Quantification of energy extraction during continuous cold therapy

A new method to evaluate bio-heat build-up in tissue?

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Content

Preface

Dit voorwoord wil ik graag in het Nederlands schrijven zodat iedereen die ik wil bedanken het zeker begrijpt. Allereerst wil in mijn proefpersonen bedanken, zonder hun had ik deze thesis nooit kunnen schrijven, want geen vrijwilligers betekende geen resultaat. Natuurlijk zou het ook moeilijk geweest zijn zonder stageplaats, daarom wil ik de mensen van EMC medical instruments bedanken voor de kans die zij aan mij gegeven hebben, om als allereerste dit soort onderzoek uit te voeren. Raf, Koenraad en Els een dikke merci! Vervolgens zijn er enkele mensen die een cruciale rol hebben gespeeld in het vervolmaken van deze thesis bedanken; Nic, voor het maken van het sensorenkastje en de bijbehorende software; papa en Miel voor het bouwen van het experimentele koeltoestel en Lut Daniels die mij de huidplooimeter leende. Zonder hun bijdrage zou het allemaal veel moeilijker geweest zijn om deze stage en bijhorende thesis te maken.

Abbreviations & Symbols

c:	Specific heat capacity
E:	Total energy [J]
ECM:	Extracellular matrix
E_x:	Effect
h:	Convective heat transfer coefficient [J.°C/s.m^2]
IL-1:	Interleukine-1
l:	Thickness of the material [m]
m:	Mass [kg]
MMP:	Matrix metalloproteinases
NMR:	Nuclear magnetic resonance
NO:	Nitric oxide
NOS:	Nitric oxide synthesis enzyme
NTC:	Negative temperature coefficient
PDGF:	Platelet derived growth factor
q:	Flow rate [m^3/s]
Q:	Amount of thermal energy [J]
$\dot{Q}$:	Rate of heat transfer [J/s]
Q":	Rate of heat flux [J/s. m^2]
R:	Resistance [Ω]
ROS:	Reactive oxygen species
T:	Temperature [°C]
TGF-β:	Transforming growth factor beta
TNF-α:	Tumor necrosis factor alpha
VC:	Vasoconstriction
VD:	Vasodilatation
VEGF:	Vascular endothelial growth factor
ΔT:	Temperature difference
λ:	Heat conductivity [J/s.°C.m]
ρ:	Density[g/m^3]

Abstract

Introduction: Cryotherapy is used in medicine to acutely treat closed soft tissue injuries, post-operative pain and oedema formation. Several studies indicate that the extraction of thermal energy can reduce the inflammatory response in the damaged tissue. Secondary damage due to the inflammatory reaction is minimized by cooling and the healing process knows a quicker start. There are however no clinical guidelines physicians can use to assure beneficial clinical effects and the application relies on the experience of the treating physician. Knowledge about individual characteristics that can influence the effectiveness of the cryotherapy could lead to better, more personalized treatment protocols that increase clinical benefit. The goal of this master thesis was to investigate the amount of thermal energy that could be extracted from healthy tissue and to relate this to characteristics, such as age, weight, BMI, skinfold thickness on the thigh and, skin temperature change of the individual under test.

Materials and methods: First, an experimental set-up of a continuous cold cryotherapeutic agent was assembled. Temperature sensors were calibrated and build into the agent to allow measurement of thermal energy transferred from a heat reservoir to the cooling agent. Second, normal knee tissue of 65 healthy volunteers, men and women, was used as a model for heat extraction. During a 10 minute energy extraction procedure (cooling), the heat transfer from the knee tissue to a therapy pad was measured. Subjects were asked to fill in a questionnaire and their skinfold thickness was measured. Using multiple regression, individual characteristics were correlated to the total amount of energy that could be extracted in 10 minutes.

Results and discussion: Validation experiments with the experimental cooling agent showed that it is possible to measure the energy transfer to the cooling agent. There is however still room for improvement of accuracy of the measurement. . The mean amount of energy extracted from human knee tissue in 10 minutes was 29,839 ± 5298 J. Mean starting skin temperature was 29 ± 1°C and reduced to 20 ± 4°C after 10 minutes of cooling. Sex, weight, BMI, and the interaction between pre- and post-cooling skin temperature and these variables were comprised in a prediction model for energy extraction.

Conclusion: The proof of concept, energy transfer from the human body to a cooling agent can be measured, has been delivered. Also an energy extraction prediction model was generated. This model correlates physical characteristics of an individual to the amount of energy extracted in 10 minutes time. Data generated in this master-thesis provides the basis for an extended clinical trial in which energy extraction from pathologic tissue is also included.

Abstract (NL)

Inleiding: Cryotherapy wordt in de medische wereld vooral gebruikt als acute behandeling bij verstuikingen en om pijn en oedeemvorming na een operatie te verminderen. Er is bewijs in de literatuur dat warmteonttrekking de ontstekingsreactie in het beschadigde weefsel kan beperken. Hiermee wordt secundaire weefselschade beperkt en kent het eigenlijke genezingsproces en snellere start. De manier waarop de koude wordt aangebracht en de duur van de behandeling is echter niet vastgelegd in strikte protocols maar berust vooral op de ervaring van de behandelende kinesist of arts. Een standaardprotocol en meer informatie omtrent persoonsgebonden eigenschappen die een invloed hebben op de behandeling zouden kunnen leiden tot geoptimaliseerde klinisch diagnostische resultaten. Daarom werd in deze masterthesis onderzocht of de hoeveelheid energie die ontrokken kan worden aan gezond weefsel gerelateerd is met bepaalde gemakkelijk meetbare karakteristieken van het individu

Materiaal en Methoden: Eerst werd een *continuous cold* cryotherpeutische toestel gebouwd in een experimentele setting. Temperatuur sensoren werden gekalibreerd en ingebouwd om de thermische energie overgedragen van een warmtereservoir aan het koelsysteem te meten. In een tweede fase werd het knieweefsel van 65 gezonde vrijwilligers, mannen en vrouwen, gemeten. Tijdens een 10 minuten durende energie extractie met het experimentele cryotherapeutische toestel, werd de energie transfer van het weefsel naar de therapie pad gemeten. Er werd aan de testpersonen gevraagd om een vragenlijst in te vullen en hun dij huidplooidikte werd bepaald. Met multiple regressie werden persoonsgebonden fysieke karakteristieken gecorreleerd met de totale energie ontrokken tijdens de 10 minuten koeling.

Resultaten en Discussie: Validatie experimenten toonden aan dat het mogelijk is om de energie transfer naar een therapeutisch koelmiddel te meten is. Er is echter wel nog ruimte om te verbeteren. De hoeveel energie onttrokken van humaan knieweefsel over 10 minuten koeling was 29,839 ± 5298 J. De gemiddelde huidtemperatuur voor de meting was 29 ± 1°C. Dit zakte naar 20 ± 4°C na de 10 minuten koeling. Geslacht, gewicht, BMI en de interactie tussen de huidtemperatuur pre- en post-koeling en de voorgaande variabelen werden vervat in een predictie model voor de totale energie ontrokken.

Conclusie: Het bewijs dat energie onttrekking van warmte aan het lichaam door een cryotherapeutisch middel kan worden gemeten is geleverd. Bovendien kon de hoeveelheid energie die werd ontrokken tijdens een 10 minuten durend koeling, worden gecorreleerd aan persoonsgebonden fysieke karakteristieken De data die in deze masterthesis werden bekomen kunnen gebruikt worden als basis voor een grotere klinische studie waarin ook energie transfer van pathologisch weefels wordt gemeten.

1. Introduction

1.1 Bio-heat production and distribution

The first law of thermodynamics states that energy can neither be created nor destroyed. In a closed system, total energy is constant. A human body acquires almost all energy from ingested food, and uses this energy to operate cells and to keep body temperature constant. The gastrointestinal tract breaks it down in different kind of 'fuels' that can be used by our cells. The cells can store these fuels or oxidize them to release energy. The second law of thermodynamics states that chemical transformations always result in a loss of free energy. Some of this free energy is conserved in high-energy bonds in adenosine-tri-phosphate and can be released in a controlled manner to provide the energy for cellular functions. But there is also energy 'wasted' as heat because of inefficiencies of the biochemical reactions (1).

The thermoregulatory mechanism of the body creates an internal environment in which reaction rates are relatively high and optimal. Normal body core temperature ranges from 36°C to 38°C and depends on time of the day, physical activity, hormonal status in the menstrual cycle and age. The body's rate of heat production is closely linked to the rate of metabolism. If this heat could not be lost, physical exercise for example would be limited because excessive hyperthermia would begin to impair body function. This impairment does not occur, primarily because of the efficiency of the thermoregulatory system (2).

Heat is not uniformly created in the human body and the rate of convective heat transfer from any tissue to the blood thus depends on the rate energy production in the tissue, temperature of the tissue, temperature of the incoming blood, and blood flow through the tissue. When excessive heat is present in a tissue, blood will carry this energy to the body core. Transport of heat energy throughout the body occurs mainly by convection; only a minor amount of the body's generated heat is transferred directly from the body core to the skin by conduction (2).

Which mechanisms prevent the core from overheating? Free nerve endings with thermo-active ion-channels in the skin provide information about the temperature of the enviroment to the hypothalamic control center. The thermoregulatory system uses peripheral temperatures as an early-warning system to enable effective responses even before any deviation in core temperatures occurs (anticipatory). In contrast, thermoregulatory responses to changes in core temperature are regulated by negative feedback.

One of the efferent responses is adjusting the smooth-muscle tone of the cutaneous arterioles by the autonomic nervous system (2,3). Blood flow to the skin can change markedly (Figure 1). Upon heat challenge blood flow towards the skin can increase up to 60% of the cardiac output, by maximal vasodilatation. The vasoconstrictor response to local cooling can reduce skin blood flow to essentially zero (4). If a cold stress is sufficiently large, the body will try to increase heat production by shivering (2).

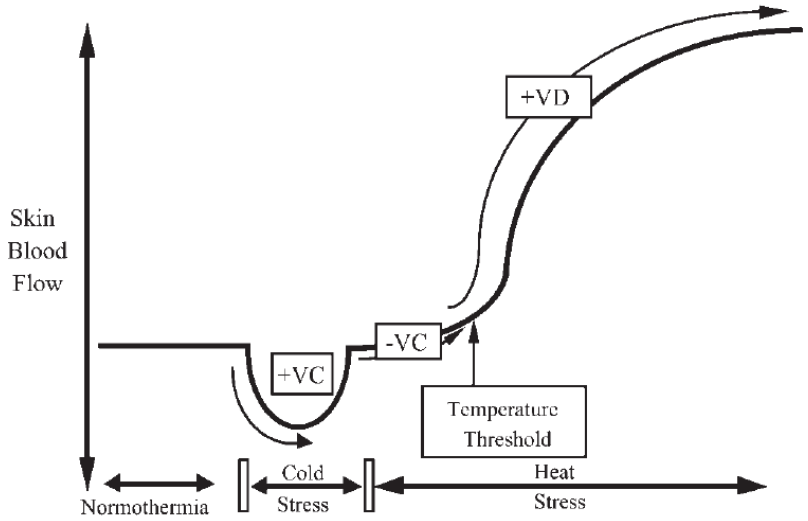

Figure 1: Skin blood flow responses to cold and heat challenge. During periods of normothermia, cardiac output towards the skin is approximately 5%. This can reduce to essentially zero during periods of hypothermia and up to 60% of cardiac output during periods of hyperthermia (DL). +VC: increased vasoconstriction, -VC: decreased vasoconstriction, +VD: increased vasodilatation, temperature threshold: start of active vasodilatation and sweating **(4)**

Heat energy at the skin is lost to the environment by radiation, conduction, convection and evaporation of water. Radiation of heat from the skin accounts for approximately 60% of heat lost when the body is at rest in a neutral thermal indoor environment. If heat stress is sufficiently great, the autonomic nervous system will activate eccrine sweat glands. Evaporation of the sweat will increase skin cooling. Water evaporation by the respiratory system also cause cooling of the body core. Convective heat loss from the skin occurs when air or a fluid touches the skin, e.g walking in the wind or swimming. Heat transfer from the skin by conduction occurs when the body touches a solid material of a different temperature. Placing a cold object on the skin, e.g. in cryotherapy as discussed below, will result in heat loss by conduction (2,3).

Heat distributions over joints have a stable pattern, while the temperature rage may vary depending on the individual's physiological or environmental changes. These patterns can be visualised using infrared thermography. Deviations from this normal pattern, such as hot spots occurring at a place that is expected to be colder, are indications of inflammation in the deeper tissue (Figure 2).

The abnormalities can be localized or distributed over the joint. Evaluation of patterns is more reliable than measurements of isolated spots, since skin temperature is affected by many internal and external factors such as circadian rhythm, activity, metabolic rate, atmospheric temperature and humidity (5).

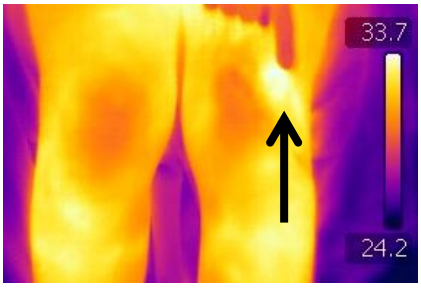

Figure 2: Infrared picture of knees at rest, the patella acts as a heat shield (5). The left knee has a normal heat distribution pattern. The right knee has a hotspot suprolateral to the patella (K. Kunnen).

1.2 Cryotherapy

Lowering of tissue temperature by withdrawal of heat energy from the body to achieve a therapeutic effect is called cryotherapy. Heat is lost through conduction of heat from the skin to the cryotherapeutic agent. Deeper tissues conduct their heat to the cooler overlying tissues and skin. Heat conduction involves the diffusion of molecular energy through matter without bulk molecular motion (6). In static conditions, thermal conduction $Q_{conduction}$ is the heat transfer per unit area [J/s.m^2]. Fourier's law governs that per unit area the transfer in a given direction is proportional to the temperature gradient:

Equation 1 (6)

$$Q''_{conduction} = \lambda . \frac{T_1 - T_2}{l}$$

with $Q''_{Conduction}$, convective heat flux per unit area [J/s.m^2]; λ, heat conductivity of the material [J/s.°C.m]; $T_1 - T_2$ is the temperature difference from one point to another on or in the material of interest[°C]; and l, the thickness of the material [m]. Different types of tissues have varying heat conductivity λ values. This value represents to which extent the tissue (or any other material) can conduct heat. Copper for instance has a heat conductivity value of 380 J/s.°C.m (7). Skin and muscle tissue approximate water (0.6 J/s.°C.m) with 0.45 and 0.50 J/s.°C.m. Adipose tissue on the other hand is, in comparison to water, a much worse heat conductor with a value of 0.27 J/s.°C.m (8). This means that subcutaneous adipose tissue acts as an isolator for cold and heat for underlying tissues. To effectively cool the deeper tissues (muscle and joints), it is important to cool the skin for extended periods of time.

Whereas conduction thus not addresses heat transfer with bulk motion of matter, convection does. Cold fluid or air moves in bulk along the body and carries the heat between the body and the environment. Continuous cold cryotherapeutic agents (water or air is allowed to flow alongside the body), such as the device developed by EMC medical instruments, uses the principle of forced convection. Convective heat transfer consists of two parts, conductive heat transfer with no bulk movement near the body surface and advection of fluid particles to regions of dissimilar regions (6).

Equation 2 (6)

$$Q''_{covection} = h \,.\, (T_s - T_\infty)$$

with $Q''_{Convection}$, convective heat flux per unit area [W/m^2]; h, convective heat transfer coefficient [$W.°C/m^2$]; T_s, surface temperature; and T_∞, bulk fluid temperature.

1.2.1 Cryotherapy in practice today

Several cooling devices are used in medicine to treat closed soft tissue injuries or to reduce post-surgery oedema formation and pain. There is also evidence that cryotherapy may reduce inflammation, and resulting secondary tissue damage (9,10,11). This would lead to a more rapid healing of the surgical wound and return to participation (12). However the results of different studies that assessed the speed of return to participation of patient who's tissue is cooled compared to patient that do not receive cooling, are not conclusive (13,14).

Most commonly used cryotherapeutic agents in clinical practice, are different forms of ice, gel packs and ice-water immersion (15,16,17). The ability of the cooling agent to undergo a phase change increases its capacity to absorb heat. Melting ice is therefore a more efficient external cooling method than gel packs because there is about 80 times more heat required to raise the temperature by 1°C than cold water that does not go through phase change (15,18). The contact temperature of a chosen agent with the skin, is hard to control and the risk of frost bites still exists (19,20). There are also reports that suggest that cooling in combination with compression is more effective than cooling as such in treating traumatic lesions (21). Unfortunately there are no standardized clinical guidelines relating to cryotherapy management (22). Method and modality of application, duration and frequency all diverge significantly between studies.

1.3 Medical relevance

Use of cooling in daily medical practice is not well supported by research, and medical scientific evidence only encompassed in case reports and rather small randomized clinical trials. Patient characteristics and knowledge about the cooling properties of cryotherapeutic agents could become determinants of in what manner and for how long the cryotherapy must be administered to become clinical beneficial.

Post-surgery cooling with the EMC medical instruments equipment, upon which this masterthesis is based, is electronically guided. Integration of an energy transfer measurement in the system could give information to the treating physician quantity of thermal energy withdrawn from the cooled tissue. This information could possibly be a assist in adjusting cryotherapy to the patients need or in diagnosing complications at an early stage, before serious damage is allowed to take place.

1.3.1 The inflammatory reaction and healing

Acute inflammation is the body's natural response against infection but also occurs as response to surgical induced trauma. The goal of this inflammatory reaction is to clear the damaged, necrotic tissue due to the trauma and to allow good healing. During the induction of trauma, damaged cells and blood vessels will start to produce pro-inflammatory signals. Due to these signals, changes in the vascular tonus and cellular events are observed (23,24). The most important events on the vasculature are summarized in Figure 3.

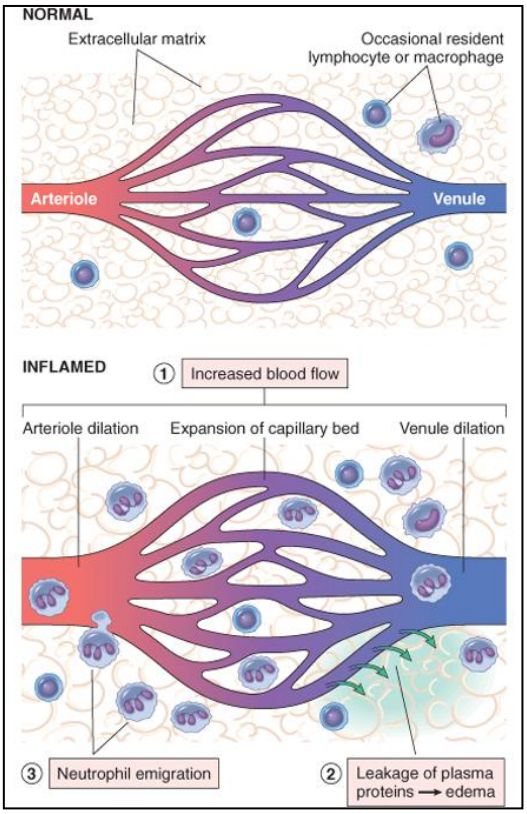

Figure 3: Important vascular and cellular events at the start of an inflammatory reaction. Inflammatory mediators cause local arteriolar and venular dilatation that result in an increased blood flow [1]. Endothelial cell contraction allows leakage of plasma and proteins into the extracellular space and causes oedema [2]. Stasis due to increasing blood viscosity enables neutrophils to margin and transmigrate across the vessel walls [3] (23).

Mast cells respond to the trauma by releasing histamine that gives an immediate local arteriolar vasodilatation and induces increased vascular permeability. Histamine together with serotonin, a vasodilatator, is also released by platelets during aggregation (23,25). This results in a increased local blood flow that causes a calor, and redness of the skin. Endothelial cell contraction and activation, elicited by pro-inflammatory agents such as tumor necrosis factor α (TNF-α), interleukine-1 (IL-1), leukotrienes and neutrophils, leads to the leakage of protein-rich fluid into the interstitium. The net result is the accumulation of fluid in the extravascular space, called oedema (23,26).

Furthermore, stasis in the small vessels due to increasing blood viscosity, allows blood monocytes and neutrophils to margin to the vessel wall. These cells will transmigrate through the vessel wall towards the inflammation. During this process the cells are activated by interactions with cytokines and by cytosol and membrane debris of necrotic or apoptotic cells. Chemokines, secreted by activated tissue macrophages and leukocytes that already reached the inflammation site, will guide the cells further into the tissue (27). Monocytes will differentiate into macrophages, and clear cell debris and bacteria present in the wound together with the monocytes (28). Importantly, and in the scope of the present work, these cellular events increase cellular metabolism leading to increased heat production inside the inflamed tissue.

Hypoxia inside the damaged tissue induces vascular endothelial growth factor (VEGF) secretion that stimulates angiogenesis. A soft pink tissue that contains macrophages and leukocytes, new blood vessels, proliferating fibroblasts, loose extracellular matrix (ECM) is formed. It is called granulation tissue. Scar formation is the inevitable end point of wound repair. Termination of the acute inflammatory response involves degradation of the various chemical mediators, normalisation of the vasculature permeability and cessation of the leukocyte emigration. These inflammatory cells will also start to produce anti-inflammatory mediators which will limit the reaction. Eventually when the inflammation is ceased, the vascularisation in the granulation tissue will regress and the scar matures (Figure 4). The tissue's exaggerated heat production will also attenuate (23,24).

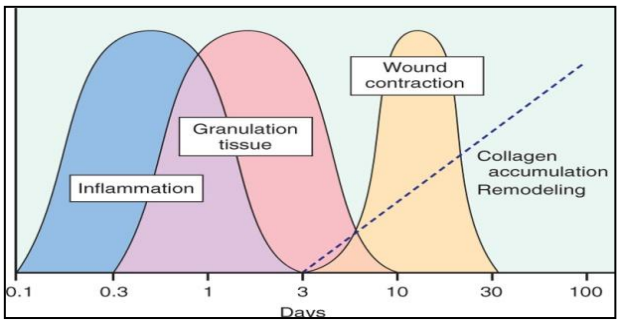

Figure 4: Succession of reactions induced by tissue trauma resulting in the healing of the damaged tissue. At time zero an injury is induced. Shortly thereafter an inflammatory reaction will occur as a response to the damage inflicted. When cell debris and present bacteria are cleared the inflammatory reaction will cease and granulation tissue is formed. When the wound is healed the scar matures and the wound will contract (23).

1.3.2 Physiological and molecular changes with cryotherapy

Although some inflammatory factors stimulate tissue repair, excessive inflammation is associated with impaired wound healing. When the balance between pro- and anti-inflammatory substances is disturbed, infiltrated leukocytes can cause secondary tissue damage by releasing reactive oxygen species (ROS), proteases and the pro-inflammatory cytokines TNF-α and IL-1 (23,27).

When secreted at low levels, ROS can increase the cascade of inflammatory mediators. At higher levels, it is responsible for tissue injury by several mechanisms, including (1) endothelial damage; (2) protease activation and antiprotease inactivation, with a net increase in breakdown of the ECM; and (3) direct injury to other cell types. The lysosomal granules of neutrophils and monocytes contain many molecules that can mediate acute inflammation. These may be released after cell death, by leakage during the formation of the phagocytic vacuole. The most important of these lysosomal molecules are enzymes. The neutral proteases, including elastase, collagenase, and cathepsin. These enzymes induce tissue injury by degrading elastin, collagen, basement membrane, and other matrix proteins (23,24). Pro-inflammatory cytokines on the other hand, are potent inducers of MMP expression (24).

Several studies have shown that local tissue cooling is able to attenuate the spread of secondary tissue damage. Cooling reduces the microcirculation dysfunction and the subsequent leukocyte adhesion to the vessel wall and tissue infiltration (10,11). Decrease in the amount of leukocytes at the inflammation site implicates a decrease in the amount of harmful mediators that are secreted. Decreased tissue temperature also causes a decrease in the metabolic rate of cells and therefore minimises damage due to hypoxia (29). This decrease in metabolism is also responsible for decreased production of ROS, proteases and their function, and cytokines.

1.3.3 Skin temperature after total knee replacement

It is been proven that after total knee replacement the overlying skin temperature stays significantly elevated for 6 months without known complications (30). The maximum temperature is most frequently measured two or three days after surgery when the patient is also pyretic. Age, sex and tourniquet time could not predict the extent of the postoperative knee temperature rise. Unfortunately complications cannot yet be predicted by measurement of the skin temperature (30,31). It can be speculated that this increased temperature represents the increased metabolism in the healing tissue.

1.4 Research question and aim

The literature indicates that absolute skin temperature is not useful in assessing the inflammatory status of the underlying tissues (30,31). And that absolute skin temperature is also a bad predictor of cryotherapy effectiveness (32). Salisburry et. al. on the other hand, showed that all joints have specific heat distribution patterns. Disturbances of these patterns are more reliable in assessing the inflammatory status in the joints (5).

Increased cellular metabolism due to an inflammatory reaction and healing of tissue generates more thermal energy than normal tissue. The blood flow towards inflamed and healing tissue is also increased due to mediators present at these sites and angiogenesis (23). Possibly, measurement of the thermal energy transfer from a tissue to a continuous cold cryotherapeutic agent, could give more information about its inflammatory status compared to ordinary infrared photography. It is expected that cessation of the inflammatory response and healing will allow the thermal energy in the tissue to gradually fall back to normal values.

The first goal of this master-thesis was to establish a validated system to measure heat transfer from the knee tissue to a continuous cold cryotherapeutic agent. This was achieved by building an experimental set-up, based upon the EMC medical instruments continuous cold device. Temperature sensors were calibrated to assure correct measurement. Influence of different settings of the experimental set-up, on the energy transfer was assed. The system was validated by comparing known heat loss from a warm water reservoir with the energy transferred to the experimental continuous cold cryotherapeutic agent.

The second goal was to assess the thermal energy that could be extracted from normal knee tissues in healthy volunteers and relate this to individual characteristics. The continuous cold cryotherapeutic agent experimental set-up, used in the validation experiments, was rebuilt to a more practical version. With this device thermal energy extractions of 10 minutes were performed on the knee tissue of the volunteers. Change in skin temperature over these 10 minutes was also monitored. The information generated by this proof of concept evaluation could be the basis for an extended clinical study in which pathologic conditions are compared to normal.

2. Materials and Methods

2.1 Calibration of the temperature sensors

Temperature registration was performed using Negative Temperature Coefficient (NTC) thermistors (Aqua Computer; Gleichen OT Benniehausen, Germany). These thermistors are ceramic semi-conductor elements made from metal oxides. Different types were used as shown in Figure 5.

Figure 5 : NTC thermistor types used in experimental heat extraction set-up. Far right; NTC thermistor build in metal shielding. Right; 'Naked' NTC thermistor. Far Left; NTC thermistor build in piece of metal tubing, screw thread on the outside. Left; same type as upper left but with screw thread on the inside. All sensor measure between 1 and 2 cm.

The electric resistance (R) of the elements changes in function to the internal temperature (T) in an inverse proportional way. If the temperature rises, the resistance in the NTC thermistor will decline. The relationship between these parameters can be visualised in an resistance versus temperature curve. The slope of the tangent to this curve is an indication about the measure of the β-value from the NTC (Eq 3) . The β-value is not a rigorous constant but is temperature dependent within a small range of operating temperatures (33). According to the manufacturer, the NTC thermistors that were used, have a standard reference resistance of 10 kOhm ± 5% at 25°C and a β-value of 3435K ± 3% between 25° and 85° Celsius.

Equation 3 (33)

$$\beta = ((T1 * T2) / (T2-T1)) * \ln (R_{T1} / R_{T2})$$

To determine the exact β-value of every NTC thermistor, the electrical resistance existing within the semi-conductor element of the NTC thermistor was measured at different temperatures. In an deionised ice bath (0°C) and deionised water baths at temperatures between of 5, 15, 25, 35 and 45°C, the resistance existing inside the NTC sensor was measured by use of a multi-meter (KLaasing Electronics; Oosterhout, The Netherlands). To assure steady measurement, the wires of the multimeter were clamped to the NTC thermistor plug (Figure 6, D'). Temperature of the ice and water bath was monitored by a two sensor thermometer (DigitMex; Hong Kong, China) which was calibrated within 1°C (Biomed, Hasselt University). The water bath was insulated with tinfoil to prevent large temperature shifts during the measurement (Figure 6, A).

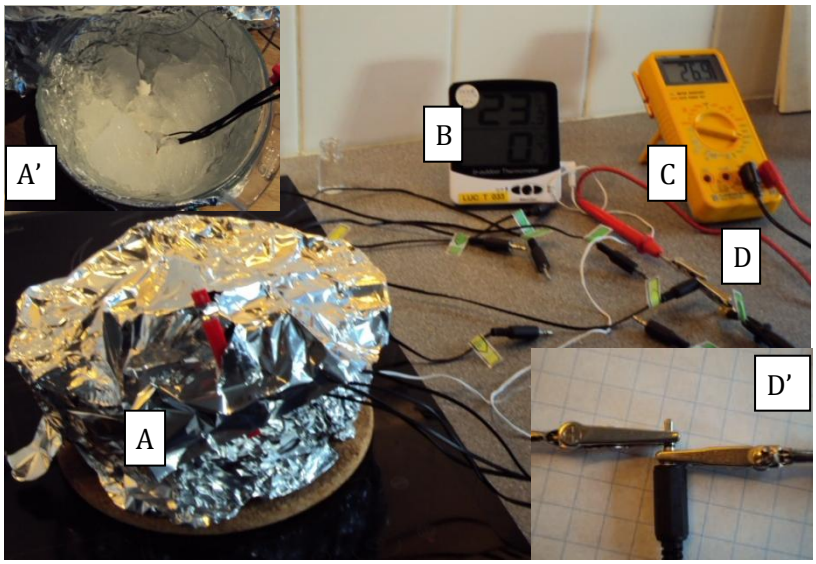

Figure 6: Experimental set-up for measurement of the electrical resistance existing within the semi-conductor element of the NTC thermistors. A basin (A and A') filled with water or ice contains the NTC thermistors. The temperature is monitored by a calibrated thermometer (B). Resistance readout is done by a multi-meter (C). Plugs of the NTC thermistors were clamped to assure steady measurement (D and D').

In following experiments the read-out of the NTC thermistors and flow meter was done by the EMCview prototype (Figure 7, A), developed especially for this study by EMC medical instruments. Eight NTC thermistors and two flow meters could be connected to the device and monitored simultaneously. The analogue electric signals were converted to a digital signal which was send to the computer every second. A flow meter, also ordered from Aqua Computer, was used to measure the flow rate through the system. A 169 rotations of the internal rotor represented 1 litre of fluid. Measurements of the flow rate were linear starting from 1.3L/min. Temperatures and flow, and also the total amount of fluid that ran through the device could be visually followed in the EMCview software during measurement. The NTC thermistor variables (reference resistance and β-value) determined for each NTC thermistor was inserted and an extra correction could be made (Figure 7, B). The EMCview software also stored the data in Windows Office Excel compatible files, which were used for further analysis.

Inter- and intra-day variability of each NTC thermistor was determined by measuring each NTC thermistor 3 times for 3 consecutive days. A 25°C deionised water bath was prepared and insulated with tinfoil. The temperature of the water was controlled by the DigitMex thermometer. The temperature, calculated by the EMCview program, could be followed easily. After 1 minute stabilisation period, the temperature given by the NTC thermistor and the control thermometer were noted and compared.

A

B

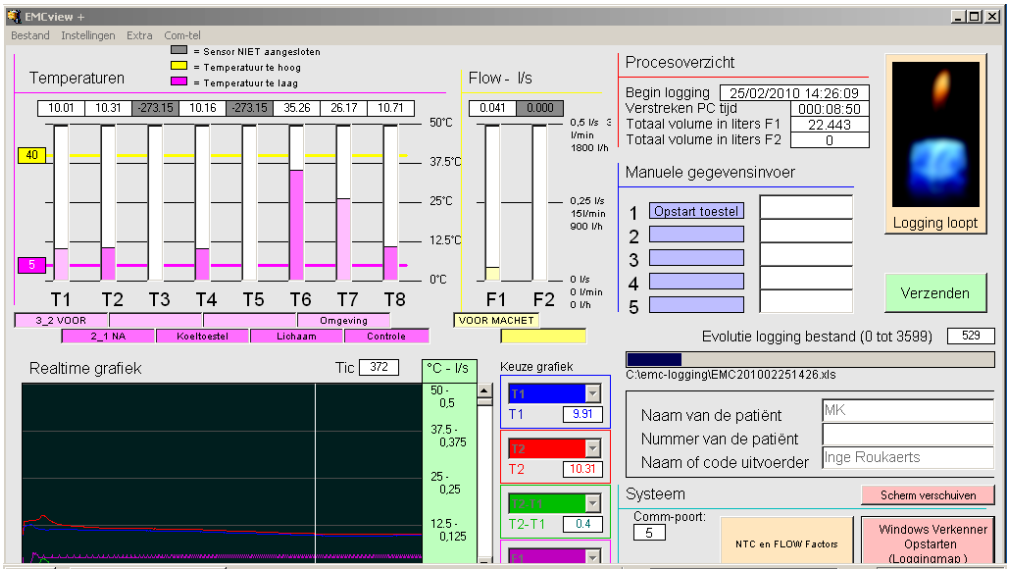

Figure 7: A. EMCview prototype, measures 15 cm wide and 10 cm deep. On the front two flow meters can be connected, on the backside eight NTC thermistors of different types can be connected. Data converted to digital signals are send to the computer and processed by the EMCview software. B. Screenshot of the EMCview software. On the upper left the temperatures of the eight inputs could be monitored, corrected and named. On the lower left temperatures and flow rates could be followed in a real-time graph. On the right side, additional comments could be made and subject information dictated. In the lower right corner in "NTC en FLOW Factors", β-values and reference resistance of every NTC thermistor could be inserted.

2.2 Optimization and validation of the energy transfer measurement

After the NTC thermistor variables were acquired and inter and intra-day variance was assumed to be acceptable low, an experimental set-up for energy extraction from human knee tissue was build. This system (Figure 8) was used to optimize the setting of different influencing variables and to validate the energy transfer.

Cold water in reservoir A (Fig. 8,A) was pumped through a therapy-pad. Flow rate of the pump (model MCP655™, Swiftech; Long Beach, USA)could be changed. The therapy-pad was hung in a second reservoir B (Fig. 8, B) containing 25L of water at a well defined temperature. The water was ultimately collected in reservoir C (Fig. 8 ,C). Water temperature was monitored in all three reservoirs, and at the entrance and exit of the therapy-pad.

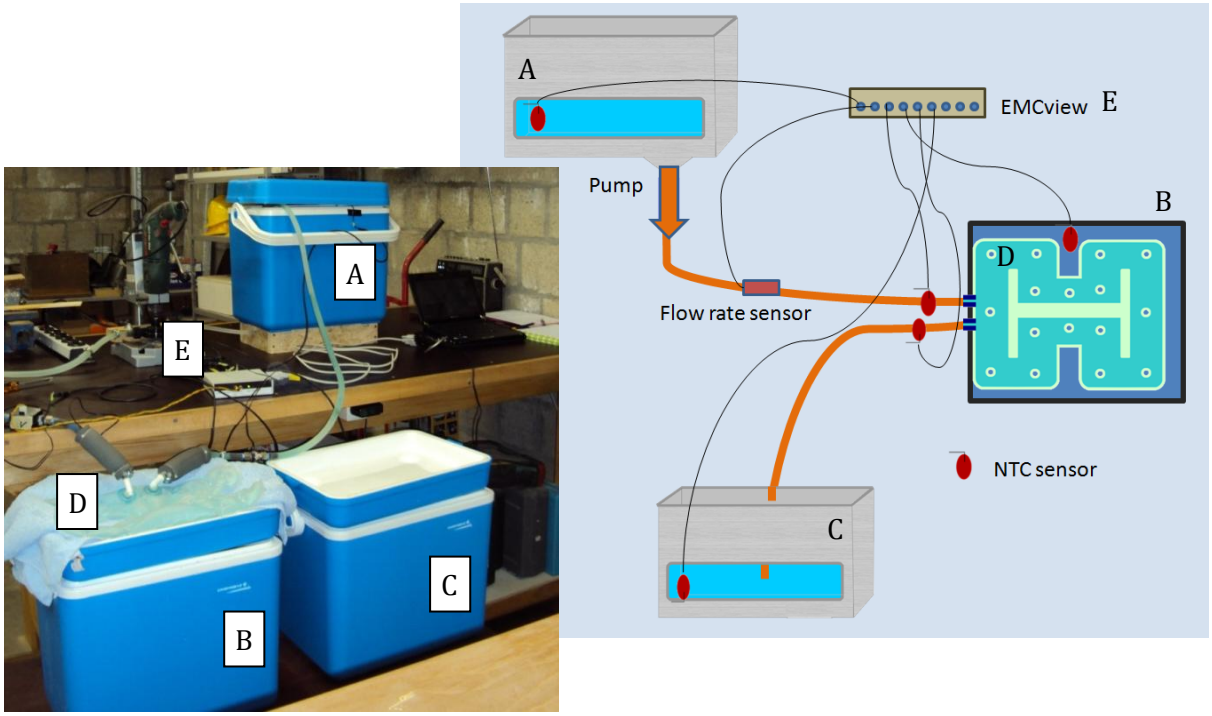

Figure 8: Set-up for implementation of the Plackett-Burman design and energy transfer validation experiments. Cold water stores in reservoir A was pumped through the therapy pad (D) that hung in a warm water reservoir B. The water was ultimately collected in reservoir C. Input of the temperature sensors was collected by the EMCview (E) and send to the computer.

2.2.1 Placket-Burman screening design

In a 12 experiment Plackett-Burman screening design the influence of different variables on the selected responses was assessed. These variables, also called factors, were: temperature of water that ran through the therapy pad, temperature of the water in reservoir B, flow rate, therapy pad condition (full or empty) upon appliance, isolation of the tubing, and duration of the pre-measurement stabilisation period (cooling of the set-up). The selected responses were: energy loss from reservoir B (Fig 8,B), energy extraction by the therapy pad (Fig 8,D) and the difference between these two responses. Calculation equations can be found in 2.2.2 Validation experiments.

The different factors were tested on two levels; +1 and -1. This meant for the different factors following value upon execution of the experiment: therapy pad temperature, 10°C, and 15°C; flow, 1.3 L/min, and 2.3 L/min; temperature of the warm water reservoir, 33°C, and 35°C; mingling of the warm reservoir, no, and yes; condition of the pad, empty, and full; isolation of the tubing, no, and yes ;stabilisation period, 3 min, and 6 min. The influence of these factors on a 10 minute energy extraction could then be assessed. For further statistical analysis 4 dummy factors were included in the design. The level of a factor for each of the 12 experiments can be found in Appendix 1.

The effect E_x of a factor on a selected response is calculated with Equation 4; with ∑Y(+) and ∑Y(-) the sums of the responses of the experiments where factor x was at levels (+) and (-) respectively.

Equation 4

$$E_x = \frac{\sum Y(+) - \sum Y(-)}{n/2}$$

The effect of a dummy factor is considered to be due to experimental error and thus *a priori* declared negligible. The mean effect of these dummies is expected to be zero. Variance of the experiments $(SE)_e$ can be calculated with the sum of the effects calculated for the dummy factors (Eq 5).

Equation 5

$$(SE)_e = \sqrt{\sum (E_{dummyi}{}^2)/n_{dummy}}$$

Significant effects ($p < 0.05$) are significantly larger than zero. This means that changing the level of the factor significantly changes the outcome of the selected response. To test this significance a student-*t*-test was used (Eq 6).

Equation 6

$$t = \frac{|E_x|}{(SE)_e}$$

2.2.2 Validation experiments

To validate the energy extraction, the dispersion between the thermal energy extracted by the therapy pad (Fig 8,D) and the thermal energy lost from the reservoir B (Fig 8,B) was calculated. This comparison was made over a time period of 10 minutes. The temperature of reservoir B was predetermined at 35°C, while the temperature of reservoir A (water that flows through the therapy pad) was varied to 10°C, 15°C, 20°C and 25°C.

It was assumed that all energy lost from reservoir B was removed as heat that was transferred to the therapy pad. The set-up could thus be described as an open thermodynamic system (Eq 7) in which loss to the environment was negligible:

Equation 7 (6)

$$E_{final} - E_{initial} = Q_{initial-final}$$

with $E_{initial}$, the amount of energy present at time zero; E_{final}, the amount of energy at the end of a defined time period; and $Q_{initial\text{-}final}$ the amount of thermal energy transferred.

The thermal energy [J] lost from the warm reservoir during the 10 minutes could be calculated (Eq 8):

Equation 8 (34)

$$\Delta Q = c \,.\, \Delta T \,.\, m$$

with the mass m [kg] water contained in reservoir B (25 k), the specific heat capacity c[J/kg .°C] (4186 J/kg .°C for water), and ΔT the temperature difference of the water in the reservoir before and after the experiment.

The thermal energy transfer rate $\dot{Q}$ to the therapy pad per second [J/s] could be calculated from: the difference in temperature ΔT of the water at the entrance and exit of the therapy pad, the flow rate q [m^3/s] of the water (0.000038 m^3/s), the specific heat capacity c and the density ρ [kg/m^3] of the water (1000 kg/m^3) (Eq 9).

Equation 9 (34)

$$\dot{Q} = q \,.\, c \,.\, \Delta T \,.\, \rho$$

The integration to time of Equation 9 gives the total energy extracted by the therapy pad over the 10 minute experiment.

2.2.3 Variability

Additionally the variability of the energy extraction set-up was determined. An experiment with the temperature of reservoir B fixed at 35°C and the temperature of reservoir A (water that runs through the therapy pad) fixed at 10°C was repeated 4 times. The flow rate was 0.000038 m^3/s. For all 4 experiments the entire protocol was executed from the start (Appendix 2).

2.3 Clinical Study

2.3.1 Subjects

The study population existed of 65 healthy volunteers, 25 men and 40 women. Ages ranged from 18 to 82, height 170±10 cm, mass 72±15 kg and anterior thigh skin fold 24±4 mm. Each subject completed a health status questionnaire and gave written informed consent before participating in the study. All subject received identical cryotherapeutic treatment.

2.3.2 Instrumentation

Cryotherapy was applied by an experimental setup (Figure 9) that consisted of an isolated fluid reservoir, a flow rate controllable pump, a therapy pad, NTC thermistors, a flow sensor, the EMCview and additional software. The insulated fluid reservoir was filled with water of 10°Celsius. The therapy pad was fixed to the subjects knee, using Surgifix tubular bandage (FRA Production SPA; Cisterna d'Asti, Italy).

2.3.3 Procedures

Subjects assumed a prone position on a normal chair. To reduce isolation of the body due to clothing, subjects were asked to wear shorts and loose upper clothing and remove shoes and socks. An anterior thigh skinfold was measured with the subject sitting at the tip of the chair, with the leg resting on the floor in a straight position. The skinfold was measured in triple with a Harpenden skinfold calliper (Baty International British indicators; West Sussex, United Kingdom), mean outcome was divided by two to become the subcutaneous adipose thickness.

The setup was prepared by filling the reservoir with water, previously cooled to 10° Celsius with ice. The tubing was cooled by running the fluid in a circular manner trough the circuit. The EMCview software was run on the notebook pc. The therapy pad was emptied before appliancation around the subject's knee.

The surface NTC thermistor was secured supromedial to the patella as described by Haidar et.al. 2006, using Hansaplast Sparadrap (Beiersdorf AG; Hamburg, Germany). The lead was taped to the tight, out of the cooling zone, to prevent the lead and sensor from being disturbed. The empty therapy pad was secured to the knee using Surgifix. The pad was insulated after filling to avoid extra pressure caused by strapping the isolation.

2.3.4 Treatment protocol

Subjects rested in a prone position for 15 minutes before the experimental treatment. Once the NTC thermistor was in place, instantaneous temperature measurements were taken every second for the duration of the experimental period (30 minutes). Baseline temperature was measured while the therapy pad was prepared and fixed to the knee. After the pump was started and the tab in the tubing opened, allowing the water to run through the therapy pad, the 10 minutes period of cooling started. During this period about 23 litre of fluid streamed through the therapy pad. Following these 10 minutes, the pad was removed but the skin surface NTC thermistor stayed in place. Rewarming was monitored for an additional 15 minutes.

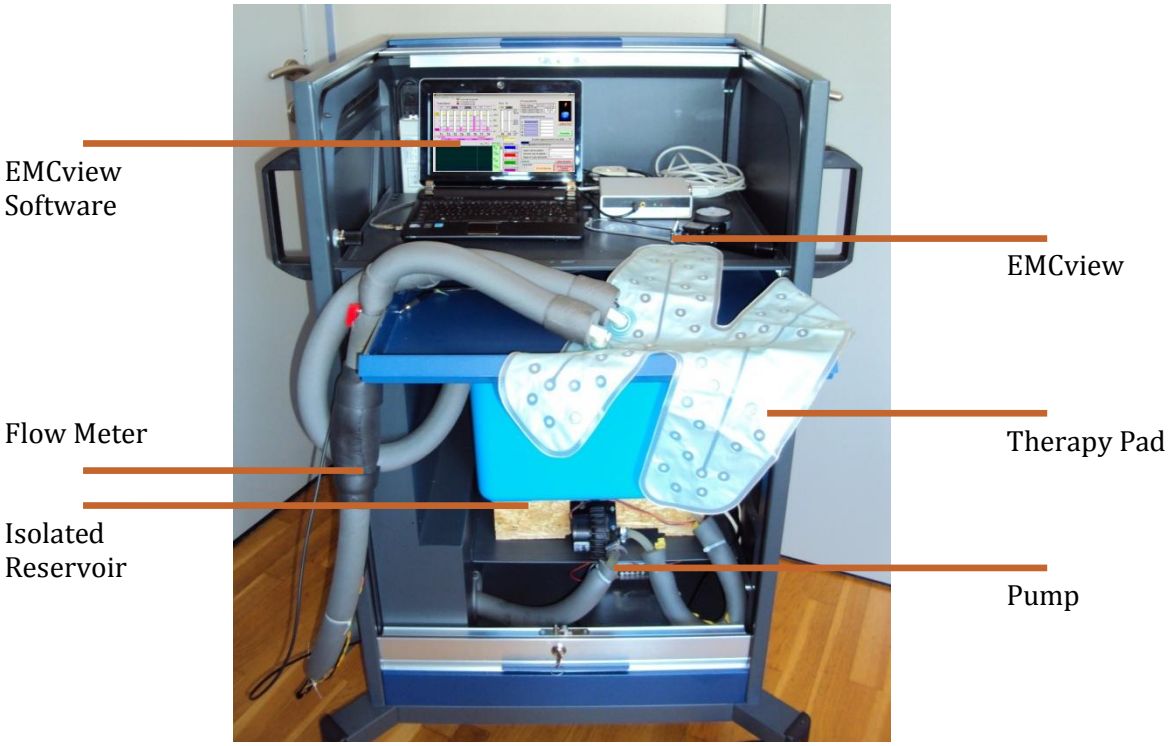

Figure 9: Experimental setup for appliance of cryotherapy based upon the original continuous cold device of EMC medical instruments. Cold water inside the insulated reservoir is pumped through the therapy pad which is applied on the subjects knee. Input from the flow meter and NTC thermistors in the reservoir, at the entrance and exit of the pad, and on the subjects knee are collected by the EMCview. This information is send to the EMCview software on the notebook and stored in excel compatible files.

2.3.5 Statistical analysis

The data were analysed in SAS® statistical analysis software (SAS Institute Inc. ; Cary, USA). A model was fit with multiple regression to identify person characteristics that were correlated to the total amount of energy transferred. The significance level was set at 95%.

3. Results & Discussion

3.1 Sensor calibration

The NTC thermistors were calibrated to get an as accurate temperature measurement as possible. The error on the β-value allowed by the manufacturer was ±3%, this would lead to an error of ±0.7°C. For this reason the exact beta-factor was determined for every NTC thermistor separately. Internal resistance of the NTC thermistor at 25°C (calibration temperature) has a product error of ±5%. This can cause a reflected temperature difference of ±1.3°C. Therefore the internal resistance at 25°C had to be measured carefully. The electrical circuit of the EMCview, the wires connecting NTC to the interface, can also cause an error in reflected temperature of 1%. By adjusting the final temperature measurement characteristics in the EMCview software most of these errors can be minimized or ruled out.

Additionally, when the signal of the interface to the EMC software is converted from analogue to digital an error can occur of plus or minus 1 bit. This is an unpredictable error that can be caused when the analogue signal value falls in between two bits. In the present electrical circuit of the EMCview, this gives an absolute temperature difference of ±0.07 at 0°C and ±0.2 at 50°C, which was neglected in the study.

3.1.1 Beta-factor and reference electrical resistance

In an ice bath (0°C) and water baths with a temperature between 5°C and 45°C with an interval of 10°C, the resistance existing inside the considered NTC thermistor was measured by use of a multi-meter (Fig 10). This was done in triple for every sensor and the mean was used in calculations.

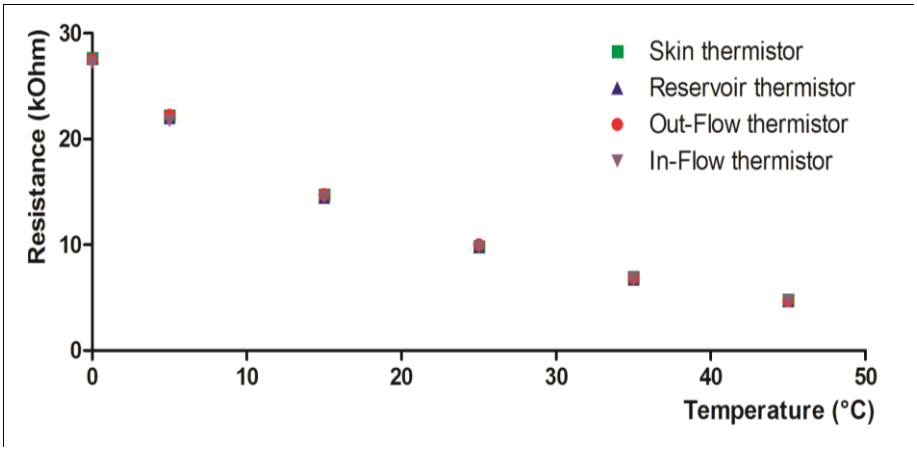

Figure 10: Temperature of the water bath in function of the electrical resistance that exists within the semi-conductor element of the NTC thermistor. The beta-factor gives an idea of the curvature.

The β-value of the used NTC thermistors was: 3,329 K; 3,367 K, 3,395 K; 3,396 K for the skin, in-flow of the therapy pad, out-flow of the therapy pad and the fluid reservoir respectively (Table 1). The mean individual electrical resistance at 25°C of each separate NTC thermistor obtained during this experiment was used as reference resistance for calculations in the EMCview software. Values were 9.80 kΩ; 9.80 kΩ; 10.02 kΩ; 9.86kΩ for the skin, in-flow of the therapy pad, out-flow of the therapy pad and the fluid reservoir respectively (Table 1).

3.1.2 Variability

The overall within- and between-day variability of the sensors in function of time (drifting) was tested at the manufactures' reference temperature of 25°C. Tests were performed three times a day for three consecutive days. There was no significant ($p>0.01$) contribution of the between-day variation to the within-day variability for the thermistors used. Considering these results, data of the three consecutive days were grouped for every thermistor to calculate variability and mean deviation from 25°C . The single or double Grubbs test was used to check for outliers. Overall variability was calculated for all sensors, being ±0.4% for the skin thermistor and ±0.3% for the reservoir thermistor. In- and out-flow thermistors both had a variability of ±0.7%, this means a maximum error of ±1.4% on the energy extraction calculations.

Setting to 25°C in the software was based upon the mean deviation from the calibrated thermometer and an empirical test. The skin thermistor had the smallest correction with +0.02°C, followed by the fluid reservoir with -0.24°C. The in- and out-flow thermistors needed the largest correction with -0.47°C and -0.55°C correspondingly. Correction values, beta-factor and reference resistance at 25°C for each thermistor can be found in table 1. All measurement data, ANOVA and Grubbs test outcomes can be found in Appendix 3.

Table 1: Settings used in the EMCview software for the NTC thermistor used in the validation experiments and clinical study.

NTC thermistor	Correction [°C]	B-factor [K]	Reference R at 25°C [kΩ]
Skin measurement	+0.02	3,329	9.80
In-flow temperature	-0.47	3,367	9.80
Out-flow temperature	-0.55	3,395	10.02
Fluid reservoir	-0.24	3,396	9.86

The variability of ±1.4% for the energy transfer measurement after calibration of the NTC thermistors was accepted. Following correction for each NTC thermistor in the EMC software, it was judged that the temperature measurement was sufficiently accurate to continue.

3.2 Optimization and validation of energy-extraction method

The primary setting of parameters was based upon experience of the EMC team and in similarity to the existing cooling device of EMC medical instruments. The influence of these factors on the end-result in an experimental setting was tested in a Plackett-Burman design. After permanent setting of the factors the system was validated and variability assessed.

3.2.1 Plackett-Burman screening design

In a 12 experiment, 2 level Plackett-Burman screening design, the influence of 7 variables on: energy loss from a water reservoir, energy transfer to the therapy pad, and the difference between these, was assessed. These variables, also called factors, were: temperature of water that ran through the therapy pad, temperature of the water in reservoir B, flow rate, therapy pad condition (full or empty) upon appliance, isolation of the tubing, and duration of the pre-measurement stabilisation period (cooling of the set-up). Values of the response and effect calculations and statistical analysis can be found in Appendix 1.

A Plackett-Burman design has a resolution III, this means that the main effects are confounded with a number of two-factor and higher-order interactions. Two-factor interactions are usually smaller than the significant main effects but they are not always so small to be completely neglected. As a consequence they will contribute to the calculated main effect. Two-factor interaction were not controlled for the factor effects, but a normal probability plot was used to scan for significant dummies. Significant dummies increase the calculated standard error and may mask significant factors in the statistical analysis. Effects that are significantly different from zero do not belong to a normal distribution and deviate from the straight line (Fig 11). Dummy 4 was omitted from the statistical analysis of the effects of the factors on the energy loss from the warm reservoir and dummy 3 for the analysis of the measured energy transfer to the therapy pad (Fig 11).

A student *t*-test was performed to find factors that had a significant effect ($p<0.05$) on the responses. None of the factors had a significant effect on the energy lost from the warm water reservoir. This could possibly be explained by the large volume (25L) of water in the reservoir, that could mask subtle changes of the total amount of energy lost during the experiment.

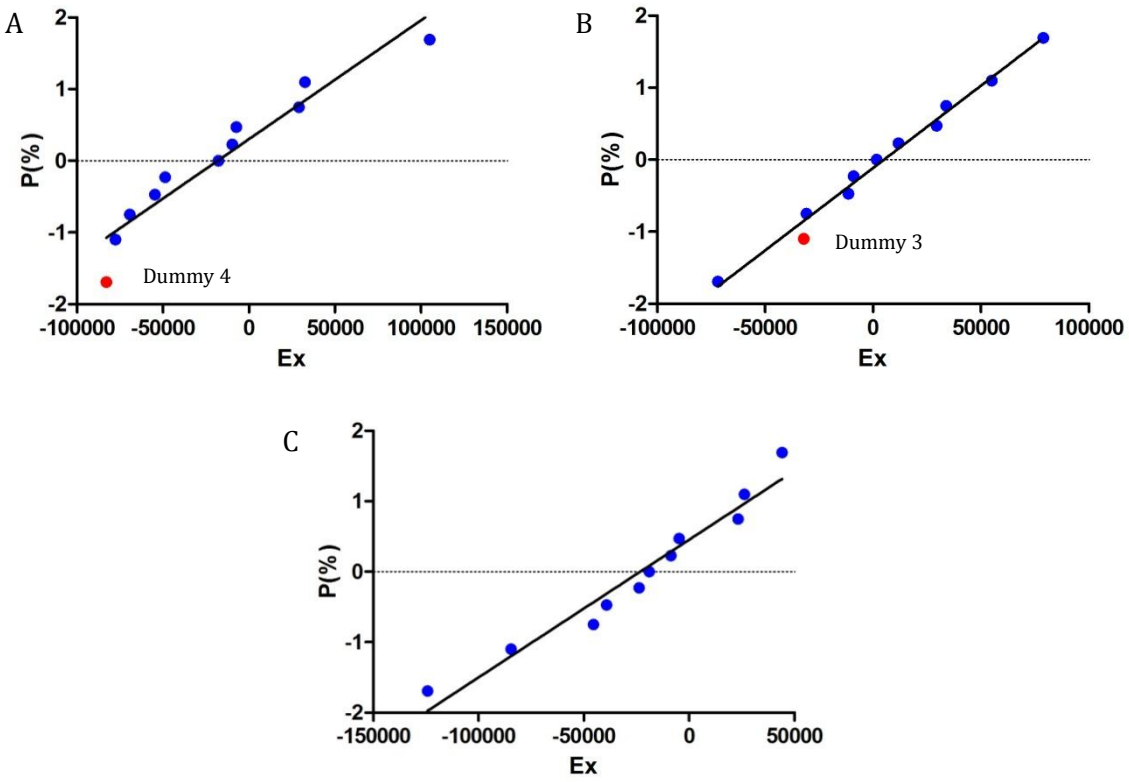

Figure 11: Normal probability plots of the calculated effects of the factors on; the energy loss from the warm reservoir (A), the energy transfer to the therapy pad (B), and the difference (C). Dummy 4 was excluded from the variance calculations for the effect of the factors on the energy loss from the warm reservoir. Dummy 3 was excluded from the variance calculations for the effect of the factors on the energy transfer to the therapy pad.

On the other hand, all factors except for duration of the pre-measurement stabilisation period, did have an effect on the measured energy transfer to the pad. This indicates that the energy transfer measurement is more vulnerable for small setting changes. There was no significant effect of the change in duration of the pre-measurement stabilisation period (p: 0.2642). However it is expected that starting the energy transfer measurement without previous cooling of the tubing and therapy pad would influence the outcome as the thermistors at the entrance and exit of the therapy pad are build into metal tubing pieces. This metal leads to delay in the temperature measurement.

The fact that temperature of the water running through the pad (p: 0.0035), temperature of the warm water (p: 0.0027), and flow rate (p: 0.0409) of the water all have a significant effect could be expected from the energy transfer equation (Eq 9, material & methods). The temperature difference is the driving force of the energy transfer from the warm water reservoir to the therapy pad, whereas flow rate determines the amount of water circulating in a defined period of time, e.g. 10 minutes, conform the set up of this experiment.

For the same reason mingling the warm water reservoir (p: 0.076) is significant. Water that has already transferred its energy to the therapy pad is replaced by warmer water.

The significant effect of the condition of the therapy pads (0.0360), full or empty when hung into the warm water reservoir, can be clarified by the filling process. If the therapy pad is empty, the warm water in the reservoir will begin transferring energy toward the pad as soon as contact is made. When the therapy pad fills, the energy has already been transferred will be convected to the water filling it. Because of this the will be warmer at what time of exiting the therapy the first few minutes, as it would be if the therapy pad was already full while bringing it into the warm water reservoir. Also turbulence and mingling of the water upon filling may disturb energy transfer in the first minute of measurement (Fig 12).

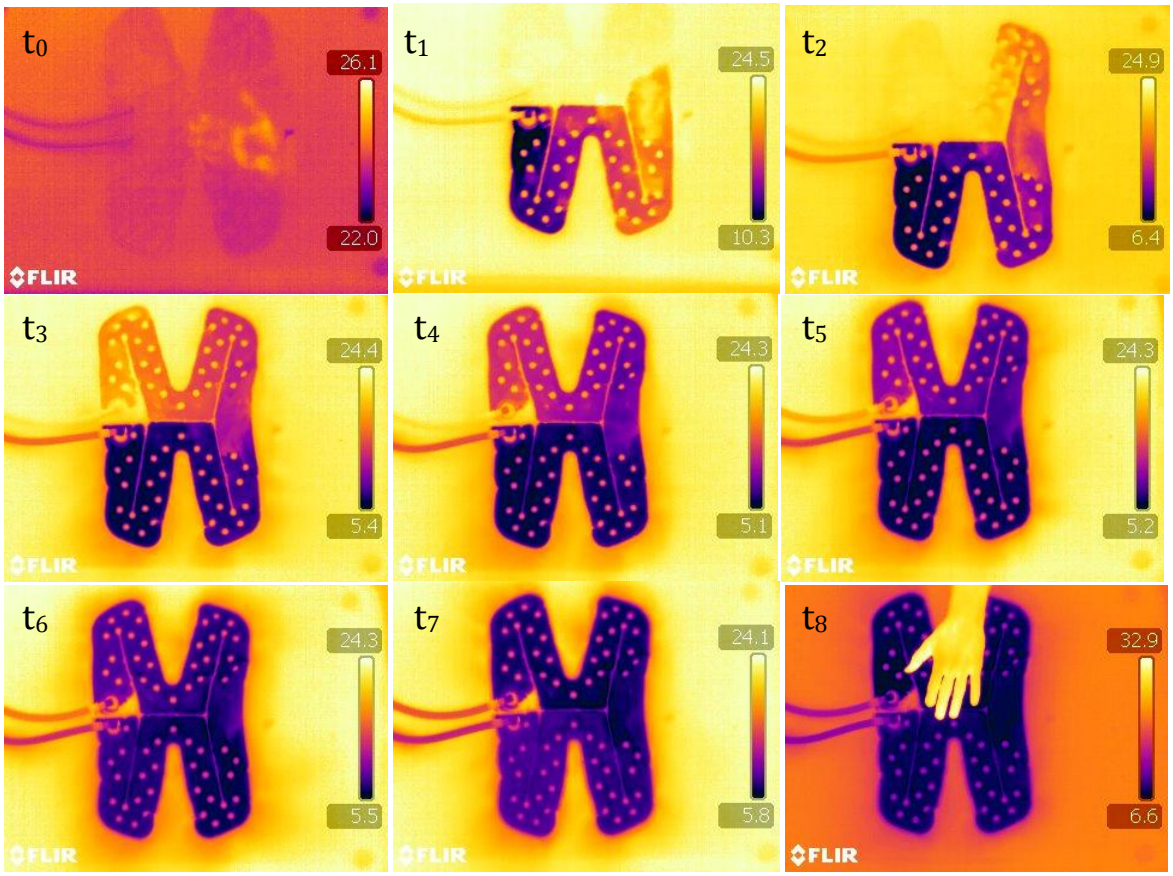

Figure 12: Infrared pictures of the filling of the therapy pad, time span of 1 minute. t_0: therapy pad at room temperature.

Isolation of the tubing (p: 0.0286) prevents energy transfer from warmer surroundings to the cold water running through the tubing. It also prevents incorrect temperature measurement by the thermistors due to a warm, cold or humid environment.

This screening provided a basis for setting of the factors for the following validation experiments and the clinical study. Tubes were permanently isolated and flow rate was fixed at 2.3L/min. The therapy pad was empty upon appliance. It was chosen to keep a stabilisation period of 6 minutes before the measurement. The temperature of the cold water was set at 10°C. This is a therapeutic temperature that is well tolerated by patients, whereas 5°C is too cold and cause pain after prolonged application (35). This is also a temperature that has been used with success by the EMC team. Temperatures higher than 10°C were not found in the current literature.

3.2.2 Validation

The energy extraction was validated by changing the temperature of the water that streams through the therapy pad, whereas the temperature in the warm water reservoir (25L) was fixed. The energy lost from the warm water reservoir was compared to the energy transferred to the therapy pad. The temperature of the cold water in reservoir A was varied from 10°C to 25°C with intervals of 5°C, the warm water was fixed at 35°C ±0.1%. Duration of the energy extraction was 10 minutes. In ideal conditions the amount of energy lost from the warm water reservoir would equal the amount of energy transferred to the therapy pad (Eq. 4, materials and methods). Energy calculations were started when water started running out of the therapy pad.

Energy lost from the warm reservoir and extracted by the therapy pas decreased when the temperature difference between reservoir and therapy pad decreased. Except for the first two minutes in which more energy was lost than extracted, differences between energy loss and extraction vary around the same values as can be seen on Figure 13.

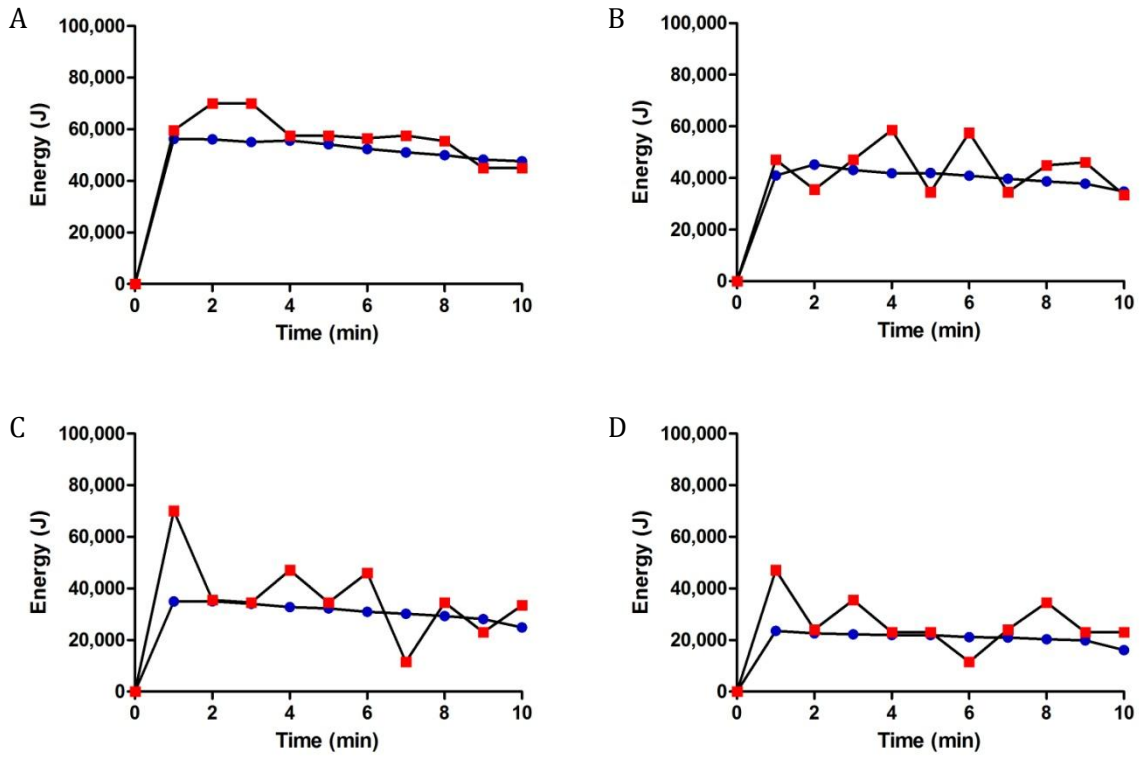

Figure 13: In red the absolute energy lost from the warm water reservoir (35°C). In blue, the energy extraction by the therapy pad. A; Water in the therapy pad 10°C (ΔT 25°C). B Water in the therapy pad 15°C (ΔT 20°C). C; Water in the therapy pad 20°C (ΔT 15°C). D; Water in the therapy pad 25°C (ΔT 10°C).

The experiments were executed at a room temperature of 13°C. The additional energy lost from the warm water reservoir might be explained by loss of energy to the cold environment. Opening of the lid from the warm water reservoir to hang in the therapy pad, together with filling of the pad, may explain the lager difference in the first two minutes. The dispersion between energy loss and extraction over the 10 minutes is lager when the water in the therapy pad is warmer (20°C and 25°C). Energy extraction is in direct proportion to the temperature difference between the warm water in the reservoir and the water in the therapy pad (Eq 9, materials and methods). When the temperature difference is smaller, the energy will not be transferred as easily, causing less energy to be extracted. Because the temperature difference with the environment was lager, heat was more easily lost to the surrounding as to the therapy pad.

Based upon the Plackett-Burman screening and this validation, the total amount of energy lost and transferred will be calculated with exclusion of the first two minutes, making a total of 8 minutes for the following experiments. This will be done to exclude any experimental error from the first two minutes due to thermistor delay, tubing and isolation temperature, and filling of the pad.

3.2.3 Variability

For all measurements the entire protocol was repeated. This protocol can be found in Appendix 3. As with the validation experiments it must be clarified that energy loss was based upon convection and not conduction as in biological tissue. It is however hard to test variability on a person because the tissue temperature is influenced by many factors such as; circadian rhythm, activity, weather, and stress. A part of the variability measured would be due to person variability. A solution to this problem could be a mold in material that has similar heat conducting properties as tissue. Unfortunately getting the material to the desired temperature would also cause trouble.

The variability of energy extraction for the 4 identical experiments was ±4.13% (Table 2). A fraction of this variability can be explained by the energy extraction calculation which had a maximum error of ±1.7% (sensor calibration). The other part could be due to other experimental variability's, such as: flow rate steadiness, and cold (therapy pad) or warm (reservoir) water temperature variations.

Table 2: Calculated energy extraction, in the experimental setting in which the therapy pad was hanging the warm water reservoir.

Replicate	Energy transfer measured
1	501,939
2	454,190
3	473,258
4	479,672
Mean	**477,264**
Standard deviation	**19,690**

3.3. Clinical study

The goal of this clinical study was to elucidate the influences of individual demographic characteristics and the amount of energy that could be extracted from healthy tissue. To achieve this the left or right knee from 65 healthy volunteers without history of knee problems was cooled for 10 minutes. The group comprised both men and women aged 18 to 82 (Fig 14).

The energy extracted was calculated as the integration of the energy transfer per second over the 8 last minutes of the 10 minutes cooling (Fig 15). The minimum extraction was 20,340 J (4.86 kCal) and the maximum 43,344 J (10.36 kCal). The mean was 29,839 ± 5298 J.

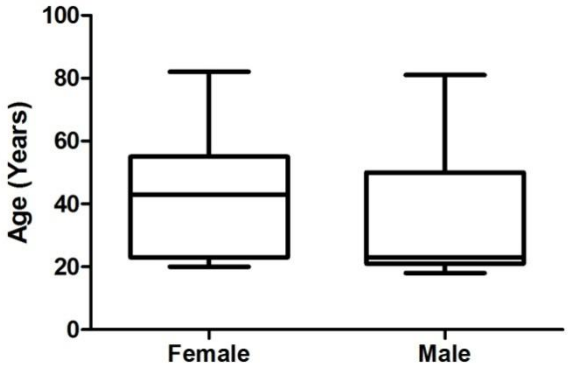

Figure 14: Age distribution per sex. The in the female group there were 41 subjects, in the male group 24. Whiskers at minimum and maximum. Box: Lower quartile, median and upper quartile.

Mean starting skin temperature was 29 ± 1°C. After 10 minutes of cooling this was reduced to 20 ± 4°C. The difference between these two values was used in the multiple regression analysis. Temperature of the skin started decreasing immediately after appliance of the therapy pad, although the cold water did not yet ran through it (Fig 16). It must however be said, that because of the stabilization period, the therapy pad was already cold. The progression of the skin cooling and rewarming in time is consistent with curves found in the literature (15,18,21,32).

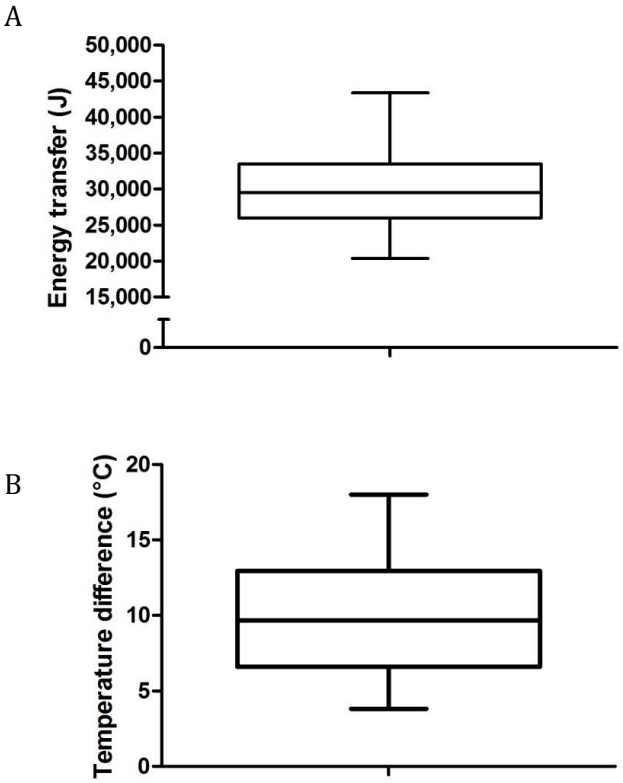

Figure 15: A. Distribution of the total amount of energy extracted in a 8 minute time span. B. Temperature difference pre- and post-cooling skin temperature.

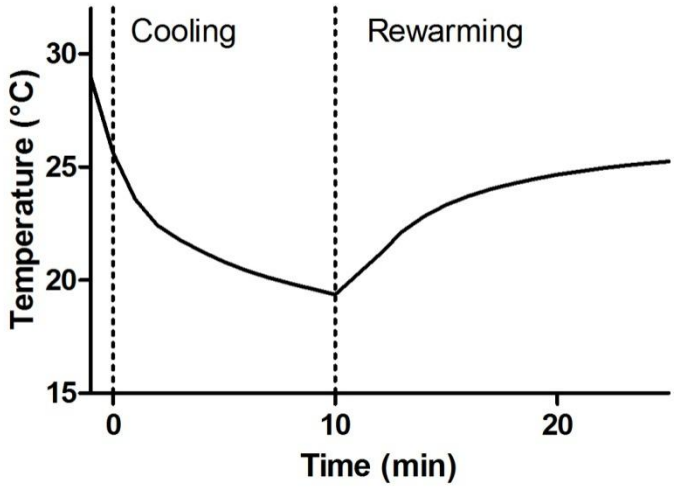

Figure 16: Time course of the skin temperature at baseline, during cooling and rewarming.

The multiple regression analysis was performed with a model that included following independent variables: age, sex, weight, body mass index (BMI), difference in skin temperature pre- and post-cooling, and the interaction between: this difference and age, difference and sex, difference and weight, difference and BMI, age and weight, weight and sex, BMI and sex. The dependent variable was total energy extraction. Sex was a class variable with female being 0 and men 1. After elimination of the non-significant interactions and variables, the final prediction model was:

Prediction model

$$Energy\ extraction = \alpha - \beta_1 Sex + \beta_2 Weight - \beta_3 BMI - \beta_4 Difference + \beta_5 Difference . Sex - \beta_6 Difference . Weight + \beta_7 Difference . BMI + e$$

with α, the intercept, and *e* an error factor normally distributed with mean 0 and variance σ^2.

Difference in pre-a and post-cooling skin temperature as a variable was not significant (p: 0.1932), but because it's interactions were significant (p: 0.0406; 0.0088; 0.0012 respectively) it had to be kept in the model. Sex, weight and BMI had a p-value of 0.0490, 0.0020, and 0.0015 respectively. Age (p: 0.5058) and skinfold thickness (p: 0.9634) or interactions with these variables were not significant.

Table 5: Estimates for the intercept and the coefficients of the different independent variables.

Independent variable	Estimates
Intercept	42,353
Sex (if 1)	-10,533
Weight	933
BMI	-3,137
Difference	-1862
Difference . Sex (if 1)	1,046
Difference . Weight	-72
Difference . BMI	276

When an individual's variables are filled in together with the estimate for its coefficients, the amount of energy that would be extracted can be predicted. Estimates for the different independent variables can be found in Table 5. For instance, the experimental energy extraction value of the subject with number *250540* was 37,220 J. This was a women of 70 years old, that weigh 78 kg, and had a BMI of 32.5. After the cooling the skin temperature was reduced with 16.5°C. The prediction model generated estimates the energy extraction at 36,930 J :

$$\text{Energy extraction} = 42{,}353 - 10{,}533 \cdot \mathbf{0} + 933 \cdot \mathbf{78} - 3{,}137 \cdot \mathbf{32.5} - 1{,}862 \cdot \mathbf{16.5} - 1{,}046 \cdot \mathbf{16.5} \cdot \mathbf{0} - 72 \cdot \mathbf{16.5} \cdot \mathbf{78} + 276 \cdot \mathbf{16.5} \cdot \mathbf{32.5} + e$$

Looking at this prediction model, it can be stated that males will have a lower energy extraction value as females with similar values for weight, BMI and temperature change. Some insight for this statement may come from studies on the influence of female reproductive hormones, estrogen and progesterone, on skin blood flow control mechanisms and core body temperature. In general, estrogens promote cutaneous vasodilatation by shifting the vasodilatation temperature threshold downwards with 0.5°C. Progesterone on the other hand inhibits active vasodilatation (36). Additional studies showed that the level of these hormones alter the release of vasoconstricting neurotransmitters (37). These reproductive hormones also affect core body temperature in women (36). The precision of temperature regulation is similar in man and women, but the set-point is a few tenths of a degree greater in women even in the luteal phase of the menstrual cycle. During the follicular phase it increases with a few tenths of a degree compared to the luteal phase (3).

Interactions of change in skin temperature pre- and post-cooling with sex, weight, and BMI appeared to be correlated to the energy extracted and might be related to the extent of vasoconstriction in the skin. As already cited in the introduction of this work, blood flow to the skin can reduce to essentially zero upon cooling (4),decreasing convective heat transfer from the core to the surface.

Local cooling causes a two phased temperature-dependent vasoconstriction; an initial reflex phase lasting a few minutes due to noradrenergic mechanisms, and a prolonged phase due to non-neural mechanisms. During this initial phase, neural vasoconstriction competes with an initial non-neural vasodilatation induces by the local cooling (4). The prolonged phase is mediated by inhibition of the nitric oxide (NO) mediated vasodilatation by inhibition of the nitric oxide syntheses (NOS) enzyme and at a site downstream for NOS, yet to be elucidated (38). There might be inter-individual differences in the responsiveness vasoconstrictor reflex or inhibition of the NOS that are related with BMI, weight and sex. In a addition to this vasoconstriction, local cooling attenuates histaminergic vasodilatation (39). This might become important when energy extraction measurements are executed in pathologic (inflammatory) conditions. Histamine secreted by platelets is a mediator in acute inflammation and vasodilatation when trauma is induced (26).

BMI and weight are also included in the model; this insinuates a role for adipose tissue. Kim YH et. al. found a significant correlation between BMI and baseline skin temperature (r: -0.72) (40), in the present study this correlation was not found. Reports on the relationship between adipose tissue, and the change in intra-muscular tissue temperature are contradicting.

Jutte et. al stated that adipose thickness (measurement of the skinfold overlying the test area) was a bad predictor of intra-muscular temperature during cooling (r = 0.37) (32). Otte JW et.al on the contrary showed that prolonged cooling is necessary for individuals with a thicker adipose layer to achieve the same intra-muscular temperature as individuals with a less thick layer (41). Energy extraction measures how much energy is transferred from the tissue to the skin and adjunct therapy pad. It can be postulated, keeping the results of Otte JW et. al in mind, that a thicker adipose layer hinders energy to easily conduct to the skin surface. This is consistent with the isolating function of adipose tissue and the low heat conductivity (λ: 0.27 J/s.°C.m) of adipose. While weight and BMI appear to be important predictors for amount of energy extracted, thigh skinfold thickness appears to be not (p: 0.9634). Possibly, measurement of the infrapatellar pad and the fat in the popliteal fossa with nuclear magnetic resonance (NMR) will have a relationship with the energy extracted. But it must be pointed out that beneath this fat no muscle tissue, in which heat can be present, is located and joints are relatively avascular as such.

4. Synthesis & Conclusion

With this proof of concept study it was aimed to show that the energy transfer from tissue that is being cooled with a continuous cold cryotherapeutic agent can be quantified, and to link the total amount of energy transferred to individual characteristics.

Validation experiments showed that it is reliable to quantify the energy transfer from a heat reservoir to a continuous cold cryotherapeutic agent. There is however still room to improve the accuracy of the temperature measurement and subsequent quantification of the energy extraction. Also heat conductive properties of the therapy pad and isolation to the environment can be improved. Physical events during the first two minutes of the energy extraction are still to be elucidated.

A prediction model of the energy extraction was fitted with several individual characteristics. This showed that sex, weight, BMI, and change in skin temperature (pre/post cooling) interactions with sex, weight, and BMI are significantly correlated to the total amount of thermal energy extracted in an 8 minute time frame. Possibly other characteristics, such as; fat tissue inside the joint, time of the day, sportsmanship, weather (season), and so on. In following studies, other important correlations will be further investigated.

These findings are the first that apply a new strategy in cryotherapy research. Energy extraction has never before been used to quantify cryotherapeutic efficiency or has been linked to individual characteristics. In the future, this kind of quantification studies can be used to individualize cryotherapy to patients needs and to apply cryotherapy in a consistent research based manner. Further prospects are to evaluate the energy extraction in a clinical relevant situation. To be more specific, to quantify energy extraction after surgery, when inflammatory processes are present. Discriminating between the energy extraction from inflamed tissue and normal tissue can give important aid, as diagnostic tool. This information could ultimately lead to a new parameter (energy extraction measured in a specific time frame) to identify normal healing tissue and the onset of complications.

References

1. Nadel E. Metabolism and nutrition. In: Boron WF, Boulpaep EL, editors. Medical Physiology. Philadelphia: Saunders; 2005. 1211-1230.
2. Nadel E. Regulation of body temperature. In: Boron WF, Boulpaep EL. Medical physiology. philadelphia: Saunders; 2005. 1231-1241.
3. Sessler DI. Thermoregulation and heat balance. In: Mayer SA, Sessler DI, editors. Therapeutic hypothermia. New York: Taylor and Francis; 2005. 1-25.
4. Kellogg DL. In vivo mechanisms of cutaneous vasodilatation and vasoconstriction in humans during thermoregulatory challenges . J Appl Physiol 2006; 100: 1709-1718.
5. Salisbury RS, Parr G, De Silva M, Hazleman BL, Page-Thomas DP. Heat distribution over normal and abnormal joints: thermal pattern and quantification. 1983; 42: 494-499.
6. Merril TL. Thermodynamics and heat transfer. In: Mayer SA, Sessler DI, editors. Therapeutic hypothermia. New York: Taylor and Francis; 2005. 265-292.
7. Atteveld JA, Berkepeis B, Beuker AJ, Booij CG, de Haan PC, Hommes WA. Polytechnisch zakboekje. 40ste Editie. Arnhem: Koninklijke PBNA bv; 1983.
8. Maruyama S, Okajima J, Komiya A, Takeda H. Estimation of temperature distribution in biological tissue by using solutions of bioheat transfer equantion. Heat Transfer - Asian Research 2008; 37(6): 374-386.
9. Westermann S, Vollmar B, Thorlacius H, Menger MD. Surface cooling inhibits tumor necrosis factor-alfa-induced microvasular perfusion failure, leucocyte adhesion, and apoptosis in the striated muscle. Surgery 1999; 126: 881-889.
10. Oliveira NM, Rainer, Salvino TF. Three intermittent sessions of cryotherapy reduce the secondary muscle injury in skeletal muscle of the rat. Journal of Sports Science and Medicine 2006; 5: 228-234.
11. Schasser KD, Disch AC, Stover JF, Lauffer A, Bail HJ, Mittlmeier T. Prolonged superficial local cryotherapy attenuates microcirculatory impairment, regional inflammation, and muscle necrosis after closed soft tissue injury in rats. American Journal of Sports. Medicine 2007; 35(1): 93-102.
12. Hubbard TJ, Denegar CR. Does cryotherapy improve outcomes with soft tissue injury? Journal of Atheltic Training 2004; 39(3): 278-279.
13. Adie S, Naylo JM, Harris IA. Cryotherapy after total knee arthroplasty: a systematic review and meta-analysis of randomized controlled studys. The Journal of Athroplasty 2009. doi:10.1016/j.arth.2009.07.010.

14. Hubbard TJ, Aronson SL, Denegar CR. Does cryotherapy hasten return to participation? A systematic review. The Journal of Atheltic Training 2004; 39(1): 88-94.
15. Dykstra JH, Hill HM, Miller MG, Cheatham CC, Michael TJ, Baker RJ. Comparison of cubed ice, crushed ice, and wetted ice on intramuscular and surface temperature changes. Journal of Athletic Training 2009; 44(2): 136-141.
16. Kennet J, Hardaker N, Hobbs S, Selfe J. Cooling efficiency of 4 commen cryotherapeutic agents. Journal of Athletic Training 2007. 42(3): 343-348.
17. Myrer JW. Temperature changes in the human leg during and after two methods of cryotherapy. Journal of Athletic Training 1998; 33: 25-29.
18. Merrick MA, Jutte LS, Smith ME. Cold modalities with different thermodynamic properties produce different surface and intramuscular temperatures. Journal of Athletic Training 2003; 38(1): 28-33.
19. Selfe J, Hardaker N, Whitaker J, Hayes C. Thermal imaging of an ice burn over the patella following clinically relevant cryotherapy application during a clinical research study. Physical Therapy in sport 2007; 8: 153-158.
20. Stevens D, D'Angelo D. Frostbite due to improper use of frozen gel packs. The New England journal of medecine 1978; 299(25): 1415.
21. Merrick MA, Knight KL, Ingersoll CD, Potteiger JA. The effect of ice and compression wraps on intramuscular temperatures at various depths. Journal of Athletic Training 1993; 28(3): 236-245.
22. Barry S, Wallace L, Lamb S. Cryotherapy after total knee replacement: a survey of current practice. Physiotherapy Research International 2003; 8(3): 111-120.
23. Kumar V, Abbas AK, Fautso N, Mitchell RN. Acute and chronic inflammation. In: Kumar V, Abbas AK, Fautso N, Mitchell RN, editors. Robbins Basic Pathology. 8th edition. Philadelpia: Saunders; 2007. 31-58.
24. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: Molecular and cellular mechanisms. Journal of Investigative Dermatology 2007; 127.
25. Lentsch AB, Ward PA. Regulation of inflammatory vascular damage. Journal of pathology 2000; 190: 343-348.
26. Ward PA, Lentsch AB. The actute inflammatory response and its regulation. Arch Surg 1999; 134: 666-669.
27. Martin P, Leibovich SJ. Inflammatory cells during wound repair: the good, the bad and the ugly. Trends in Cell Biology 2005; 15(11):599-607 .
28. Hsu SF, Niu KC, Lin MT. Brain cooling causes attenuation of cerebral oxidative stress, systemic inflammation, activated coagulation and tissue ischemia-injury during heatstroke. Shock 2006; 26(2): 210-220.

29. Stevens A, Lowe J. Skin and Breasts. In: Stevens A, Lowe J, editors. Human histology. 2nd edition. London: Mosby; 1997. 355-370.
30. Haidar SG, Charity RM, Bassi RS, Nicolai P, Singh BK. Knee skin temperature following uncomplicated total knee replacement. The Knee 2006; 13: 422-426.
31. Mehra A, Langkamer VG, Day A, Harris S, Spencer RF. C reactive protein and skin temperature post total knee replacement. The Knee 2005; 12: 297-300.
32. Jutte LS, Merrich MA, Ingersoll CD, Edwards JE. The relationship between intramusculartemperature, skin temperature, and adipose thickness during cryotherapy and rewarming . Arch Phys Med Rehabil 2001; 82: 845-850.
33. Cantherm. How to choose and work with NTC thermistors: In: Thermistors. 2010. URL: www.cantherm.com .
34. Young HD, Freedman RA. Temperature and heat. In: Young HD, Freedman, editors. Sears and Zemansky's university physics. 12th edition. San Fransisco: Pearson Education Inc.; 2008. 570-609.
35. Ohkoshi Y, Ohkoshi M, Nagasaki S, Hashimoto T, Yamane S. The effect of cryotherapy on intraarticular temperature and postoperative care after anterior cruciate ligament reconstruction. The American Journal of Sports Medicine 1999; 27(3): 357-362.
36. Charkoudian N. Skin blood flow in adult human thermoreguation: How it works, when it does not, and why. Mayo Clin Proc 2003; 78: 603-612.
37. Stephens DP, Bennett LA, Aoki K, Kosiba WA, Charkoudian N, Johnson J. Sympathic nonnoradregenic cutaneous vasoconstriction in women is associated with reproductive hormone status. Am J Physiol Heart Circ Pysiol. 2002; 282: 264-272.
38. Hodges GJ, Zhao K, Kosiba WA, Johnson JM. The involvement of nitric oxide in the cutaneous vasoconstrictor response to local cooling in humans. J Physiol 2003; : 849-857.
39. Grossmann M, Jamieson MJ, Kirch W. Histamine response and local coolling in the human skin: involvement of H1- and H2-receptors. J Clin Pharmacol 1999; 48: 216-222.
40. Kim YH, Back SS, Choi KS, Lee SG, Park SB. The effect of cold air application on intra-articular and skin temperatures in the knee. Yonsei Medical Journal 2002. 43(5): 621-626.
41. Otte JW, Merrick MA, Ingersoll CD, Cordova ML. Subcutaneous adipose tissue thickness alters cooling time during cryotherapy. Arch Phys Med Rehabil 2002; 83: 1501-1505.

Appendix 1

Table 1: Level of the different factors in the 12 experiments. Levels are used to calculate the effect of the factor on the responses. Levels for the dummies are used to calculate the effect of the dummies on the response from which the experiment variation is calculated.

Experiment	F1	F2	F3	D1	F4	F5	D2	F6	F7	D3	D4
1	+1	+1	-1	+1	+1	+1	-1	-1	-1	+1	-1
2	-1	+1	+1	-1	+1	+1	+1	-1	-1	-1	+1
3	+1	-1	+1	+1	-1	+1	+1	+1	-1	-1	-1
4	-1	+1	-1	+1	+1	-1	+1	+1	+1	-1	-1
5	-1	-1	+1	-1	+1	+1	-1	+1	+1	+1	-1
6	-1	-1	-1	+1	-1	+1	+1	-1	+1	+1	+1
7	+1	-1	-1	-1	+1	-1	+1	+1	-1	+1	+1
8	+1	+1	-1	-1	-1	+1	-1	+1	+1	-1	+1
9	+1	+1	+1	-1	-1	-1	+1	-1	+1	+1	-1
10	-1	+1	+1	+1	-1	-1	-1	+1	-1	+1	+1
11	+1	-1	+1	+1	+1	-1	-1	-1	+1	-1	+1
12	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1

Table 2: Value of the responses for the 12 experiments, used in the effect calculations. Energy is expressed in Joule.

Experiment	Energy loss	Energy transfer	Difference
1	393,484	325,178	68,306
2	592,319	507,354	84,965
3	716,852	374,137	342,715
4	628,946	494,272	134,675
5	596,505	501,155	95,350
6	463,599	314,524	149,076
7	454,432	359,655	94,777
8	497,087	358,639	138,449
9	665,574	389,226	276,348
10	617,435	487,422	130,013
11	550,459	450,983	99,476
12	671,853	384,983	286,870

Table 3: Effect of the factors on the response and the variance of the different responses calculated out of the effect of the dummy variables. For the calculation of $(SE)_e$ from the energy loss dummy 4 was excluded and form energy transfer dummy 3.

Factor	Energy loss	Energy transfer	Difference
T koud	-48,795	-71,982	23,187
q	-9,809	29,442	-39,251
T warm	104,957	78,838	26,119
D1	-17,832	-9,083	-8,750
mengen	-69,376	54,944	-124,320
Pad	-54,808	-30,926	-23,883
D2	32,483	-11,532	44,015
Isolatie	28,995	33,839	-4,844
Stabilisatie	-7,367	11,678	-19,045
D3	-77,748	-32,201	-45,547
D4	-82,981	1,604	-84,585
$(SE)_e$	**49,726**	**8,526**	**53,016**

Appendix 2

Protocol used for the validation experiments (A) and clinical study (B).

- Switching on computer and setting the NTC thermistor variables
- Filling of the cold reservoir with previously cooled water of 10° , close lid
- Notation of ambient temperature
- Stabilization of the tubing by running the water in circuit.
    B. Allow subject to fill in questionnaire
    B. Measurement of skinfold thickness
- Stop flow and emptying of the therapy pad
    B. Move device towards subject
    B. Tape NTC thermistor superomedial to the patella
- Start logging (measurement data are stored in excel compatible file)
    A. Hanging therapy pad in warm water reservoir
    B. Fixing therapy pad to the subjects knee and open tap
- Start flow for 10 minutes
- Stop flow
    A. Stop logging, therapy pad out of the warm reservoir
    B. Keep logging on for additional 15 minutes, remove therapy pad from subject's knee
- Empty therapy pad

Appendix 3

Table 1: Data from variability test for the NTC thermistor used to monitor the subjects knee temperature.

Skin NTC thermistor			
replicate	**day 1**	**day 2**	**day 3**
A	24.98	24.88	24.98
B	24.88	25.19*	24.98
C	24.98	24.88	24.98
Overall mean	**24.97**		
Variance	**0.09**		

Single Grubbs (two-sided) value for *day 2/replicate B is 2.2999. Critical value for G0.05;9 is ±2.2150 and for G0.01;9 is ±2.3870. This value is a strangler and is kept in the data-set from further variance calculations.

ANOVA on the 99% significance level. p-value: 0.9023, between day variance did not have a significant contribution in the total variance.

Table 2: Data from variability test for the NTC thermistor used to monitor the temperature of the fluid at the entrance of the therapy pad.

In-Flow NTC thermistor		
replicate	**day 2**	**day 3**
A	25.60	25.50
B	25.40	25.71
C	25.40	25.19
Overall mean	**25.47**	
Variance	**0.18**	

ANOVA on the 99% significance level. p-value: 0.0219, between day variance did not have a significant contribution in the total variance.

Table 3: Data from variability test for the NTC thermistor used to monitor the temperature of the fluid at the exit of the therapy pad.

Out-Flow NTC thermistor			
replicate	**day 1**	**day 2**	**day 3**
A	25.46	25.87	25.67
B	25.36	25.77	25.46
C	25.46	25.67	25.36
Overall mean	**25.56**		
Variance	**0.19**		

ANOVA on the 99% significance level. p-value: 1.000, between day variance did not have a significant contribution in the total variance.

Table 4: Data from variability test for the NTC thermistor used to monitor the temperature of the fluid in the insulated reservoir.

Reservoir NTC thermistor			
replicate	**day 1**	**day 2**	**day 3**
A	25.41	25.35	25.24
B	24.24*	25.24	25.24
C	25.24	25.14	25.14
Overall mean	**25.25**		
Variance	**0.09**		

Single Grubbs (two-sided) value for *day 1/replicate B is -2.5823. Critical value for G0.05;9 is ±2.2150 and for G0.01;9 is ±2.3870. This value is an outlier and is excluded for further variance calculations.

ANOVA on the 99% significance level. p-value: 0.4358, between day variance did not have a significant contribution in the total variance.

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