensory abnormalities for each parameter are displayed in Table 3. The patients were significantly different from the controls for CDT, WDT, TSL, MDT and VDT. The PHS and ALO were significantly different when comparing the nerve lesion group to controls and the amputation group to controls. CPT was significantly different between the nerve lesion group compared to the controls, MPT was significantly different in the amputation group compared to the controls, and WUR was significantly different in de hand fracture group compared to the controls.

Table 1 | Patient characteristics. Three subgroups of cold intolerance and the control group.

	Control N=15	Nerve Lesion N=11	Amputation N=15	Hand fracture N=6
Age (years) Mean (SD)	31 (11.4)	39 (11.7)	48 (15.1)	40 (15.4)
Gender (M:F)	10:5	7:4	11:4	2:4
Time after trauma Mean weeks (SD)	Not applicable	179 (103)	240 (118)	213 (26)
Mean CISS Score mean (SD)	0.1 (0.5)	53.0 (15.0)	48.4 (17.6)	37.8 (6.3)

Table 2 | QST outcome of the three patient groups and the control subjects. * Log transformed

	Controls N=15		Nerve lesion N=11		Amputation N=15		Fracture N=6	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
CDT	-1,21	0,50	-3,82	1,50	-3,74	2,34	-2,72	2,52
WDT*	0,12	0,36	1,18	0,62	1,46	0,71	0,48	0,65
TSL	4,83	1,66	10,08	4,67	10,25	5,73	7,37	6,47
PHS	0,03	0,18	0,27	0,65	0,20	0,77	0,00	0,00
CPT	11,40	6,50	19,32	6,65	14,28	8,01	13,56	10,22
HPT	42,39	2,85	42,43	2,34	44,45	3,30	42,82	3,66
MDT*	-1,44	0,42	1,34	1,23	0,65	1,36	-1,14	0,66
MPT*	3,70	0,87	4,39	1,43	4,08	1,28	3,78	1,06
MPS*	0,25	1,40	-0,68	1,87	-0,53	1,05	-0,10	1,18
ALO	0,00	0,00	0,01	0,04	0,02	0,09	0,00	0,00
WUR*	2,43	1,03	2,81	1,12	2,88	1,68	3,31	1,69
VDT	6,87	0,38	5,27	1,23	5,88	0,74	6,33	0,92

Table 3 | Frequency of Quantitative sensory abnormalities for each QST parameter in the subgroups.

* Log transformed

	Control vs nerve (P-value)	Control vs amputation (P-value)	Control vs fracture (P-value)	A Delta fibers	A Bèta fibers	C fibers
CDT	0,00**	0,00**	0,00**	X		
WDT*	0,03	0,00**	0,02			X
TSL	0,00**	0,00**	0,00**	X		
PHS	**0,00****	**0,02**	0,37	**X**		
CPT	**0,01**	0,45	0,25	**X**		X
HPT	0,61	0,55	0,38			X
MDT*	0,00**	0,00**	0,03		X	
MPT*	0,07	**0,03**	0,27	**X**		
MPS*	0,50	0,11	0,63			
ALO	0,00**	0,00**		X	X	
WUR*	0,47	0,10	**0,03**			X
VDT	0,00**	0,00**	0,01		X	

Discussion

To investigate patterns of somatosensory changes in patients with posttraumatic cold intolerance, we applied QST to patients with cold intolerance from three different subgroups. Overall, we found that in each pathology group the QST outcome was disordered. While the QST abnormalities did not differ systematically between the three patient groups, overall, both the number of abnormal QST variables as well as the magnitude of the differences was the most prominent for the nerve injury group. The hierarchical cluster analysis indicated two different patterns of sensory abnormalities and inspection of the groups suggested that Cluster 1 is characterized by strongly abnormal C-fiber activity. Variables of the different clusters do not relate to the different patient subgroups.

In the present study, we found that the values of the control subjects were in line with previous studies of Magerl et al. and Rolke et al.[35,36] In the patient group we found abnormal QST outcome in all three patient groups and the pattern of QST abnormalities indicate that in all three groups both the A and C fibers can both be disordered. We discovered that all fibers are disordered, and therefore all fibers may be associated with the symptoms of cold intolerance. To our knowledge, this is the first study using QST to quantify sensory abnormalities in patients with cold intolerance. Our findings, however, are in line with studies on patients with small fiber neuropathy due to diseases, such as amyloidosis, diabetes, Guillain-Barre syndrome, HIV, leprosy, sarcoidosis and systemic lupus erythematosus,[37-45] which show that the complaints are associated with abnormalities in the functioning of C-, Aδ- and Aβ- fibers. In contrast with this, in Fabry patients,[46] it was found that only the Aδ fibers are affected.

A number of limitations are present in this study. The relatively small number of subjects, especially compared to multicenter studies of the DFNS[49] is the first limitation. Despite of this, large and statistically significant differences were seen between patients and controls, even when comparing the specific subgroups with the patient group. Another limitation was that we included only patients with cold intolerance. For future studies, based on the present results, it may be valuable to compare post traumatic patients with and without cold intolerance. A limitation may also be that the trauma may have resulted in various microvascular and neurological damage in different patients. Therefore, both within and between the different subgroups, the specific pathology may differ. It would be of interest to perform a long-term follow-up of different patient groups to establish how cold intolerance develops in relation to changes in the QST.

In this study, QST setup has proven its ability to assess and quantify sensory nerve function in a noninvasive manner in great detail. Therefore, this setup may be beneficial to assess somatosensory abnormalities in patients with (posttraumatic) cold intolerance; to identify specific subgroups of neurological dysfunction; to follow the developments of the somatosensory disorders of cold intolerant patients in time or to evaluate possible responses to (experimental) therapy. The specific information has provided additional insight in the pathophysiology of cold

intolerance and has reinforced the hypothesis that patients with cold intolerance are neurological pain disordered. Unfortunately, no satisfactory treatment is yet available for cold intolerance and avoidance of cold presently seems to be the most practical solution for patients.

The pathophysiology of cold intolerance is presently poorly understood. In a number of recent published studies,[50-52] it was shown that while some patients with cold intolerance have abnormal thermoregulation, this abnormal thermoregulation might not be the main cause of cold intolerance since cold intolerance can also be found in patients and animal models with normal thermoregulation.[50,51,53] As an alternative to abnormal thermoregulation, the underlying pathophysiology of cold intolerance may be primarily neurological. One of the possible underlying mechanisms of cold intolerance could be disordered function of the thermosensitive potential channels (TRP), TRPM8 and TRPA1. TRPM8 is the receptor that senses innocuous temperatures (< 28°C) and TRPA1 is activated at noxious temperatures (< 17°C). Previous reports have shown that an increase in TRPM8 and TRPA1 channels and/or alterations in threshold activation on the injured and adjacent uninjured nerves contribute to cold intolerance in both neuropathic and inflammatory animal models. One of the future perspectives are the investigation of TRPA1 and TRPM8 channels in skin biopsies and to correlate these findings with the QST outcome of the affected location, which might give an insight in the underlying skin neuropathies.

In conclusion, this study indicates that quantitative sensory testing can assess and quantify sensory nerve function in a noninvasive manner and that it can be valuable in assessing somatosensory abnormalities in patients with cold intolerance. The three different subgroups of patients with cold intolerance were found to have somatosensory abnormalities and we found that all A- and C- fibers were affected in patients with posttraumatic cold intolerance. We found that patients with a nerve lesion are the most effected subgroup; that Aβ- and Aδ- fibers were generally the most disordered; and different QST outcome profiles may be present, characterized by the presence or absence of C-fiber abnormalities.

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Chapter 8

Disordered conditioned pain modulation system in patients with posttraumatic cold intolerance

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Abstract

Background

Conditioned pain modulation (CPM) is a phenomenon of 'pain inhibiting pain' that is important for understanding idiopathic pain syndromes. Because the pathophysiology of posttraumatic cold intolerance is still unknown but could involve similar mechanisms as idiopathic pain syndromes, we evaluated the functioning of the conditioned pain modulation system in patients with posttraumatic cold intolerance compared to healthy controls.

Methods

Fourteen healthy controls and 24 patients diagnosed with cold intolerance using the Cold Intolerance Symptom Severity questionnaire were included. Of the 24 patients with cold intolerance, 11 had a nerve lesion and 13 an amputation of one or more digits. To quantify the CPM, pain threshold for mechanical pressure was measured at the affected region as a baseline measure. Then, the contralateral hand received a cold stimulus of ice water to evoke the noxious conditioning. After the cold stimulus, the pain threshold for mechanical pressure was determined again.

Results

The absolute and relative changes in algometer pressure (CPM effect) between pre and post conditioning were significantly smaller in the cold intolerance group compared to the control group (absolute P=0.019, relative P=0.004). The CPM effect was significantly different between the control group and the subgroups of nerve lesion (P=0.003) and amputation patients (P=0.011).

Conclusions

In this study, we found a CPM effect after a cold stimulus in both controls and patients. A significant weaker CPM effect compared to the controls was found, as in other chronic pain conditions. The conditioned pain modulation system within patients with cold intolerance is altered.

Introduction

Cold intolerance is a disabling chronic pain disorder that occurs in the first months after a trauma to the upper extremity, and generally does not diminish over time.[1-7]

It is defined as abnormal pain, with or without discoloration, numbness, weakness or stiffness of the hand and fingers after exposure to mild to severe cold.[8-11] Although this condition is a frequent consequence of upper extremity trauma, the incidence is particularly high following upper extremity nerve injury and amputation to one or more digits (56-100%) and also predominant in patients with a fracture of the hand (38%).[1,2,5,6,12-16] Posttraumatic cold intolerance is a bothersome and disabling symptom, affecting both work and leisure activities.[4-6,17]

Unfortunately, the exact pathophysiology of cold intolerance is still unknown. Recent studies have shown that the presence and severity of posttraumatic cold intolerance cannot solely be explained by disordered thermoregulation[6,18-21] as demonstrated by Duraku et al.[20] who found no direct correlation between cold intolerance and re-warming patterns in different peripheral nerve injury models. Based on this, Duraku et al. suggested that treatments focussing on vaso-regulation may therefore not diminish symptoms of cold intolerant patients. It is thought that more neurological mechanisms play an important role in the development of cold intolerance.[6,7] Until now, however, it is not well understood why only a select number of the patients develop cold intolerance while other do not.[6,7,12,22]

An explanation why some patients develop more cold intolerance than others could relate to the interpersonal variability in pain modulation.[23] Especially variability in how 'pain inhibits pain' in individual patients could play a role. This 'pain inhibiting pain' can be evaluated by quantifying the conditioned pain modulation (CPM) effect.[24] The CPM effect can be examined by measuring the pain intensity during a stimulus before and after the application of a noxious 'conditioning' stimulus at another body location. A reduction in the magnitude of the 'test-pain' in response to the 'conditioning stimuli' is considered the CPM effect.

With the CPM effect the function of the central descending inhibitory system can be assessed.[24,25] This central descending inhibitory system is of interest because it can inhibit or facilitate transmission of noxious information, a number of recent studies have shown that this system is involved in the pathophysiology of chronic pain[25-27] and that pain modulation patterns are reduced in various patients groups with idiopathic acute and chronic pain syndromes.[28-34] To our knowledge, the CPM effect has never been investigated in patients with cold intolerance.

The primary objective of this study was to evaluate the functioning of the conditioned pain modulation system in patients with posttraumatic cold intolerance and to compare it with a group of healthy controls. Throughout this article the authors will use the terminology of CPM as advised by Yarnitsky et al.[23,35]

Methods

Participants

Patients with a nerve lesion to the median and/or ulnar nerve or an amputation of one or more digits were included when suffering cold intolerance. Cold intolerance was diagnosed with the Cold Intolerance Symptom Severity (CISS) questionnaire that has a minimum score of 0 and a maximum score of 100 points.[3,36] Based on the range of Dutch normative values, a patient with a score of 30 or higher is defined as having abnormal cold tolerance.[3,36] Patients and controls were included when they were 18 years or older and had sufficient knowledge of Dutch language to follow the instructions of the researcher and be able to complete the CISS questionnaire. The trauma was at least 10 months or older. Medication, smoking and patient characteristics were registered. The study was approved by the Medical Ethical Committee of our hospital (MEC 2009-363) and all patients provided written informed consent.

Measurements

Patients were evaluated using a standardized CPM protocol. The assessments were always performed in the same order by the same researcher in the same room with monitored surrounding temperatures of 23°C (±1.0). Patients first acclimatized to the room temperature for 30 minutes. Results were reported, stored and statistical calculations were performed using SPSS for windows version 17.0.

The pain threshold for mechanical pressure was measured at the affected region using an algometer. A pressure stimulus was applied on the location were the patient experienced the most complaints of cold intolerance and was repeated 3 times with a 1-minute time interval. Pain intensities were logged using a numbered rating pain score NRS from zero (no pain at all) to ten (worst pain imaginable). To achieve a sufficient noxious stimulus the NRS pain score must be at least six points.

The most commonly known noxious conditioning paradigm to evoke a CPM like effect was used,[24] which is a cold stimulus. In this test, the contralateral hand was immersed in ice water of 1.8 degrees Celsius (SD 0.4) for at least 30 seconds and with a maximum duration of 3 minutes to prevent cold injury. The patient was instructed to immerse the hand until the pain was unbearable, with a minimum pain score of at least six for a sufficient noxious conditioning. After cold noxious conditioning, again, the pain threshold for mechanical pressure was determined and the NRS score and pressure were logged.

Data analysis

Means were calculated for age, time after trauma, CISS score, algometer pressure and pain score's. The patients group was compared with the controls. In addition, subgroups were created for nerve lesion patients and amputation patients, which were both independently compared with the control group.

The CPM effect was quantified by calculating the absolute and relative changes in pain threshold pressure before and after the noxious stimulus. To test for the presence of a CPM effect in each group, we used a one-sample t-test to determine if the absolute changes differed significantly from zero. Independent t-test was used to test for differences in absolute and relative changes between patients and controls. Differences were considered to be significant if $P < 0.05$ was obtained.

Results

Fourteen healthy controls and 24 patients diagnosed with cold intolerance using the CISS questionnaire were included. Of the 24 patients with cold intolerance, 11 had a nerve lesion and 13 an amputation of one or more digits. Patient characteristics are displayed in Table 1.

The relative differences (CPM effect) between pre conditioning and post conditioning are displayed in Figure 1 for the controls, the patient group and both subgroups. Controls immersed the hands in ice water longer to achieve a sufficient pain score (P=0.036, see Table 2). The absolute and relative changes in algometer pressure (CPM effect) between pre and post-conditioning were significantly smaller in the cold intolerance group compared to the control group (absolute change, P=0.019, relative change P=0.004). The CPM effect was also significantly different between the control group and the subgroups of nerve lesion patients (P=0.003) and amputation patients (P=0.011). A significant CPM effect, that is, an increases in stimulus needed to reach the pain threshold, was found both in the control group ($P < 0.001$) as in patients with posttraumatic cold intolerance ($P < 0.001$).

Table 1 | Patient characteristics.

	Control	Cold Intolerance	Cold Intolerance	
			Nerve Lesion	Amputation
Number	14	24	11	13
Age (years) Mean (SD)	32 (11.4)	45 (14.4)	39 (11.7)	49 (15.2)
Gender (M:F)	0.57 8/6	0.66 16/8	0.64 7/4	0.69 9/4
Time after trauma (weeks, (SD))		208 (111)	179 (103)	233 (115)
Mean CISS Score (SD)	0.1(0.5)	52 (15.6)	53 (15.0)	52 (16.7)

Age gender and time after trauma are comparable within the subgroups; mean Cold Intolerance Symptom Severity (CISS) score within the patient subgroups is also comparable

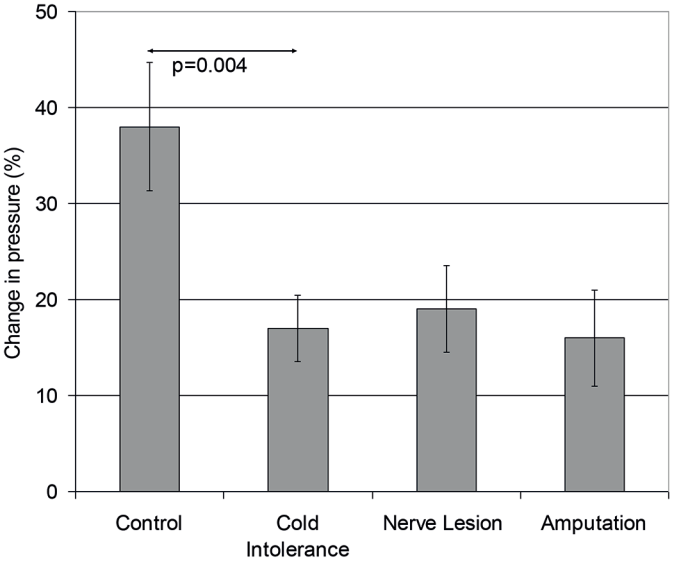

Figure 1 | Relative differences. The relative differences between pre and post condition are displayed for the control, patient group and both subgroups (nerve lesion and amputation). A significant (P=0.004) reduced Conditioned Pain Modulation (CPM) effect is seen in the patients with cold intolerance when compared to the control group.

Table 2 | Results.

	Pre conditioning stimulus		Conditioning stimulus		Post conditioning stimulus		Change in algometer pressure	
	Algometer pressure (kPa)	**NRS during algometer pressure**	**Duration in ice water (seconds)**	**NRS in ice water**	**Algometer Pressure (kPa)**	**NRS during algometer pressure**	**Absolute (kPa)**	**P-value**
Controls (SD)	8.0 (2.8)	6.0 (1.4)	103 (66)	7.4 (1.7)	10.8 (3.3)	6.4 (1.3)	-2.8 (1.5)	**< 0.001****
Cold Intolerance (SD)	8.8 (3.0)	6.0 (2.1)	58 (50)	7.8 (2.1)	10.3 (3.6)	6.0 (2.1)	-1.5 (1.5)	**< 0.001****
Nerve Lesion (SD)	8.3 (3.8)	6.4 (2.4)	39 (26)	8.4 (1.2)	9.9 (4.3)	6.5 (2.3)	-1.6 (1.3)	**0.003***
Amputation (SD)	9.2 (2.1)	5.7 (1.9)	73 (61)	7.4 (2.6)	10.6 (2.9)	5.7 (2.0)	-1.5 (1.7)	**0.011***
P-value (controls vs cold intolerance)	0.429	0.912	**0.036***	0.538	0.685	0.540	**0.019***	

Controls immersed the hands in ice water longer to achieve the same pain score (P=0.036). Pain intensities were logged using a numbered rating pain score (NRS) from zero (no pain at all) to ten (worst pain imaginable).A significant CPM effect in the control group as in patients with posttraumatic cold intolerance was found (P < 0.001). The absolute changes in pre vs. post conditioning were in the entire patient group significantly smaller compared to the control group (absolute P=0.019, relative P=0.004). The level of pain modulation was significantly higher when comparing control with the nerve lesion group (P=0.003) and the amputation group (P=0.011). The relative differences between pre and post condition are displayed in Figure 2 for the control, patient group and the subgroups.

Discussion

The objective of this study was to evaluate the function of the conditioned pain modulation (CMP) system in the central descending inhibitory system in patients with posttraumatic cold intolerance. Compared to healthy controls, we found that the CPM effect was reduced in patients with cold intolerance, although in both controls and patients with posttraumatic cold intolerance a CPM effect was measured.

The finding that the CPM effects in the cold intolerance group was smaller than in the control group is similar to findings in other chronic pain syndromes.[28-32] The reduced CPM effect indicates that the function of the central descending inhibitory system is altered.[24,25] Because the central descending inhibitory system can inhibit or facilitate transmission of noxious information, it has been suggested to be involved in the pathophysiology of chronic pain.[25-27]

It has been reported that alterations of the CPM system can be a predictor of chronic postoperative pain. Yarnitsky et al. measured CPM effects in pain-free patients before a thoracotomy.[37] These patients were followed over time to study the development of chronic postoperative pain. Patients with a smaller CPM effect and chronic postthoracotomy pain showed higher pain scores. The findings of this study suggest that the pain modulation pattern might be essential in developing chronic pain.[23,37] Possibly, CPM testing could be a diagnostic tool to predict the probability of developing clinical cold intolerance, which could be of great interest to inform and educate patients about the chances of developing cold intolerance.

The present study has a number of limitations. The first limitation is that the number of subjects is relatively small. Despite of this small number of patients, we found strong significant differences comparing patients and controls, indicating relatively large effects. It should be noted, however, that due to the small sample size and the relatively large SD of some of the group values (Table 2), the true size of the (significant) differences between the groups could not be estimated very precisely. A second limitation was that the patient group has a higher percentage of males (66%) compared to the control group (57%). Although data indicate that there are differences in the size of the CPM effect between males and female, this relatively small different cannot account for the large differences in CPM effect between groups.[38] Another limitation was the inclusion of patients with only cold intolerance. Based on the present findings, it may be valuable for future studies to compare posttraumatic patients with cold intolerance to patients without cold intolerance to determine the predictive value of the CPM test.

The patient group with cold intolerance we described in this study could have benefit of the research performed by the group of Yarnitsky et al.[39] The group tried to improve drug selection in patients with painful diabetic neuropathy, Yarnitsky et al. hypothesised that if an individual's pain modulation system is malfunctioning, then a drug that rectifies the mechanism would be most beneficial in alleviating the pain. Therefore they expect patients with less efficient CPM such as

patients with cold intolerance, to benefit more from the Selective Serotonin Norepinephrine Reuptake Inhibitor (SSNRI) than those with a well-functioning CPM.[39]

In conclusion, similar to findings in other chronic pain syndrome patients, we found that the CPM is reduced in patients with cold intolerance, indicating an altered function of the central descending inhibitory system in this patient group. This could be of interest that patients with cold intolerance may benefit from SSNRI's. Secondly, the CPM effect can be a predictive for the development of chronic pain such as cold intolerance for instance in patients who have had a nerve lesion.

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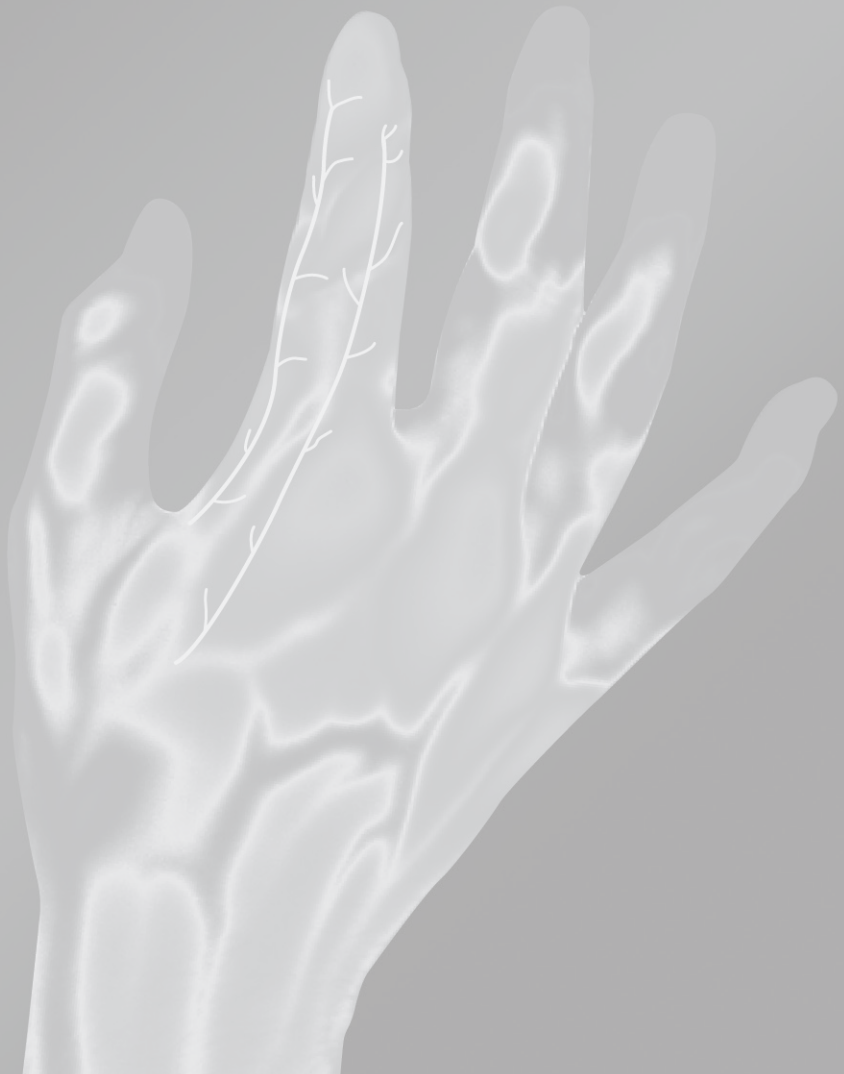
Chapter 9

General discussion



The purpose of this thesis was to study the pathophysiology of cold intolerance. Two hypotheses are often proposed to explain the origin of cold intolerance. 1) A disordered vascular system leading to an impaired thermoregulation of the extremities and the skin, and/or 2) A disrupted neural regulation leading to a changed neural response to cold stimuli that causes the symptoms of intolerance to cold. In the previous chapters we have tested both hypotheses and the results have been presented. The outcome of these chapters will help us to accept or reject these hypotheses that were presented in the introduction of this thesis.

Investigating the clinical problem

Complaints of cold intolerance in patients with a hand fracture have not been reported extensively prior to our study. This patient group is interesting because no major nerve or blood vessel has been damaged. We investigated the prevalence and severity of cold intolerance in 129 patients with one or more hand fractures using the CISS questionnaire. Patients with distinct nerve or vascular lesions were excluded. We found that 38% of the patients with a hand fracture had symptoms of cold intolerance. In addition, significant correlations were found between CISS score and the number of fractures, number of hospital visits, number of rehabilitation visits and absence of work due to rehabilitation.[1] This indicates that cold intolerance is an important problem in patients after a hand fracture, but also that posttraumatic cold intolerance can occur without the presence of a lesion to one of the major nerves of the hand. The findings of this chapter should create awareness that these complaints can occur in these patients and they create better recognition at the outpatient clinic.

The reason why this patient group develops cold intolerance could be because of the involvement of C and A-delta nerve fibers that can cause different types of bone pain.[2] However, how these nerve findings change during a trauma/fracture is unknown and therefore should be investigated.

Vascular Origin Hypothesis

Cold intolerance has been related to low skin temperature, discolored fingers and a poor vascular function. In literature a poor vascular function has been suggested to be a key factor in the development of cold intolerance,[3-5] however the exact pathophysiology is unknown. To study the vascular origin of cold intolerance, we used two different techniques to assess the thermoregulation: 1) measuring the rewarming after a cold stress test and 2) measuring the cold induced vasodilatation (CIVD) reaction during long-lasting cooling.

Rewarming

By applying a cold stress test to patients that recovered from a hand fracture, we determined if the rewarming of the fractured finger was disordered. Via thermographic measurements we found no differences in the rewarming patterns between the affected and non-affected hand of the patients, and between patients and controls. In addition, we found no relation between the severity of cold intolerance and rewarming patterns.[6] In contrast to our expectations a number of patients with relatively high CISS scores demonstrated normal rewarming patterns. The lack of a relation between a dysfunctional rewarming and cold intolerance may have important implications for understanding cold intolerance since it indicates that problems with thermoregulation are not the origin of cold intolerance. It also puts the findings of Ruijs et al.[5] in a different perspective. They found a relation between abnormal rewarming and cold intolerance in nerve injury patients. While the study of Ruijs et al. suggests that abnormal rewarming, and therefore abnormal thermoregulation, may lead to cold intolerance, our findings indicate that they may both be symptoms of a nerve injury but that cold intolerance is not the direct result of a disordered thermoregulation.

While these findings suggest that dysfunctional thermoregulation may not directly cause cold intolerance, the complexity of upper extremity trauma like in hand fractures with collateral tissue damage, such as micro lesions to the vessels, makes it impossible to reject the vascular origin hypothesis. Therefore we selected an animal model to explore the rewarming pattern in rats after very specific and isolated nerve injuries. The rats received different types of peripheral nerve injuries: spared nerve injury, complete sciatic nerve lesion and a sham operation. A cold stress was performed and the rewarming patterns were measured using thermocouples that were attached on the dorsal side of the hind paw preoperatively, three, six and nine weeks postoperatively. Despite the fact that these rats developed cold intolerance, which was objectified with a cold plate test, the results of this study showed that after peripheral nerve injury the rewarming was not altered.[7]

Cold induced vasodilatation

A second technique, in addition to measure rewarming patterns, to assess the quality of the thermoregulatory system in rats after different types of peripheral nerve injuries is to measure the cold induced vasodilatation (CIVD) reaction. The role the sympathetic nervous system plays in the magnitude of CIVD is still undisputed, it is still debated if the sympathetic system is initiating the CIVD response or if peripheral triggers are responsible. The aim of chapter 5 was to determine the role of the sympathetic system in initiating a CIVD response. The same rats as in the previous chapter were used for this study. The rats had undergone different types of peripheral nerve injury, the hind limbs were cooled and skin temperature was recorded (pre-, three, six and nine weeks postoperatively) to evaluate the presence of CIVD reactions. Again, the presence of cold intolerance was determined using a cold plate and the Von Frey test to measure mechanical allodynia.

In chapter 5, we found no significant differences in CIVD reactions between groups (SNI, CSL and Sham) and no differences were evident when comparing the CIVD reactions at the different measurement points (preoperatively, three, six and nine weeks postoperatively). The outcome of this study leads to different conclusions. First, because the CIVD reactions seem not to be effected by any type of peripheral nerve lesion, the sympathetic system probably does not play a major role in the initiation of a CIVD reaction in the hind limb of a rat. Peripheral triggers are more likely to be responsible for initiating a CIVD reaction. Triggers that regulate vascular control and that can function without peripheral nerve innervation, such as norepinephrine[9] and nitric oxide that can activate vasodilatation.[10] Second, because no substantial changes in the CIVD reaction after peripheral nerve injury were observed, the origin of cold intolerance after a traumatic nerve injury could be initiated by local factors. These local factors could be over-active skin nerve fiber endings that fire too rapidly causing pain, or the local transient receptor potential channels that can behave like microscopic thermometers and can sense hot or cold environments.

In conclusion, based on the above-mentioned findings, along with the conclusions of chapter 3 and 4, it is unlikely that cold intolerance is caused by a disordered vascular function based on an impaired thermoregulation of the extremities. As described in chapter 3 it could be that there is a disordered thermoregulatory system however it does lie at the root of the complaints of patients with cold intolerance. Therefore the first hypothesis, in which a disordered vascular system can lead to an impaired thermoregulation of the extremities and the skin can be rejected. For the second part of this thesis, we were interested in changes of the small nerve fiber endings and the ability to modulate pain in a patient with cold intolerance.

Neural Origin Hypothesis

To test the presence of a neural dysfunction, two sub-hypotheses were tested, 1) there are somatosensory changes in patients with cold intolerance 2) there is a difference in pain modulation between groups of control and patients with cold intolerance after hand injury. When we could identify a neural dysfunction in patients with cold intolerance, it would be possible to concentrate future research on the exact mechanism of cold intolerance with a possible result that locally acting medication could be developed.

Quantitative Sensory Testing

To test the first sub-hypothesis of somatosensory changes in patients with cold intolerance, we designed a measurement setup where the behavior of the different types of small nerve fibers (Aβ-, Aδ- and C) was investigated in patients with cold intolerance. By using a quantitative sensory testing device, in a non-invasively manner, the behavior of the different types of small nerve fibers (Aβ-, Aδ- and C) can be measured.

Within the cold intolerant patient group, three subgroups were tested: 1) median and or ulnar nerve injury patients, 2) patients with an amputation of one or more fingers, and 3) hand fracture patients. Cold intolerance was determined by the cold intolerance symptom severity (CISS) questionnaire. The patients were subjected to the QST using a Medoc TSA-II Neurosensory analyzer and were measured at the dermatome where they experienced most complaints. We demonstrated that patients with cold intolerance significantly differed from the healthy control group on their cold detection threshold, warm detection threshold, thermal sensory discrimination, mechanical detection threshold and vibration detection threshold. The outcome of this study indicates that the peripheral sensory channels of A-delta and C fibers are disrupted in patients with cold intolerance. The QST abnormalities did not differ systematically between the three patient subgroups; however the number of abnormal QST variables as well as the magnitude of the differences was the most prominent for the nerve injury group.

Taking previous findings into account, this chapter shows that the most peripheral part of the nervous system, the small nerve fiber endings, are dysfunctional in patients with posttraumatic cold intolerance, which is consistent with the hypothesis that a neural origin is at the root of the pathophysiology of cold intolerance.

Conditioned Pain Modulation

To test the second sub-hypothesis, that there are somatosensory changes in patients with cold intolerance, we designed a measurement setup using the phenomenon 'pain inhibits pain'. An explanation why only some patients develop cold intolerance after a trauma to the hand could relate to interpersonal variability in pain modulation. In cold intolerant patients and in healthy controls, we measured the conditioned pain modulation (CPM) effect. In this chapter, fourteen healthy controls and 24 cold intolerant patients participated. Of the 24 patients, 11 had a nerve lesion and 13 an amputation of one or more digits. We found a significantly weaker CPM effect in the patients compared to the controls, as also reported in patients with other chronic pain conditions. These results indicate that the conditioned pain modulation system in patients with cold intolerance has been altered, suggesting an altered function of the central descending inhibitory system.

In conclusion, based on the past two chapters, but with the knowledge of the entire thesis, we believe that the most peripheral part of the nervous system is disordered in patients with cold intolerance. More specifically the small nerve fiber endings are not functioning well in these patients. Secondly the ability to modulate pain is disordered, indicating that the accompanying central descending inhibitory system does not function to its fullest extend in patients with posttraumatic cold intolerance. Therefore the second sub-hypothesis is not rejected and there is a difference in pain modulation between groups of control and patients with cold intolerance after hand injury. The pathophysiology of cold intolerance seems to find its origin in disordered small nerve fiber endings, where disordered thermoregulation can accompany the complaints.

Clinical case continued

What Happened to Mr. Beukers?

At the outpatient clinic the CISS questionnaire was completed, Mr. Beukers scored 49 points. According to the criteria of Dutch normative population, Mr. Beukers scored more than the cut off value of 30 points and is diagnosed with cold intolerance. In addition, the rewarming pattern, quantitative sensory testing measurement and the conditioned pain modulation effect were examined. With the use of an infrared temperature measurement after a cold stress test, an abnormal rewarming pattern was seen in the stump. QST showed that all nerve fibers were disordered and the CPM effect was reduced.

Unfortunately, at this moment, the hand surgeon could only conclude that the most effective way to prevent cold intolerance would be to avoid exposure to cold. However, with the results of the questionnaire, Mr. Beukers felt recognized in his complaints. The pain in the cooling cell was genuine. Through the measurement of an abnormal rewarming pattern, to protect the stump of cooling, gloves were advised.

Limitations and shortcomings of this thesis

There are a number of limitations throughout the studies that are important to mention. In this section, we will not repeat what has been stated in the discussion section of each chapter. However, with a "bird's eye view", the limitations of this thesis will be discussed.

While we used the cold intolerance symptom severity (CISS) questionnaire and found that it was an appropriate tool to diagnose cold intolerance and measure the severity of the complaints, we do believe that the CISS has shortcomings. The outcome of the questionnaire is useful to test the "subjective" complaints of the patient and measures, in general, the disability in the daily life activities. We believe that the questionnaire can assess the complaints of cold intolerance in a patient population. However a measurement setup such as quantitative sensory testing should be used to more specifically assess the severity of cold intolerance in an individual. A questionnaire that does not focus solely on the disability in daily life but that also takes the range of pain sub-modalities in account should be developed. This additional information will be useful in a clinical surrounding and in a research setting, where the cold intolerant patient will be described in a more specific manner.

Throughout this thesis we have struggled with the fact that in each subgroup 1) patients with cold intolerance after a fracture to the hand, 2) peripheral nerve injury and 3) an amputation of one or more digits, not a single patient has sustained the same type of trauma. The impact and the mechanism of the trauma are of influence on the type and severity of collateral damage. This

inter- and intra-subgroup variability's lead to high variability in outcome and makes it difficult to conclude on mechanisms of cold intolerance. However, on the other hand, in the animal study designs (where there is a homogeneous trauma mechanism) it is difficult to objectify the severity of the cold intolerance complaints that the rat experiences. Unfortunately these issues are inherent on working in a posttraumatic clinical or animal setting on the complaints of a painful temperature.

In chapter 3 and 4, we had difficulty with the definition of a rewarming pattern. In literature there is no consensus when a rewarming period starts and stops. We used an algorithm to determine the start and ending of the active rewarming and, in the cases where the algorithm failed, we manually selected start and stop of the active rewarming. Especially in situations of abnormal rewarming, difficulties in defining these events can be predicted. We suggest that a commonly accepted algorithm should be created for future studies, where standard start and stop moments can be calculated based on the acceleration and deceleration of the rewarming. Such an algorithm would also allow for comparison between studies.

In line with the previous shortcoming, we had no exact (mathematical) guidelines to define a CIVD reaction in rats. Our observed cyclic changes after cooling the rat hind limbs are strongly indicative of the CIVD phenomenon and appear similar to CIVD reactions reported in rats and human's.[15,16] In our opinion, CIVD research is hampered by the absence of a uniform quantification of a CIVD response. Again we would like to stress that studies should try to develop a schematic algorithm to define a CIVD reaction which takes different variables such as core temperature, subject (type of animal), cooling temperature, amplitude, and surrounding temperature into account. By means of a commonly accepted algorithm, comparisons within previous and future studies can be made.

To fully investigate our two hypotheses of a vascular and neural origin of cold intolerance, a solely vascular injury model should be designed. We did not measure cold intolerance in a patient with solely a vascular injury without any type of nerve injury. In a clinical setting these types of injury usually have additional nerve injury. However a suitable study design would be an animal vascular injury model without additional nerve injury. It could be that a rat has sustained an injury to a major artery in a paw, in which measurements are performed such as described in chapter 4 and 5. In this model, cold intolerance, mechanical allodynia, rewarming patterns and a CIVD reaction should be measured and compared to the control, sham and nerve injury group. The outcome of such a study could possibly elucidate the neural hypothesis stated in this thesis.

Despite the fact that the cold plate test can detect cold intolerance in an animal model, the test has it shortcomings and cold intolerance could be tested in a more sensitive manner. An objective measurement tool of noxious cold where animals are in their natural habitat could earn the attention of a researcher on cold intolerance that has knowledge of research on animal behaviour. This will make long-term measurements of the behaviour of an animal in its natural habitat more accurate. It is possible that a reflex is measured in the cold plate test therefore a more sensitive study design should be designed.

In the chapters 7 and 8 we did not examine the complaints of the patients to the fullest extent, resulting that the term cold intolerance could not be defined in a more specific manner. Patients with cold intolerance were included; we measured the presence/absence of mechanical allodynia in the QST assessment, however, a questionnaire (such as the DN4 or the McGill[17,18]) to formally diagnose patients with neuropathic pain could contribute to the discussion of the terminology of cold intolerance. The term cold intolerance after nerve injury can be difficult to distinguish from neuropathic pain. Neuropathic pain is defined by the International Association for the study of Pain, as pain initiated or caused by a primary lesion, dysfunction or transitory perturbation in the peripheral or central nervous system. We believe with the knowledge of this thesis that if the IASP definition is followed, patients who underwent a lesion of a nerve and develop cold intolerance have a sub-form of neuropathic pain however a more precise definition should be stated for the cold intolerant patient group.

Clinical implications

In this thesis we have found that 38% of the patients with a fracture to the hand have abnormal cold tolerance. Proving cold intolerance in hand fracture patients can improve awareness and recognition for the patient.

When a doctor wants to treat a cold intolerant patient at the outpatient clinic, choice of medication is limited. In chapter 3 we found that the rewarming patterns in the fractured hand of patients with and without cold intolerance showed no correlation with the CISS score, indicating that the severity of cold intolerance in hand fracture patients is not related to rewarming patterns. In addition, in nerve injured rats, no direct correlation between cold intolerance and rewarming patterns was seen. These findings are of interest in developing treatment for cold intolerance, since treatment focusing on vaso-regulation may not help diminish symptoms of cold intolerant patients.

In this thesis, the QST setup has proven its ability to assess sensory nerve functions in cold intolerant patients in a noninvasive manner and in great detail. This setup can be used in a clinical setting to assess somatosensory abnormalities in patients with cold intolerance, to identify specific subgroups of neural dysfunction and to follow development of somatosensory changes over time to evaluate possible responses to (experimental) therapy. In addition, this thesis has provided additional insight in the pathophysiology of cold intolerance and has reinforced the hypothesis that a neural origin could lie at the root of the complaints of patients with posttraumatic cold intolerance, which is of interest for possible developments of locally acting medication.

The last conclusion of this thesis, that the CPM is reduced in patients with cold intolerance, indicating an altered function of the central descending inhibitory system is of clinical relevance, similar to findings in other chronic pain syndrome patients. In the search for a well functioning and specific drug for chronic pain syndromes different targets can be used to reduce the

sensation of pain. Based on previous performed research it is suggested that in patients with diabetic neuropathy and a malfunctioning CPM, the patients benefitted more from Serotonin-Noradrenalin Reuptake Inhibitors (SNRI), which augments the endogenous pain inhibitory capacity (via the descending inhibitory pathways).[14] In contrast with these findings patients with a well functioning CPM did not benefit of SNRI's. Based on these findings we expect patients with cold intolerance to have a less-efficient CPM system. It may be that this patient group would benefit more from the Serotonin Norepinephrine Reuptake Inhibitor (SNRI) than those with a well-functioning CPM.[14] However additional research of this mechanism in this specific patient group should be performed to confirm or reject this assumption.

Future perspectives

Based on this thesis, we can reject the first hypothesis that it is a disordered vascular system that leads to an impaired thermoregulation of the extremities and the skin, thereby causing cold intolerance while our findings support the second hypothesis that a neural disorder leads to a changed neural response to cold stimuli that result in cold intolerance. Based on these findings, it is possible to give recommendations on a focus of future research on cold intolerance, which in general, should focus on nerve innervation. More specifically, we can make the following recommendations:

– More details are needed on the development of small nerve fiber function and of central nervous system pain modulation after hand trauma in patients that develop cold intolerance and patients that do not. To do so, larger scale and prospective longitudinal studies could be designed. The gathered information could provide detailed information of the influence of trauma on small nerve fibers and pain modulation. A prospective cohort study can be designed were small nerve fibers and pain modulation system can be assessed previous to the trauma, the subjects that are prone to undergo a trauma to the hand are followed. The outcome of this study will elucidate if patients with cold intolerance develop symptoms of cold intolerance because previous to the trauma they had a disordered CPM or that they develop a disordered CPM because of the trauma. These findings will make it possible to predict and then inform patients if they are at risk to create cold intolerance.
– The part of the central descending nervous system in cold intolerance patients which is disordered should be narrowed down. In addition it could be that a disorder in CPM effect is caused by two possibilities 1) as a consequence of the trauma peripheral nerve changes are present that influence the activation of small nerve endings 2) central regulation of received action potentials are disordered. To investigate which part of the central descending nervous system is not functioning well, a study could be designed were the different parts of the central descending system are blocked. Distally the peripheral nervous system could be tested by performing a peripheral nerve block, in contrast, by administering different types of medication that blocks the cranial/central part of the nervous system the effect on the central descending nervous system can be investigated. However it could be that in the central descending nervous system each different part contributes to a disordered CPM effect. Therefore it is of great interest to understand the CPM effect in a more detailed manner.
– Further research on the pathophysiology of cold intolerance should concentrate on local biochemical changes and their effects on small nerve fiber endings. Alternative local mechanisms that could explain the complaints that are experienced in patients with cold intolerance could be Transient Receptor Potential channels. It is known that Transient Receptor Potential channels (TRP) register ambient temperature, and that deregulation

of the properties or the number of these channels could possibly contribute to cold intolerance[11]. It has been suggested that an up-regulation of TRP channels after peripheral nerve trauma may cause cold intolerance.[12,13] Research at the effect of a trauma on the TRPA1 channels, and a possible antagonist can be developed which could diminish the complaints of patients with posttraumatic cold intolerance. A local medication such as mustard oil that can be applied as a cream could give substantial pain relief to this patient group.

- A mathematical algorithm should be designed that defines a rewarming pattern and a CIVD reaction. The algorithm should take into account all the different variables that are of influence on a CIVD reaction. The algorithm should be applicable to humans which makes it possible to compare studies. Ideally the algorithm will be applicable to compare the outcome of studies performed on human as well as animal CIVD reactions.
- A measurement setup should be designed to test cold intolerance in animals. The animals should be tested in their natural surroundings without stress, possibly a floor with different temperatures could be designed were a reflex reaction of the animal is avoided.
- The definition and description of cold intolerance should be redesigned. The new cold intolerance definition should include the knowledge of the present literature and probably will be a subgroup of the neuropathic pain patient group. A suggestion could be: Pain initiated or caused by a primary lesion, dysfunction or transitory perturbation in the peripheral or central nervous system.

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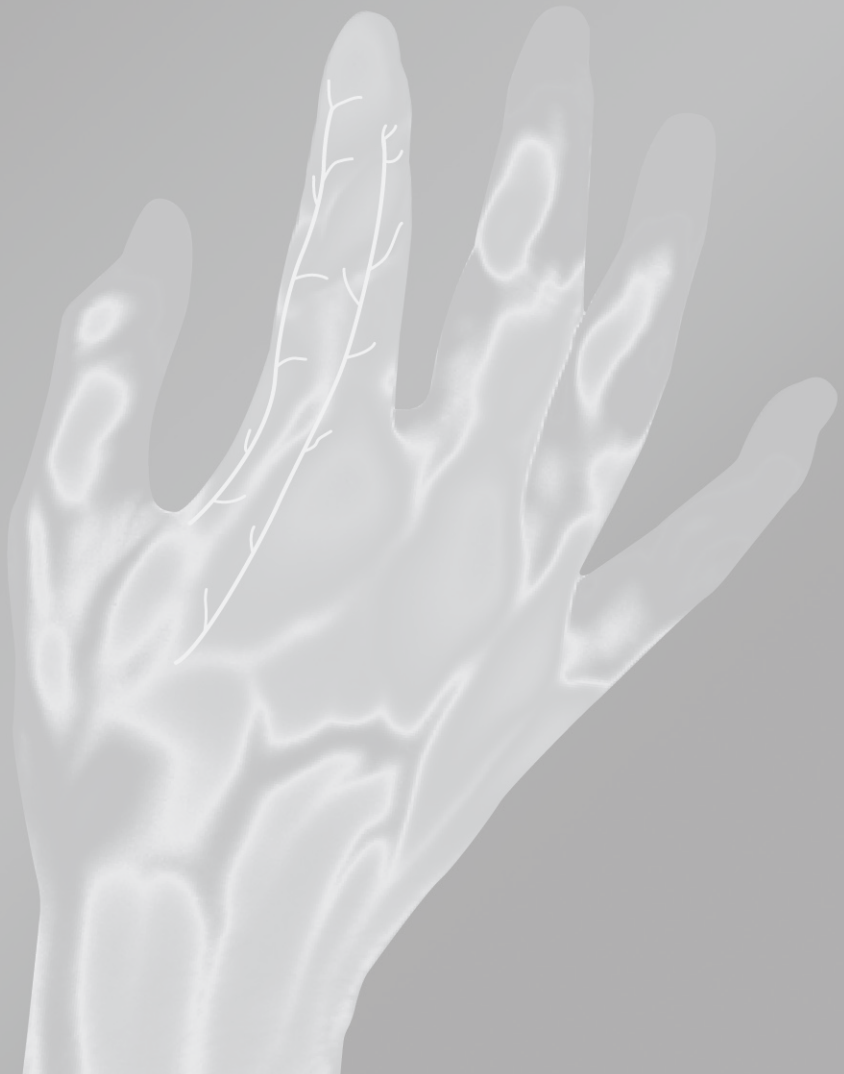
Chapter 10

Summary



Summary

The aim of this thesis was to further understand the pathophysiology of cold intolerance. From our own clinical experience and from literature we know that cold intolerance is commonly seen in patients after a trauma to the hand, for instance a nerve injury, an amputation, or the hand and arm vibration syndrome. The prevalence of cold intolerance in patients with a hand fracture, however, was unknown. In order to become fully informed of the severity of the **clinical problem** we investigated a patient population of hand trauma patients. In **Chapter 2**, we performed a retrospective study to determine the prevalence and severity of cold intolerance in patients who sustained a fracture to the hand. This patient group was of interest to us because no major nerves or blood vessels were damaged. The widely accepted cold intolerance symptom severity (CISS) questionnaire was used to measure the severity of cold intolerance in 129 patients with one or more hand fractures. Patients with additional major nerve or vascular lesion were excluded. With a response rate of 59%, we found that 38% of the patients with a hand fracture had cold intolerance. In addition, significant correlations were found between CISS score and number of fractures, number of hospital visits, number of rehabilitation visits and absence of work due to rehabilitation, indicating that cold intolerance is an important problem in patients after a hand fracture, but also that posttraumatic cold intolerance can occur without the presence of a lesion to one of the major nerves or blood vessels of the hand.

Part I was designed to investigate if a vascular damage is the origin of cold intolerance. In **Chapter 3,** we investigate the rewarming patterns in patients with cold intolerance. With an average of 30 months after patients recovered from a hand fracture, we performed a cold stress test. Temperature during the rewarming phase was measured using infrared videothermography. We found no significant differences in the rewarming patterns comparing the affected and non-affected hand of the patient. Analyzing the data and comparing the rewarming pattern of the dominant and non-dominant hand of the control subjects, no significant differences were found. Also no significant differences were found comparing the patients and controls. The results of Chapter 3 revealed no relation between the severity of cold intolerance and rewarming patterns after cold stress testing. This suggests that the thermoregulation of the hands in cold intolerant patients may not be responsible for the complaints, at least not after a cold stress test. Suggesting therefore that disrupted thermoregulation may not directly cause cold intolerance.

The complexity of upper extremity trauma during a hand fracture with accompanying tissue damage makes it is impossible to solely reject the understanding that a vascular origin is the cause of cold intolerance. Therefore, we selected an animal model to further explore the relation between a vascular or thermoregulatory dysfunction and the origin of cold intolerance.

The animal study in **Chapter 4** was designed to further investigate the relation between disordered thermoregulation and cold intolerance. Twenty-four male Wistar rats were divided into three equal groups. The rats underwent different types of peripheral nerve injury: the

first group was operated on the left hind limb using the spared nerve injury model, and the second group received a sham operation, while the third group had the complete sciatic lesion operation. A cold stress test was performed to test the presence of cold intolerance, and a Von Frey test was performed to test mechanical allodynia. Rewarming patterns were measured via thermocouples that were attached on the dorsal side of the hind paw. All measurements were performed preoperatively at 3, 6 and 9 weeks postoperatively.

Despite the fact that after peripheral nerve injury these rats developed cold intolerance, the results of this study showed that the rewarming was not altered. This chapter demonstrates that peripheral thermoregulation can still be intact after a peripheral nerve injury in a rat model. Local receptors that innervate thermoregulation via vasoconstriction and dilatation in a cold intolerant paw/hand should be investigated.

The aim of **Chapter 5** was to determine the role of the sympathetic system in initiating a cold induced vasodilatating (CIVD) response. Cold Induced Vasodilatation (CIVD) is a cyclic regulation of blood flow during prolonged cooling of protruding body parts. It is generally considered to be a protective mechanism against local cold injuries after peripheral nerve injury. Changes in the CIVD reaction have been described in patients with cold intolerance after a traumatic peripheral nerve injury. It has been suggested that there is a relation between CIVD and the development of cold intolerance.

The role of the sympathetic system in initiating a CIVD response in patients with cold intolerance could help us to find the origin of cold intolerance. Therefore, the same rats were used from Chapter 4. In this study the rats have sustained different types of peripheral nerve injury, followed by cooling of the hind limbs and recording skin temperature at pre-, three, six and nine weeks postoperatively to evaluate the presence of CIVD reactions. Again, cold intolerance was determined using a cold plate and mechanical allodynia with the Von Frey test. We found no significant differences between groups (Speared Nerve Injury (SNI), Complete sciatic lesion (CSL) and Sham) also no significant differences were found comparing the number of CIVD's preoperatively and three, six and nine weeks postoperatively. The results of the cold plate and Von Frey test showed that the SNI and CSL rats developed cold intolerance and mechanical hypersensitivity. The findings of this chapter indicate that 1) the underlying mechanism that initiates a CIVD reaction is not affected by damage to a peripheral nerve, 2) the sympathetic system does not play a major role in the initiation of CIVD in the hind limb of a rat, and 3) no substantial changes in the CIVD reaction after peripheral nerve injury were observed.

Chapter 6 was written as a response to a published article on the use of the term cold induced vasodilatation. The authors interpreted the data when the cooling had ended, while we believe that a CIVD reaction should be measured per definition, during continuously cooling. In addition, no cyclic oscillation was described. Therefore, most likely, an active rewarming pattern was seen as described in chapter 3 and 4 of this thesis instead of a CIVD reaction.

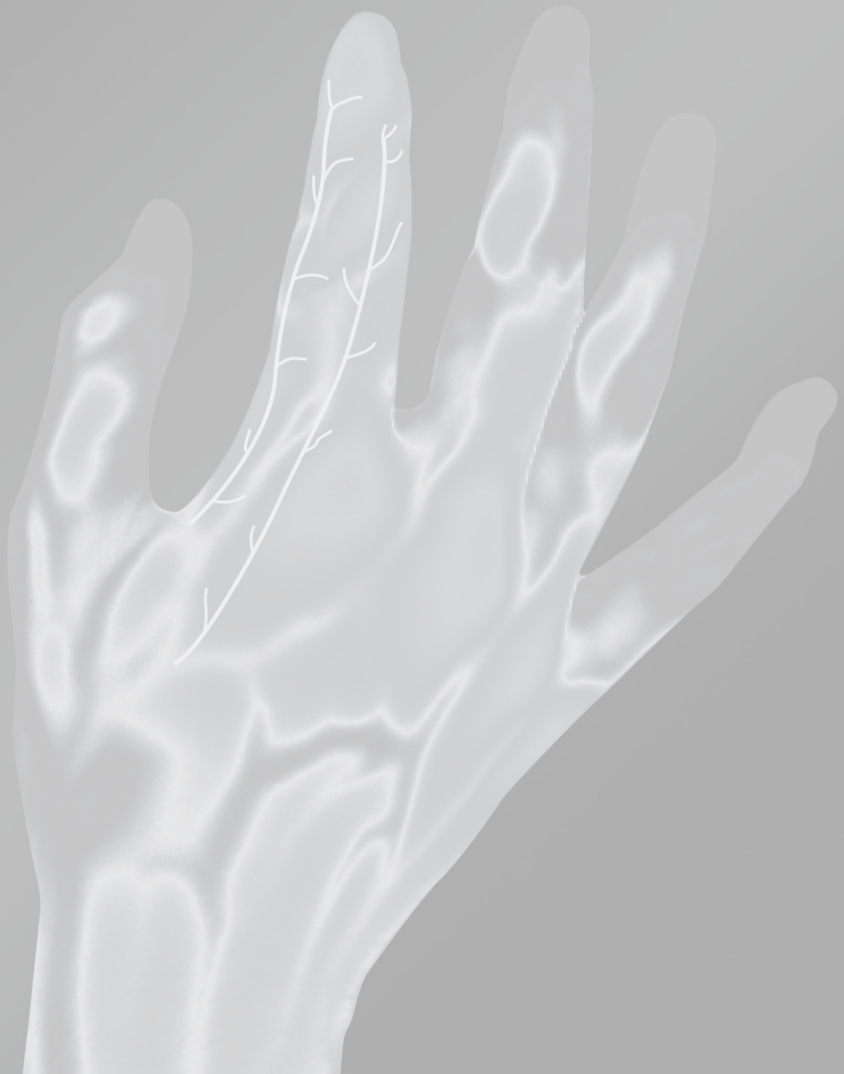
Part II: was designed to investigate if a neural origin is the basis of the pathophysiology of cold intolerance. In **Chapter 7** we used a quantitative sensory testing device to investigate if neural dysfunction is a predominant mechanism of cold intolerance. Quantitative sensory testing is specially designed to interpret, in a non-invasive manner, the behavior of the different types of small nerve fibers (Aβ-, Aδ- and C) abnormalities in patients with cold intolerance. In addition, the possibility of quantitative sensory testing device as a diagnostic tool for cold intolerance was evaluated. In the study, 15 healthy controls and 32 patients with cold intolerance participated. Eleven patients had a median or ulnar nerve injury, fifteen patients an amputation of one or more fingers and six patients a hand fracture. Cold intolerance was determined by the cold intolerance symptom severity questionnaire. All patients were subjected to quantitative sensory testing using a Medoc TSA-II Neurosensory analyzer and subjects were measured at the dermatome where they experienced the most complaints. We found that patients with cold intolerance were significantly different from the healthy control group. For the cold detection threshold, warm detection threshold, thermal sensory discrimination, mechanical detection threshold and vibration detection threshold the data was significant different. The outcome of this study indicated that the peripheral sensory channels of A-delta and C fibers were disrupted in patients with cold intolerance. When comparing the three patient groups, we found that while the QST abnormalities did not differ systematically between the three patient subgroups and that, overall, both the number of abnormal QST variables as well as the magnitude of the differences was the most prominent for the nerve injury group. This study shows that the most distal part (small nerve fiber endings) of the nervous system is disordered in patients with cold intolerance, which is in line with the hypothesis that a neural origin lies at the root of the pathophysiology of cold intolerance. This study also suggests that the quantitative sensory testing device is a useful and accurate noninvasive diagnostic tool to evaluated cold intolerance.

The goal of **chapter 8** was to test if differences between patients in developing cold intolerance then others may partially be related to differences in the interpersonal variability in pain modulation. In the study design we used the phenomenon of 'pain inhibits pain' to measure a conditioned pain modulation (CPM) effect. This CPM effect has proven to be useful to understand idiopathic pain syndromes. In this study, 14 healthy controls and 24 patients diagnosed with cold intolerance using the Cold Intolerance Symptom Severity questionnaire were included. Of the 24 patients with cold intolerance, 11 had a nerve lesion and 13 an amputation of one or more digits. To quantify the CPM, pain threshold for mechanical pressure was measured at the affected region as a baseline measure. Then, the contralateral hand received a cold stimulus of ice water to evoke the noxious conditioning. After the cold stimulus, the pain threshold for mechanical pressure was determined again. We found a CPM effect after a cold noxious stimulus in both controls and patients. However, a significantly weaker CPM effect was found in the patients compared to the controls, as also in other chronic pain conditions. These results indicate that

the conditioned pain modulation system in patients with cold intolerance is altered, suggesting an altered function of the central descending inhibitory system.

Chapter 9 The main results from the previous chapters and their implications are discussed. Conclusions are made, research questions are answered, the main objective evaluated and possibilities for future research are shared.

Nederlandse samenvatting



Samenvatting

Het doel van dit proefschrift was om inzicht te verkrijgen in de belangrijkste werkingsmechanismes van koude intolerantie. Vanuit de wetenschappelijke literatuur weten we dat koude intolerantie kan optreden na een trauma aan de hand. Voorbeelden hiervan zijn: een zenuwletsel, een amputatie van één of meerdere vingers, handen die veel zijn blootgesteld aan trillingen door machines of aan extreme koude. Tot op heden is het percentage patiënten dat na een fractuur aan de hand koude intolerantie ontwikkelt, onbekend. Om volledig inzicht te verkrijgen in de ernst van het **klinische probleem** van koude intolerantie hebben we patiënten met een hand trauma onderzocht.

In **hoofdstuk 2** hebben we een retrospectieve studie uitgevoerd om de prevalentie en de ernst te bepalen van koude intolerantie bij patiënten met een fractuur in de hand. Deze specifieke patiëntengroep was van belang voor ons omdat bij hen geen grote zenuwen of bloedvaten beschadigd waren. Patiënten met daarnaast ook een letsel aan een zenuw of bloedvat werden dan ook uitgesloten voor de studie. De algemeen bekende 'cold intolerance syptom severity' (CISS) vragenlijst werd gebruikt om de ernst van koude intolerantie te meten bij 129 patiënten met één of meerdere fracturen in de hand. Van de patiënten beantwoordde 59% de vragenlijst. In deze groep bleek 38% koude intolerantie te hebben. Tevens hebben we significante correlaties gevonden tussen de CISS score en het aantal fracturen, het aantal ziekenhuis bezoeken, het aantal bezoeken aan fysiotherapeut en werkverzuim door revalidatie. Dit geeft aan dat koude intolerantie niet alleen een belangrijk probleem is voor patiënten na een fractuur in de hand, maar ook dat posttraumatische koude intolerantie kan ontstaan zonder de aanwezigheid van een letsel aan een van de grote zenuwen of bloedvaten van de hand.

Deel 1 van dit proefschrift werd opgezet om te onderzoeken of beschadiging van een bloedvat de oorzaak is van koude intolerantie. In **hoofdstuk 3** hebben we de opwarmingspatronen van het aangedane gebied bij patiënten met koude intolerantie onderzocht in vergelijking met de niet aangedane hand en vergeleken met controle patiënten zonder koude intolerantie. Gemiddeld 30 maanden na herstel van een fractuur in de hand hebben we een koude stress test uitgevoerd. De temperatuur werd gemeten tijdens de opwarmingsfase door gebruik te maken van een infrarood videothermografische camera. We hebben geen significante verschillen gevonden in de patronen van opwarming tussen de beschadigde en niet beschadigde hand van de patiënten groep. Ook bij de vergelijking van de patronen van opwarming tussen de dominante en de niet dominante hand bij patiënten met koude intolerantie en controle patiënten werden geen significante verschillen gevonden. De resultaten van hoofdstuk 3 lieten geen verband zien tussen de ernst van koude intolerantie en de patronen van opwarming na het testen met koude stress test. Dit suggereert dat de thermoregulatie van de handen bij patiënten met koude intolerantie niet primair verantwoordelijk is voor de klachten. Echter, op basis hiervan kan een vasculaire oorzaak van koude intolerantie niet worden uitgesloten. Een trauma van de bovenste extremiteit

met een fractuur in de hand is immers complex en leidt tot veel weefselschade. De complexiteit van een trauma aan de bovenste extremiteiten met een hand fractuur leidt tot dusdanig veel weefselschade, dat het onmogelijk is om een vasculaire oorzaak van koude intolerantie uit te sluiten. Daarom is gekozen voor een vervolgonderzoek naar de oorsprong van koude intolerantie met proefdieren.

Het dieronderzoek in **hoofdstuk 4** werd opgezet om de relatie te onderzoeken tussen een mogelijk verstoorde thermoregulatie en koude intolerantie. Mannelijke Wistar ratten (24) werden opgesplitst in drie gelijke groepen. De ratten werden geopereerd en kregen verschillende soorten perifeer zenuwletsel: de eerste groep onderging een operatie waarbij ze een partieel zenuwletsel werd aangedaan(SNI) aan de linker achterpoot, de tweede groep onderging een operatie waar geen zenuwletsel werd gemaakt (Sham), bij de derde groep werd de zenuw volledige doorgenomen(CSL). Een koude stress test werd uitgevoerd om de aanwezigheid van koude intolerantie te testen tevens werd er een Von Frey test uitgevoerd om mechanische allodynie te testen. De opwarmingspatronen werden gemeten door middel van thermokoppels die werden aangebracht aan de dorsale zijde van de achterpoot. Alle metingen werden preoperatief en vervolgens 3, 6 en 9 weken postoperatief uitgevoerd.

Ondanks het feit dat na het perifere zenuwletsel deze ratten koude intolerantie ontwikkelden, toonden de resultaten van deze studie aan dat het patroon van opwarming niet veranderde, waaruit we kunnen concluderen dat perifere thermoregulatie nog steeds intact kan zijn na een perifeer zenuwletsel in een rat model.

Het doel van **Hoofdstuk 5** was om de rol te bepalen van het sympathische systeem in het initiëren van een 'cold induced vasodilatating' (CIVD) response. Cold Induced Vasodilatation (CIVD) is een cyclische regulatie van de bloedstroom gedurende langdurige afkoeling van de perifere lichaamsdelen. Het wordt algemeen beschouwd als een beschermend mechanisme tegen lokaal koudeletsel bij koude situaties, waarbij de kern van het lichaam altijd het langst warm blijft, zonder dat er schade ontstaat aan de ledematen. Veranderingen in de CIVD reactie zijn omschreven bij patiënten met koude intolerantie na een traumatisch perifeer zenuwletsel. Er is verondersteld dat er een relatie bestaat tussen CIVD en de ontwikkeling van koude intolerantie. Meer kennis over de rol van het sympathische systeem in het initiëren van een CIVD respons bij patiënten met koude intolerantie kan ons helpen bij het vinden van de oorsprong van koude intolerantie.

Voor deze studie werden dezelfde ratten gebruikt als in Hoofdstuk 4. In deze studie zijn de ratten onderzocht op de aanwezigheid van een CIVD reacties door na hun operatie de achterpoten af te koelen en de huidtemperatuur te meten, ook hier weer preoperatief en 3, 6 en 9 weken postoperatief. Wederom werd koude intolerantie gevonden bij het gebruik van een koude plaat en mechanische allodynia met de Von Frey test. We hebben geen significante verschillen gevonden in de CIVD reactie tussen de drie groepen. Ook werden geen significante verschillen gevonden bij het vergelijken van het aantal CIVD's preoperatief en 3, 6 en 9 weken postoperatief, terwijl

de resultaten van de koude plaat en de Von Frey test aantonen dat de SNI en CSL ratten koude intolerantie en mechanische hypersensitiviteit ontwikkelden. De bevindingen van dit hoofdstuk geven aan dat 1) het onderliggend mechanisme dat een CIVD reactie initieert niet beïnvloed wordt bij beschadiging van een perifere zenuw, 2) het sympathisch systeem geen grote rol speelt bij het initiëren van CIVD in de achterpoot van een rat, en 3) geen substantiële veranderingen in de CIVD reactie na een perifeer zenuw letsel werden waargenomen.

Hoofdstuk 6 werd geschreven als antwoord op een gepubliceerd artikel met betrekking tot het gebruik van de term 'cold induced vasodilatation'. De schrijvers interpreteerden de data op het moment dat de afkoeling was beëindigd , terwijl wij van mening zijn dat een CIVD reactie per definitie gemeten dient te worden gedurende koeling. Bovendien werden geen cyclische oscillaties omschreven. Daarom werd waarschijnlijk een actief opwarmingspatroon waargenomen zoals omschreven in hoofdstuk 3 en 4 van dit proefschrift in plaats van een CIVD reactie.

Deel 2 van dit proefschrift werd opgezet om te onderzoeken of een neurale origine de basis is voor de pathophysiologie van koude intolerantie. In **Hoofdstuk 7** hebben we een kwantitatief sensorische test gebruikt om te onderzoeken of neurale disregulatie het belangrijkste mechanisme is van koude intolerantie. Kwantitatief sensorisch testen (QST) is een techniek die speciaal ontworpen is om, op een niet-invasieve wijze, de activiteit van de verschillende types van kleine zenuw vezels te meten, zoals de Aß, Aδ en C-vezels. Het gebruik van QST kan daarmee mogelijk diagnostische informatie geven over patiënten met koude intolerantie. In de studie namen 15 gezonde controles en 32 patiënten met koude intolerantie deel. Elf patiënten hadden een zenuwletsel van de nervus medianus of ulnaris, vijftien patiënten een amputatie van een of meerdere vingers en zes patiënten een fractuur in de hand. Koude intolerantie werd bepaald door de koude intolerantie symptoom vragenlijst (CISS). Alle patiënten en een controle groep werden onderworpen aan een kwantitatieve sensorische test. De dermatoom waar de patiënten de meeste klachten hadden werd gemeten. Patiënten met koude intolerantie bleken significant verschillend ten opzichte van de gezonde controle groep en zowel de A-delta en C vezels waren verstoord bij patiënten met koude intolerantie. Bij het vergelijken van de drie patiëntengroepen, zagen we dat de afwijkingen niet systematisch verschillend waren tussen de drie groepen. In het algemeen waren zowel het aantal afwijkende QST variabelen als de grootte van de verschillende verschillen met gezonde data het meest afwijkend in de patiënten met een zenuwletsel. Deze studie toonde aan dat het meest distale gedeelte van het zenuwstelsel verstoord is bij patiënten met koude intolerantie, wat overeenkomt met de hypothese dat de oorzaak van koude intolerantie een neurale origine heeft. Deze studie geeft ook aan dat het kwantitatief sensorisch testen (QST) een geschikt en accuraat non-invasief diagnostisch middel is om koude intolerantie te beoordelen.

Het doel van **Hoofdstuk 8** was om te testen of patiënten met koude intolerantie verschillen van anderen op het gebied van inter-persoonlijke variatie in pijnmodulatie. In deze studie hebben we gebruik gemaakt van het verschijnsel dat pijn ergens op het lichaam de pijnsensatie op een ander

gedeelte van het lichaam remt. Dit effect wordt het geconditioneerde pijn modulerend systeem genoemd. Dit pijn modulerend systeem wordt in de literatuur beschreven als mede verklarend voor het ontstaan van pijnsyndromen. In ons onderzoek zijn 14 gezonde controles opgenomen en 24 patiënten met koude intolerantie die zijn gediagnosticeerd door middel van de CISS vragenlijst. Van de 24 patiënten met koude intolerantie hadden 11 een zenuwletsel en 13 een amputatie van één of meerdere vingers. Om het pijn modulerend systeem te kwantificeren werd de pijndrempel voor mechanische druk gemeten in het betrokken gebied als basismeting. Daarna kreeg de contralaterale hand een pijnlijke koude stimulus met ijswater. Na de koude stimulus werd de pijndrempel voor mechanische druk opnieuw gemeten. We vonden bij zowel de controles als patiënten een pijn modulerend systeem. De effectiviteit van het pijn modulerend systeem echter, was significant lager bij patiënten in vergelijking met de controles. Dit komt overeen met resultaten bij andere chronische pijnsyndromen en toont aan dat het pijn modulerend systeem bij patiënten met koude intolerantie ontregeld is. Dit zou kunnen wijzen op een veranderd effect ten aanzien van reacties van patiënten op verschillende types pijn medicatie.

In **Hoofdstuk 9** werden de belangrijkste resultaten van de vooorgaande hoofdstukken en hun implicaties besproken. Conclusies werden getrokken, onderzoeksvragen werden beantwoord, het hoofddoel geëvalueerd en de mogelijkheden van toekomstig onderzoek gedeeld.

List of Publications

Prevalence and severity of cold intolerance in patients after hand fracture; ***E.S. Smits****, T.H.J. Nijhuis, J.B. Jaquet, F.J.T. Van Oosterom, R.W. Selles and S.E.R. Hovius:* European Journal of hand Surgery. 2010 May;35(4):306-11

Re-warming patterns in hand fracture patients with and without cold intolerance; ***E.S. Smits****, T.H.J. Nijhuis, F.J. Huygen, R.W. Selles, S.E.R. Hovius and S.P. Niehof*: American Journal of Hand Surgery. 2011 Apr;36(4):670-6.

Thermoregulation in Peripheral Nerve Injury Induced Cold Intolerant rats; ***E.S. Smits****, L.S. Duraku, S.P. Niehof, S.E.R Hovius, E.T. Walbeehm and R.W. Selles*: Journal of Plastic, Reconstructive & Aesthetic Surgery. 2012 Jun;65(6):771-9

Cold-induced vasodilatation in cold-intolerant rats after nerve injury.; ***E.S. Smits****, L.S. Duraku, S.P. Niehof, H.A.M. Daanen, S.E.R. Hovius, R.W. Selles, and E.T. Walbeehm:* Journal of Plastic, Reconstructive & Aesthetic Surgery. 2013 Sep;66(9):1279-86.

Comments to the term “cold-induced vasodilatation” in “laser doppler perfusion imaging of skin territory to reflect autonomic functional recovery following sciatic nerve autografting repair in rats”. ***E.S. Smits****, L.S. Duraku, S.P. Niehof, F.J. Kusters, S.E.R. Hovius, H.A.M. Daanen, R.W. Selles, and E.T. Walbeehm:* Microsurgery. 2013 Jan;33(1):83-4

Quantitative Sensory abnormalities in patients with posttraumatic cold intolerance; ***E.S. Smits***, E.T. Walbeehm, F.J.P Huygen, E.A.M. Van Bodegraven-Hof, S.E.R Hovius, and R.W. Selles: (under review) Am J Hand Surgery

Disordered conditioned pain modulation system in patients with posttraumatic cold intolerance; ***E.S. Smits****, R.W. Selles, L.S. Duraku, F.J.P. Huygen, S.E.R. Hovius and E.T. Walbeehm*: accepted Journal of Plastic, Reconstructive & Aesthetic Surgery

Ultrasound-guided needle positioning for near nerve electromyography of the gastrocnemius muscle in a rat model Accepted Journal of Neurosurgery *T.H.J. Nijhuis,* ***E.S. Smits****, J.H. Blok, J.W. van Neck, J.M. Hekking, E.T. Walbeehm, G.H. Visser and S.E.R. Hovius* Journal of Neurosience methods. 2011 Jan 15;194(2):283-6.

Rotterdam Advanced Mutiple Plate: A novel universal device to measure objectively cold hyperalgesia and allodynia in freely behaving neuropathic pain rats. *L.S. Duraku, S.P. Niehof, M. Everaers, Y. Misirli, S. Hoendervangers,* ***E.S. Smits****, R.W. Selles, E.T. Walbeehm* under review Neuroscience

PhD Portfolio

Summary of PhD training and teaching

Name PhD student: Ernst Steven Smits Erasmus MC, University Medical Center Department: Plastic, Reconstructive and Hand Surgery	PhD period: September 2008 – January 2014 Promotor: Prof.Dr. S.E.R. Hovius Supervisor: Dr. R.W. Selles Dr. E.T. Walbeehm

PhD training

	Year	Workload (Hours)
Teaching		
General courses		
– Principles of Research in Medicine and Epidemiology	2010	33 hrs
– Introduction to Data-analysis	2010	33 hrs
– Biomedical English Writing	2010	12 hrs
Specific courses		
– Microsurgery; Mrs. J.M. Hekking Skills lab - Plastic and Reconstructive surgery	2008-2012	250 hrs
– TEDx “CEO for one day” of the Erasmus MC. Part of TEDx Rotterdam research and leadership transformation program to spread ideas	2011	80 hrs

	Year	Workload (Hours)
Oral Presentations		
– Presentation at the annual research meeting of the Dutch Society of Plastic Surgery Amsterdam, The Netherlands	2013	28 hrs
– Presentation at the FESSH XVII (Federation of the European Societies for Surgery of the Hand) EFSHT XIII (European Federation of Societies for Hand Therapy) Congress Antwerp, Belgium	2012	28 hrs
– Presentation at the annual meeting of the Dutch Society for Hand Surgery (NVvH) Amsterdam, The Netherlands	2011	28 hrs
– Presentation at the annual research meeting of the Dutch Society of Plastic Surgery 's-Hertogenbosch, The Netherlands (awarded best presentation)	2011	28 hrs
– Presentations and Chairman of the Nerve Injury session at the 11th Triennial Congress of the International Federation of Societies for Surgery of the Hand Seoul, South-Korea	2010	112 hrs
– Presentation at the annual research meeting of the Dutch Society of plastic Surgery (NVPC) Maastricht, The Netherlands	2009	28 hrs
– Presentation at the European Conference of Scientists and Plastic Surgeons Rotterdam, The Netherlands	2009	28 hrs
– Presentation at the FESSH XIV (Federation of the European Societies for Surgery of the Hand) Poznan, Poland	2009	54 hrs
– Presentation at the FESSH XIII (Federation of the European Societies for Surgery of the Hand) Lausanne, Switzerland	2008	28 hrs
Other		
– Organizing "Annual Erasmus MC Plastic Surgery Meeting"	2008-2012	80 hrs
– Organizing the 17th Esser Course: Dupuytren's Contracture in Rotterdam	2011 2013	60 hrs 250 hrs
– Organizing the 20th Esser Course: Masterclass Neuropathic Pain		

Teaching

	Year	Workload (Hours)
Lecturing		
– Course "Anatomy of the Arm en Hand" (3^{rd} and 4^{th} year students)	2009-2012	60 hrs
Supervising practical's and excursions		
– Supervision Microsurgery course Skills lab, Erasmus MC, Rotterdam	2008-2011	100 hrs
– Course "Basics in suture techniques" (3^{rd} year students)	2009-2011	80 hrs
– Supervising 4^{th} year medical students, keuze onderzoek, Rashidi Rellum, and Sieske Hoendevangers	2009-2011	1400 hrs
Grants		
– Stichting Erasmus Fonds Pijnbestrijding (€32.000)		
– Grants received from the Gerrit Jan Mulder stichting and the Erasmus Trust fund to visit congresses		

Dankwoord

De totstandkoming van deze promotie was niet mogelijk geweest zonder de bijdrage van een aantal mensen.
In de eerste plaats zijn dit natuurlijk de behulpzame patiënten. Zonder deze patiënten is klinisch en experimenteel onderzoek onmogelijk. Graag wil ik u allen hiervoor danken.

Geachte Professor Hovius, prof, profesör,

Dank voor het gevoel van vertrouwen dat u mij altijd heeft gegeven. Dit vertrouwen heeft mij gesterkt in het uitvoeren van onderzoek, schrijven van artikelen en het organiseren van congressen. U heeft mij hierbij altijd het gevoel gegeven achter mij te staan, zelfs terwijl mijn "*schrijfweize veel geduldt en begrip heefdt*" gevraagd.
Voor mij bent u een professor in de volledige betekenis van het woord. Ik heb respect voor uw adequate kennis van de wetenschap, uw betrokkenheid bij de kliniek en uw verworven waardering bij collega afdelingshoofden zowel binnen als buiten het Erasmus MC.
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Dr. R.W. Selles, beste Ruud

Jouw begeleiding is op alle vlakken goed! Werken met statistiek, gestructureerd schrijven, de helikopterview aannemen bij discussies, relativeren en pragmatisch problemen oplossen zijn een aantal lessen die je mij hebt geleerd. Je bent een erg prettige en toegankelijke copromotor waar ik met veel plezier mee heb gewerkt. Dank hiervoor.

Dr. E.T. Walbeehm, beste Erik

Jouw enthousiasme, voor zowel de wetenschap als de kliniek is in de afgelopen jaren aanstekelijk geweest. Met name jouw klinische passie zal ik gaan missen. Daarnaast heb ik ook de samenwerking gewaardeerd. Zowel de late avonden waarin wij onder het genot van een goed glas whisky over de wetenschap hebben gefilosofeerd horen daarbij, als ook de ochtenden waarin we de volledige anatomie van de onderarm doornamen op de OK. Dank voor deze lessen.

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Prof. Dr. Patka,
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Carin,
Het is fijn dat er altijd een deur openstaat voor vragen en hulp. Dank hiervoor.

Sjoerd Niehof, Emmy van Bodegraven, beste Sjoerd en Emmy
Sjoerd; ik heb enorm veel respect voor jouw praktisch inzicht. Jouw kwaliteit om doordachte oplossingen te verzinnen is bewonderenswaardig.
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Esther Fijneman, Ineke Hekking, beste Esther en Ineke
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Stafleden en assistenten van de afdeling plastische chirurgie in het Erasmus MC Iedereen op de 15de, veel dank voor jullie steun en interesse. Het was leuk om als collega's samen te werken op de verdieping, niet te vergeten de vele lunches en koppen koffie. Het was nog leuker om als vrienden een biertje te kunnen drinken bij een congres of op de piste.

Stafleden en assistenten van de afdeling chirurgie in Onze Lieve Vrouwe Gasthuis
Bedankt voor jullie steun en begrip tijdens de afgelopen maanden. Deze nieuwe werkomgeving is voor mij onlosmakelijk verbonden met een nieuwe stad, waardevolle nieuwe collega's en biertjes in de Ysbreeker.

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Stijn van der Ploeg, beste Stijn

Stijn, het is fantastisch dat het is gelukt om op dezelfde dag te promoveren en samen een feest te organiseren. Dat hebben we maar mooi voor elkaar gekregen!

Liron Duraku, beste Liron

Collega, of was het nou vriend? We hebben een mooie tijd gehad, zowel op de universiteit als daarbuiten. Het was leuk om onderzoek met je te doen, maar nog leuker om je unieke verhalen aan te horen. We gaan nog vele mooie avonturen beleven.

Tim Nijhuis, beste Tim

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Marlies

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Papa en Mama

Het is bijzonder als ouders mogelijkheden creëren waar kinderen de vruchten van kunnen plukken. In het geval van mij en Marlies is dat gebeurd. Ik weet zeker dat dit boekje niet tot stand was gekomen zonder jullie steun en de kansen die jullie mij hebben gegeven. Ik denk dat wij, aan het eind van de basisschool, geen van allen hadden gedacht dat ik ooit zou promoveren. Veel dank voor de onvoorwaardelijke steun.

Curriculum vitae

Ernst Steven Smits was born on January 23th 1985 in Rotterdam, the Netherlands. Ernst grew up in Denmark and the United Kingdom. He was nine when he and his family moved back to the Netherlands permanently.

After graduating athenaeum in five years, he enrolled in medical school at the Erasmus University in Rotterdam in 2003. During his studies he worked as a "Forgeron" at the Emergency Room in the Ikazia hospital in Rotterdam under supervision of Dr. Veen. He also initiated research projects at the department of Plastic and Reconstructive and Hand Surgery of Professor Hovius, which led to the start of his PhD. In 2008, he stayed in Cape Town to be a medical student observer at the department of Trauma Surgery and the department of Orthopedic Hand Surgery in the The Grootte Schuur Hospital.

He finished the theoretical part of his studies in 2009. Consequently he started doing full-time research to work on his PhD for 1.5 years. In 2011, the TedX leadership transformation program 'CEO for a day' allowed Ernst to substitute professor Büller (chairman of the executive board) for a day. In this program, Ernst experienced the managerial side of a large University Medical Center. He completed his Medical Degree in December 2012.

In January 2013, Ernst started as a resident (AGNIO) at the department of Plastic and Reconstructive and Hand Surgery at the Erasmus MC. In the same year, he organized 'Masterclass Neuropathic Pain', a congress in which several medical specialists centralized around neuropathic pain.

In July 2013, Ernst was accepted as a resident in training (AIOS) at the department of Plastic and Reconstructive and Hand Surgery in Rotterdam. He started his preliminary part at the department of General Surgery at the 'Onze Lieve Vrouwe Gasthuis' in Amsterdam, under the supervision of Dr. Gerhards. He currently works there.