SUPSI

Originally in German, translated into English

A randomized, controlled, double-blind study to investigate the effectiveness of cooling on performance and recovery

Clinical Trial Protocol

Short titel

Effectiveness of cooling on recovery and performance

Study Type:	Clinical Observation-Study with a medical device Study
Categorisation:	Clinical Observation-Study the Category A after Swiss Ordinance on Clinical Trials in the Human research (ClinO: Art. 20)
Study Registration:	**NCT02506283**
Study Identifier:	
Sponsor, Sponsor-Investigator or Principal Investigator:	**University of Applied Sciences and Arts Southern Switzerland Physiotherapy Graubünden** Weststrasse 8 7302 Landquart Phone: +41 81 300 **01** 70 ***Contact/ Director of Studies*** **Dr. Clijsen Ron,** PhD University of Applied Sciences Southern Switzerland Physiotherapy Graubünden Weststrasse 8, 7302 Landquart Phone: +41 81 300 **01** 75 ron.clijsen@supsi.Ch
Investigational Product:	Zamar Therapy Certified Medical Device after European Medical Devices Act (ISO: 13485:2012; DIRECTIVE 93/42/EEC)
Protocol Version and Date:	Protocol - Version 01 -13.02.2015

SUPSI

CONFIDENTIALITY

The information in this protocol is confidential and the property of the University of Applied Sciences Southern Switzerland, Physiotherapy Graubünden in Landquart-CH. The contents of this protocol should not be forwarded, reproduced, published or passed on to others - neither in full nor in parts - without the written consent of the head of the institute. Excluded from this are the statements in the subject information and the declaration of consent of the study participants.

SUPSI

SIGNATURE OF THE PERSON RESPONSIBLE FOR THE STUDY

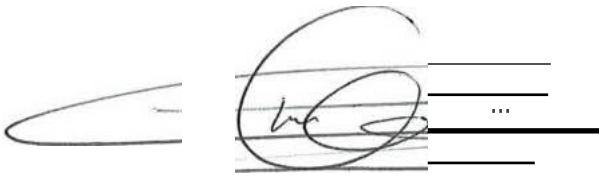
Study number

Study Title One Randomized, controlled double-blind study the Investigation of the effectiveness of cooling on recovery and performance

Sponsor Investigator:

I confirm herewith the To carry out experiments,. how in the present protocol, version 01 of the 13.02.2015, and comply with the World Medical Association Declaration of Helsinki, ICH-GCP Guidelines, the ISO 14155 standard (where applicable), as well as the locally applicable Swiss Human Research Ordinance HFV

Sponsor Investigator
Dr. Clijsen Ron, PhD
University of Applied Sciences
and Arts Southern Switzerland
Physiotherapy Graubünden
Weststrasse 8, 7302 Landquart
Phone: +41 81 300 01 75
ron.clijsen@supsi.Ch



Landquart, 13.02.2015

Place/Date Signature

SUPSI

TABLE OF CONTENTS

SUPSI

Study Synopsis

(ClinO, Appendix 3, 1.1, 2.1, 3.1, 4.1; Appendix 5, 2b; AGEK Summary)

Sponsor / Sponsor Investigator	**University of Applied Sciences and Arts Southern Switzerland** **Department of Business Economics, Health and Social Care** Physiotherapy Graubünden e.g.H. Dr. Ron Clijsen Weststrasse 8 CH-7302 Landquart
Study Title:	A randomized, controlled, double-blind study to investigate the effectiveness of cooling on performance and recovery
Short Title / Study 10:	Effectiveness of cooling on recovery and performance
Protocol Version and Date:	Version 01 from 13.02.2015
Trial registration:	Cantonal Ethics Committee Zurich
Study Category and Rationale:	Observational study Cat. A according to the Lesson Ordinance (Art. 20) The test subjects are not given medication or tissue samples are taken. No invasive methods are used.
Clinical Phase:	-
Background and Rationale:	External cooling applications have been known in elite sports for years and are already being used there. This non-invasive intervention is also gaining popularity in popular and amateur sports. Cooling is said to have positive effects before and after sporting activity. For example, cooling after sporting activity is said to contribute to improved regeneration, while pre-activity cooling is supposed to increase performance.Literature on post- and pre-activity cooling is already available. However, the results are inconclusive and more randomized controlled trials need to be conducted to clarify the results.

SUPSI

Objective(s):	This study will investigate the effectiveness of cooling and provide new insights into the sensible use of this form of application. The study is divided into different sub-projects: A- Effect of post-activity cooling on vertical jump height and assessment of subjective muscle soreness in the legs and perceived general fatigue during 72 hours of recovery time. As an exhaustive measure, the subjects must complete maximum vertical jumps for 1 min. B- Effect of preactivity cooling on maximum voluntary isometric muscle contraction of the thigh muscles and the assessment of subjective muscle soreness in the legs and perceived general fatigue during 72 hours of recovery time. As an exhaustive measure, subjects must isometrically contract and relax their ventral thigh muscles to 60% of their maximum voluntary ventral thigh contraction capacity for 1 min.
Outcome(s):	***Primary OutComes*** Recovery parameters and performance parameters - Jump height (cm) - Subjective muscle soreness in the legs (VAS scale 0 -10) - Subjective information on general exhaustion (BORG scale 0 - 10) Maximum voluntary isometric muscle contraction of the thigh muscle (mV) ***Secondary Outcome*** None
Study design:	Randomized, controlled, double-blind study

SUPSI

Inclusion / Exclusion criteria:	**Inclusion Criteria** Young, healthy adults aged 18 - 30 years No surgical interventions on the musculoskeletal system in the trunk area and lower extremities Injuries to the trunk and/or lower extremities that occurred more than 11 years ago and no longer cause symptoms • Anti-conception drugs **Exclusion criteria** Current injuries of any kind affecting the trunk and/or lower extremities Injuries to the torso and/or lower extremities that occurred less than 1 year ago Injuries to the trunk and/or lower extremities that occurred more than 1 year ago and are still causing symptoms Fear of cooling intervention Taking medication of any kind (even self-purchased) Pacemakers & cardiac arrhythmias Known circulatory problems Pregnancy Diagnosed skeletal static deviations appendectomy less than 2 years ago. Raynaud's syndrome

SUPSI

Measurements and procedures:	*Jump height measurement* The jump height after the load is determined with a jump height measuring plate designed for this purpose (Just Jump Plate, Probotics Inc., Huntsville, USA). The test subjects assume a standing position on the plate. The test subjects are allowed to start from the squatting position (countermovement jump) to measure the jump height. The arms must be placed at the hips during the entire movement. The jump height is measured in cm and takes place directly, 24 hours, 48 hours and 72 hours after the interventions. *Subjective muscle soreness* The subjects reported their subjective feeling of muscle soreness on a visual analogue scale (VAS). The scale will be scaled from "0" (no muscle soreness present) to "10" (largest imaginable muscle soreness) in cm increments. The measurement takes place directly, 24 hours, 48 hours and 72 hours after the interventions. *Subjective information on general exhaustion* The subjects reported their subjective general feeling of exhaustion on a BORG scale (0 - 10). The scale will be scaled from "0" (no general exhaustion) to "10" (greatest conceivable general exhaustion) in cm increments. The measurement takes place directly, 24 hours, 48 hours and 72 hours after the interventions. *Measurement the Maximum Arbitrary Isometric thigh/muscle contraction (MV/C)* Muscle activity is monitored with the help of the wireless surface electromyogram system sEMG active sensors from PLUXwireless bodysignals S.A. (physioplux.com), bandpass filtered between 25- 500Hz and full-wave rectify. MVIC is measured on the Cor 1 - ergometer chair in 90° knee flexion. The raw data of muscle activity is processed with the software of PLUXwireless biosignals S.A. The electrodes are placed according to the internationally recognized SENIAM guidelines (SENIAM project 2013). **Further processing of the data** MATLAB® will be used for further data analysis, and Excel from Windows® will be used for data pooling and for the graphical representation of the end data. *Determination of 60 % of MV/C* 60% of MVIC is performed on the Cor 1 - ergometer chair for biomechanical measurements V1.0 (www.otbioelettronica.(it). All measurements related to MVIC are measured in the Cor 1 in a squat position of 90°.

SUPSI

Study Product / Intervention:	***ZAMAR Therapy*** Zamar Therapy is a certified medical device according to European Directives (Directive 93/42/EEC and ISO 13485:2012). The temperature can be for this device from -5.0 °C to +45 °C. The maximum adjustable treatment time is 1 hour. Ensure cuffs the Optimal Contact with the corresponding Localization. One Coolant circulates continuously between the device and the Cuff. In this study, temperatures of + 8 °C (cooling) and thermoneutral temperatures (+ 32 °C) are used for 20 minutes.
Control Intervention (if applicable):	In order to obtain high-quality stemresults, the subjects were exposed to only one experimental condition at a time. Two experimental conditions (cooling vs. thermoneutral application) are compared. This applies both to subproject Aals on subproject 8. The division into the 2 possible groups is randomized. The data collected in this way is processedby outcome or group pooled.
Number of Participants with Rationale:	For financial reasons , the number of subjects is limited to a total of 40 participants. The planned number of 20 subjects (ProjectA), or 20 Subjects (Project B) also correspond to the group sizes of the comparable Kühl1 studies (cf. (Ascensao et al. 2011, Bailey et al. 2007, Crystal et al. 2013, Rowsell et al. 2009, De Pauw et al. 2014, Guilhem et al. 2013, Jakeman et al. 2009, Howatson et al. 2009, Montgomery et al. 2008, Rupp et al. 2012, Yanagisawa et al. 2003, u.a.).

Study Duration:	April 2015 - February 2016
Study Schedule:	April 2015: planned start of measurements
	February 2016: End of measurements

SUPSI

Investigator(s):	Examiner - Dr. Ron Clijsen1.[2,3] - Prof. Dr. Jean-Pierre Baeyens1•3•4 - Prof. Dr. Peter Clarys3 - Dr. Ursula M. Küng1 - Hohenauer Erich, MSc1.[2] *1Uuiversily College Physiolherapy 11,im 11an der Laan, Weslstrasse 8, 7302 Landquar/* *[1] Fachhochsc/111/e Siidschweiz, Departement of Business Eco110111ics, Heafthand Sociaf Care, Physiolherapie Gra11bü11de11, Weststrasse 8, 7302 Landuart* *[3] Vrije Universileit Bmssels, Facufty of Physical Ed11Cf1tion and Physical 111erapy, Plei11/a1111 2, 1050 Brusse/s* *' University of A11twe1p, Faculty of Appsong E11gi11eeri11g, Prinsslraat 13, 2000 A111we1p* ***Contact/ Director of Studies*** **Dr. Clijsen Ron,** PhD University of Applied Sciences Southern Switzerland Physiotherapy Graubünden Weststrasse 8, 7302 Landquart +41813000175 ron.clijsen@supsi.anyway
Study Centre(s):	Single Centre Study: Thim van der Laan University College Physiotherapy Weststrasse 8 7302 Landquart

SUPSI

Statistical Considerations:	The effectiveness of cooling on recovery and performance are tested on: Repeated measures analysis of variance (MANOVA) factors: Protocol A - Task (Cooling vs. Thermoneutral application) - Time (These factors are measured immediately after cooling and 24 hours, 48 hours and 72 hours after the interventions) Protocol B Task (cooling vs. thermoneutral application) Time (These factors are measured immediately after cooling and 24 hours, 48 hours and 72 hours after the interventions) The significance level is $P < 0.05$, the statistical data analysis is carried out with SPSS 22.
GCP Statement:	This study will be conducted in compliance with the protocol presented here, the current version of the Declaration of Helsinki, the ICH-GCP, ISO EN 14155 (as far as authoritative), and the Swiss Human Research Ordinance HFV.

SUPSI

Overview of the study

Cold therapy, such as cold water immersions and ice packs, has long been known as post-activity recovery strategies. Sports with different levels of intensity lead to different levels of exhaustion of the body's musculoskeletal, neuronal and metabolic systems. Exercise can also lead to microscopic injuries in muscle tissue, colloquially known as muscle soreness. In sports physiotherapy and rehabilitation, cold therapy has long been used as a post-activity intervention to alleviate these subjective symptoms. This is also confirmed by the current scientific knowledge base (Ascensao et al. 2011, Banfi et al. 2010, Crystal et al. 2013, Delextrat et al. 2013, u.a.). Cold therapy is usually referred to as a procedure which aims to alleviate pain symptoms and inflammatory processes.

This mechanism is attributed to the vasoconstrictive effect of cooling, which affects the Inflammatory response reduced by decreased cell metabolism (White et al. 2013). A recovery period that is too short or not rewarding can result in an athlete not being able to complete their training at the required intensity. Furthermore, a complete recovery is important in order to achieve optimal competition performance. A recently published meta-analysis by Leeder et al. (2012) was able to show that cold water immersions are an effective strategy to reduce muscle soreness symptoms after exhausting and strenuous physical exercise. These results are in line with those of Bleakley et al. (2012), who were able to show in their meta-analysis that cold applications reduce subjective stress sensations by up to 72 hours after cold application compared to passive interventions. However, cold applications are not only used to positively influence recovery times, but are also used.a. used in elite sports to increase the performance of athletes. The authors, who have already dealt with these topics in the literature, clearly point out the importance of publishing further study results on these topics in order to confirm or refute the results known so far. Looking at the methodological quality of the individual studies, it also becomes clear how important it is to deliver high-quality study results in this area.

The question now arises as to whether cooling with the Zamar Therapy device can have a positive effect on performance or the subjective feeling of recovery.

Aim of the study

In this study, the effect of a 20-minute cooling application (+8 °C) on the subjective muscle soreness and general fatigue data, as well as the objectively measurable, maximum isometric muscle contractions of the ventral thigh muscles and the jump height after strenuous physical exertion will be investigated. The effects of cooling are compared to a thermoneutral application (+32 °C} and compared over 72 hours (immediately after, 24 hours, 48 hours and 72 hours after the interventions). The aim is to make recommendations for cooling applications in the field of recreation and performance and to substantiate the state of scientific knowledge. This study can benefit all those who work with amateur and elite athletes.

SUPSI

Abbreviations

CA	**Competent Authority (e.g. Swissmedic)**
CEC	**Competent Ethics Committee**

SUPSI

Study Schedule

(AGEK 4.2; SPIRIT #13; I IT 6.4.2)

The completion of the measurement series is planned for December 2015, and the completion of the entire project for December 2016.

Table 1: Schedule

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Okt	Nov	Dez
'15				Data collection Subproject A			Statistics Subproject A Writing the article Subproject A			Verfassen des Artikels Teilprojekt A		
'15				Data Collection Subproject B			Statistics Subproject B Writing the article Subproject B			Verfassen des Artikels Teilprojekt B		

SUPSI

1. STUDY ADMINISTRATION

1.1 Sponsor, sponsor Investigator

University of Applied Sciences and Arts Southern Switzerland
Department* of *Business Economics, Health and Social Care
Physiotherapy Graubünden

Dr. Ron Clijsen, PhD

Weststrasse 8
7302 Landquart

+41 81 300 01 75
ron.clijsen@supsi.ch

1.2 Examiner

Director of Studies

Dr. Ron Clijsen[1,2,3] *(PhD, GCP Module 1- III & GCP Basic Course)*

Selection of subjects, obtaining consent to participate in the study, study administration, carrying out the measurements - collecting the data, analyzing the data, interpreting the data, writing the report

University of Applied Sciences and Arts Southern
Switzerland Research Manager University of Applied
Sciences and Arts Southern Switzerland Weststrasse 8,
7302 Landquart
+41 81 300 01 73
ron.clijsen@supsi.ch

SUPSI

Other examiners

Prof. Dr. Jean-Pierre Baeyens[2,3,4]

Data analysis, interpretation of the data

Prof. Dr. Peter Clarys [3]

Study Monitoring/Monitoring

Dr. Ursula **M.** Küng[2] *(PhD, GCP Module 1- III & GCP Basic Course)*

Selection of test subjects, data analysis, interpretation of the data, writing of the report

Hohenauer Erich[1,2], MSc *(MSc, GCP Module I - III & GCP Basic Course)*

Selection of the subjects, execution of the measurements - collection of the data, data analysis, interpretation of the data, writing of the report, obtaining consent to study participation, study administration

[1] University of Applied Sciences and Arts Southern Switzerland, Department of Business Economics, Health and Social Gare, Physiotherapy Graubünden, Weststrasse 8, 7302 Landquart

[2] University College Physiotherapy Thim van der Laan, Weststrasse 8, 7302 Landquart

[3] Vrije Universiteit Brussels, Faculty of Physical Therapy, Pleinlaan 2, 1050 Brussels

[4] University of Antwerp, Faculty of Applied Engineering, Printstraat 13, 2000 Antwerp

Other employees

none

1.3 Statistician

Prof. Dr. Jean-Pierre Baeyens
Vrije Universiteit Brussels
Faculty of Physical Education and Physical Therapy
Pleinlann 2
1050 Brussels
+32-2-6292722

University of Antwerp
Faculty of Applied Engineering
Printstraat 13
2000 Antwerp
+32-3-265-41-11

jean-pierre.baeyens@vub.ac.mal

SUPSI

1.4 Laboratory Manager

Dr. Ron Clijsen, PhD
Head of Research
Laboratory
University of Applied Sciences Southern Switzerland, Department of Business Economics, Health and Social Care Physiotherapy Graubünden

Weststrasse 8
7302 Landquart
+41813000175
ron.clijsen@supsi.Ch

1.5 Monitoring the study

Prof. Dr. Peter Clarys

Vrije Universiteit Brussels
Faculty of Physical Therapy
Pleinlaan 2, 1050 Brussels

Peter.clarys@vub.ac.mal

1.6 Privacy

All data obtained from study participants will be coded and not passed on to other people. Personal data and personal details are stored in paper form in a locked filing cabinet. Only the study supervisor, the head of the research laboratory and the study director at the same time have access here, with the management of the documents being the responsibility of the head of the research laboratory.

All digital data is anonymized and encoded. No conclusions can be drawn about individuals. The digital data is stored and archived on in.stituts' internal computers and is not released to outsiders.

Employees involved in data processing have no insight into the personal data and the coding of it.

1.7 Other committees or institutes involved

The study is being conducted in the research laboratory at the University College Physiotherapy Thim van der Laan, Weststrasse 8, CH-7302 Landquart.

The test products and measuring equipment are all located in this room and therefore all measurements and training can be carried out at the same location. The room offers enough space to be able to comply with all the safety aspects described below without any problems.

At present, no further studies with this arrangement are taking place in this experimental laboratory. Currently, another study is taking place in our institute (KEK-ZN-Nr.2014-0107 to investigate the effectiveness of slackline training). However, the measurements of these two studies do not take place at the same time and do not hinder or influence each other. Thus, the utilization of the infrastructure is not excessive and is justifiable.

SUPSI

There are no other institutes or committees involved in this study. approval or monitoring of the study.

2 ETHICAL AND REGULATORY ASPECTS

2.1 Study Registration

The implementation of the present study is the responsibility of the Zurich Cantonal Ethics Committee. An application for admission is submitted. Any further conditions of the responsible ethics committee must be implemented in the study protocol.

When the study protocol is submitted to the Cantonal Ethics Committee in Zurich, the study is also registered with the Swiss Coordination Office for Research on Human Subjects.

2.2 Categorization of the study

Observational study Cat. A (ClinV Art. 20)

The test subjects are not given medication or tissue samples are taken. No invasive methods are used. Trials are carried out with CE-certified medical devices in accordance with their instructions for use.

2.3 Competent IEthics Committee (CEC)

Human research in the Canton of Graubünden falls under the remit of the Cantonal Ethics Commission Zurich. Changes to the study protocol are not permitted without the prior consent of the CEC, except for the immediate elimination of obvious hazards to the participants. These are according to Chapter 2.10 of the CEC.

An early termination or interruption of the study must be reported to the CEC within 15 days, and the regular end within 90 days. The final report must be submitted within one year of the end of the programme. Corrections are in accordance with Chapter 2.1O.

2.4 Competent Authorities (CA)

The present study falls under the remit of the Zurich Cantonal Ethics Committee. Obligations and deadlines can be found in the previous point.

2.5 Ethical Leadership of the Study

The study will be conducted in accordance with this protocol and the current version of the Helsinki Declaration, the ICH-GCP, the European Medical Devices Directive 93/42/EEC and the ISO standard 14155 and ISO 14971 (where applicable), as well as the Swiss Human Research Ordinance and thus Swiss law.

The CEC receives an annual report and is informed about the course of studies.

2.6 Conflict of interests

There is no conflict of interest of a financial nature on the part of the sponsor investor (head of studies), nor is there a corresponding relationship of dependency.

2.7 Patient information and informed consent

The examiners will explain to each participant the nature and goal of the study. This also includes the expiration of the protocol, the approximate duration, possible risks and benefits. The participant is informed that participation is voluntary and can be withdrawn from the study at any time without further personal consequences of any kind. The participant is further informed about who has access to the personal data and how data protection is guaranteed.

Each participant receives written information about the test subject and the declaration of consent with

SUPSI

the description of the study with all the information necessary to decide whether to participate in the study. He or she will also be given sufficient time to read this written information and to ask questions to the responsible examiner.

The subject information and the informed consent form are submitted to the CEC, where it must be reviewed and approved.

The signed declaration of consent must be submitted before the start of the study procedure. The participant should read and check them before signing. The responsible examiner then also signs the declaration of consent. Finally, the participant receives a copy of the declaration of consent. The signed declaration of consent is kept in the original as a study document in a locked filing cabinet.

2.8 Confidentiality & Privacy

The examiners ensure that the privacy of the participant is maintained. In particular, data protection and confidentiality are guaranteed and no personal data is presented or published. The signed declaration of consent, as well as the completed questionnaire with the other personal details, are kept in the original as a study document in a locked filing cabinet.

The digital data will be anonymized and treated confidentially, and access to the personal data will not be allowed to third parties. The digital data collected is stored solely on the institute's own computers and is not passed on to any external persons or transferred to other computers.

Direct access to the personal data is only available to the head of the research laboratories (study leader), the study supervisor and authorized persons of the CEC.

2.9 Early termination of the study

The sponsor investor (principal investigator) may discontinue the study early if/if ethical

concerns arise

insufficient number of subjects

the safety of the participants cannot be guaranteed

Findings from clinical practice a continuation the Making the study pointless

early evidence or harm of the experimental intervention has been proven

2.10 Protocol Adjustments

Adjustments and changes to the current study are only permissible after review by the CEC.

Short-term changes to the protocol prior to an audit by the CEC may be made to ensure the rights and safety of participants. However, such adjustments must be documented and reported to the CEC as soon as possible.

Extensive adjustments are only permissible after a review by the CEC. Small adjustments must be notified to the CEC as soon as possible and listed in the annual report.

SUPSI

3 BACKGROUND AND RATIONALE

3.1 Background and justification

Cold therapies have long been used as a treatment after strenuous exertion (Costello et al. 2012). In sports medicine, cold therapy is a common intervention, the effect of which has already been investigated in many studies. Cold therapy applications are used to alleviate the symptoms of subjective muscle soreness and general fatigue (Ascensao et al. 2011, Bailey et al. 2007, Costello et al. 2012, Crystal et al. 2013, Delextrat et al. 2013, Elias et al. 2012, Eston et al. 1999, Goodall et al. 2008, Guilhem et al. 2013, Hausswirth et al. 2011, Howatson et al. 2009, Ingram et al. 2009, Jakeman et al. 2011, King et al. 2009, Kuligowski et al. 1998, Minett et al. 2012, Paddon-Jones et al. 1997, Pointon et al. 2011, Pointon et al. 2012, Pournot et al. 2011, Rowsell et al. 2009, Rupp et al. 2012, Sellwood et al. 2007, Stanley et al. 2013, Tseng et al. 2013) . Furthermore, cold therapy is used to determine objective parameters, e.g.creatine kinase, lactate, various interleukins or C-reactive proteins (Crowe et al. 2007, Leal et al. 2011, Tucker et al. 2012, Pointon et al. 2012, Vaile et al. 2088, Heyman et al. 2009, Bastos et al. 2012, De Pauw et al. 2014, King et al. 2009, Howatson et al. 2009, Guilhem et al. 2013). However, cryotherapy is not only used to optimize subjective and objective recovery parameters, but also to improve objective performance (Duffield et al. 2009, Boegard et al. 2010, Brade et al. 2014, Wegmann et al. 2012). Cold therapy is described as a procedure that relieves pain and specifically reduces inflammatory reactions after injuries and overuse. As a mechanism of action of cooling, it is said to have the vasoconstrictive effect, which reduces inflammatory reactions by reducing cell metabolism. Banfi et al. (2010) recently published results according to which whole-body cooling would not have a negative effect on athletes. However, only little evidence could be found here that favored whole-body cooling over "non-cooling". Leeder et al. (2012), on the other hand, was able to show that cold water immersions are an effective strategy to alleviate subjective symptoms. These results are consistent with those of Bleakly et al. (2012). Wegmann et al. (2012)
were able to show in their meta-analysis, that Prrä activity cooling effectively has a positive effect on the endurance capacity. The application of external refrigeration applications was preferred to internal refrigeration applications in terms of the potential performance-enhancing effect.

Aim of the studies

In this study, the effect of a 20-minute cooling application (+8 °C) on the subjective muscle soreness and general exhaustioninformation, as well as the objectively measurable maximum isometric muscle contractions of the thigh muscle and the jump height after strenuous bodyStresses and strains are examined. The effects of cooling are compared with a thermoneutral application (+32 °C) and applied directly, 24 hours, 48 hours and 72 hours after the interventions. The aim is to, To make recommendations for cooling applications in the field of recovery and performance and to substantiate the state of scientific knowledge. This Studie is thus intended to provide trainers, therapists and provide doctors with in-depth insights.

SUPSI

The study is divided into two sub-projects:

Post-activity cooling (subproject A) - In this subproject, the influence of post-activity cooling compared to thermoneutral application on recovery is determined. It is investigated whether the maximum jump height changes between these interventions after maximum performance in the form of vertical jumps (1 min) and 20 min of sustained cooling or thermoneutral application (direct, 24 hours, 48 hours, 72 hours after the interventions). To investigate this, the test subjects are tested before the maximum performance (1 min vertical jumps) and again directly, 24 hours, 48 hours and 72 hours after cooling/thermoneutral application. In order to be able to determine the baseline and measure the effect of cooling compared to the thermoneutral application , the test subjects must each perform 3 vertical, maximum jumps. The maximum vertical jump height is used as an objective recovery factor in this subproject. Weilers, this subproject investigates the extent to which the subjective muscle soreness and general exhaustion data of the test subjects differ within the cooling group and thermoneutral
group. To this end, before and inach the maximum performance (1 min vertical jumps) measured the subjective information of the test subjects (direct, 24 hours, 48
hours, 72 hours after cooling/thermoneutral application). A 0 - 10 VAS and 0 - 10 BORG scale is used. The VAS and BORG data are used as a subjective recovery factor in this subproject.

Preactivity cooling (subproject B) - In this subproject, the influence of preactivity cooling compared to thermoneutral application on performance is determined. It is investigated whether the MVIC of the ventral thigh muscle changes between these interventions after submaximal performance in the form of 60% MVIC (1 min) of the ventral thigh muscle and preceding 20 min of cooling or thermoneutral application. In order to determine the baseline and to investigate the effect of cooling on the MVIC, MVIC is determined via an EMG measurement and on the Cor 1 before loading. Following the MVIC determination, 60% of the MVIC is determined. Afterwards , the subjects will perform a submaximal isometric contraction (60% MVIC) for 1 min. Before this submaximal load, the test subjects receive cooling or a thermoneutral application. Following the submaximal load and 24 hours, 48 hours and 72 hours after the load, MVIC is tested again. MVIC will be
subproject as objective performance factors. Furthermore, this study examines the subjective Muscle soreness as well as the general state of exhaustion before and after cooling (directly, 24 hours, 48 hours, 72 hours after the interventions). This is done with a 0-10 VAS and a 0-10 BORG scale. The VAS and BORG data are used as subjective performance factors in this subproject .

3.2 Medical product and indication

Zamar Therapy

Zamar Therapy is a certified medical device according to European directives (Directive 93/42/EEC and ISO 13485:2012). The temperature can be reduced from -5.0 °C to +45 °C. The maximum adjustable treatment time is 1 hour. Cuffs ensure optimal contact with the corresponding body region. A coolant circulates continuously between the device and the cuff.

This device is used for the following indications: treatment of the musculoskeletal system (contusions, contractures, grade III lesions, hematomas), tendinogenic system (tendinitis, tenosynovitis, tendinopathy), bone-cartilage system (contusions, post-mortem lesions) fractures), capsuloligamentary system (sprains, contusions of ligaments), postoperative, aesthetic surgery and to accelerate recovery time.

SUPSI

In this study, temperatures of + 8 °C and thermoneutral temperatures (+ 32 °C) are used for 20 minutes .

Fig. 1: Zamar Therapy (www.zamarmedical.com)

There is no written agreement with Zamar Medical to provide the Zamar Therapy for this study.

3.3 Preclinical evidence

(ICH/E6 6.2.2; SPIRIT #Ga)

ICH: A summary of findings from clinical studies that potentially have clinical significance .....

Various studies have already shown that cooling has been able to achieve a positive influence on performance and recovery capacity compared to non-cooling measures . These positive effects could be observed both on a subjective level (Ascensao, 2011; Bailey, 2007; Minett, 2012; Ingram, 2009, Delextrat, 2013; Elias, 2012), as well as on an objective level (Tseng, 2013; Pournot, 2011; Ascensao, 2011; Pointen, 2011; Tucker, 2012, Heyman, 2009). On the other hand, there are studies that question the effectiveness of cooling in terms of its effectiveness (Leal, 2011; Hassan, 2011; Jakeman, 2011)

3.4 Current clinical evidence

(ICH/E6 6.2.2; SPIRIT #6a)

ICH: A summary of findings from ... and from clinical trials that are relevant to the trial.

Current research results regarding the cooling effects, on which the partial protocols of this study are based, have been presented in chap. 3.3.

Castle et al. (2005) were already able to observe a significantly increased peak power output after the application of cold packs to the thigh muscles compared to passive interventions (p<0).05). A systematic review by Ross et al. (2013) also showed that most studies were able to show a success of cooling on performance. Similar results can be observed with regard to cooling and recovery (Ascensao et al. 2011, Bailey et al. 2007, Costello et al. 2012, u.ä.).

The main problem with this type of results is that there is a lack of high-quality studies that include blinding subjects and examiners. Only these types of studies will be able to explain the actual effect of cooling as the number of studies increases. Broatch et al. (2014) already point to this problem. They discuss whether the effect of cooling actually takes place on a physiological or more on a psychological level.

SUPSI

Currently, there is no research taking place in the research laboratory of the Thim van der Laan University College Physiotherapy with the product Zamar Therapy or any other device from Zamar Medical.

According to information and knowledge of Zamar Medical (Porec, Croatia), no further research is currently taking place with Zamar Therapy.

3.5 Medical product: Type of use in the study (ICH/EG6.2.4; SPIRIT #Ga)

ICH: Description of and justification for the route of administration, dosage, dosage regimen, and treatment period(s).

Zamar Therapy from Zamar Medical is used in this study. This thermotherapy device is used as part of the intended application. The examiners apply cold or thermoneutral temperatures. The applied temperatures do not pose a danger to the test subjects at any time. The Zamar Therapy can be used for a maximum of one hour and can generate temperatures from - 5°C to + 45°C. In this study, we will use temperatures of +8 °C (cold application) and + 32°C (thermoneutral application) in subproject A and subproject B. Doctors and physiotherapists can opt in their treatment for cold treatments to e.g.B. pain relief. However, these professional groups not only work with patients, but also with hobbyists and top athletes, or provide them with individual care. The question of whether the recovery phase can be shortened, or the performance can be increased by means of cooling applications is a legitimate question. These two parameters include not only objective values but also subjective information from the athlete.

3.6 Comparison group

(AGEK 11.3; SPIRIT #6b)

Cooling vs. Thermoneutral application

Two groups are compared to each other in order to compare the effects of cooling compared to thermoneutral application. In this way, the efficiency of cooling should be reduced to the ability to recover or performance. The cooling temperature within the cooling group will be +8 °C for both the recovery and performance tests . The temperature of the thermoneutral application will be + 32 °C for both the recovery and performance tests.

Randomization

In order to produce a high-quality study, the test subjects are each assigned to an experimental group, which are later compared with each other.

The classification is carried out randomly, and the data collected in this way are pooled accordingly according to application.

3.7 Risk/ Benefits

(ClinV, Appendix 4, 3.5; Type 25d2; ICH/EG6.2.3; AGEK 11.1; SPIRIT #Ga; MD: ISO 14155 Annex A & 150 14971)

ICH: Summary of the known and potential risks and benefits, if any, to human subjects.

For the subjects, there is a minimal risk of overload during the maximum jumping exercise for 1 min and the MVIC 60% of the ventral thigh muscles (1 min). The rehearsals are expressly informed that they have reached the maximum or submaximal load at any time. Furthermore, 2 examiners will be next to the subject during the 1 min jump load to support him/her in case of circulatory problems

SUPSI

to be able to absorb it. A fully equipped emergency kit is in the examination room at all times during the examinations. In the case of circulatory problems due to hypoglycaemia, the examination room also includes dextrose, chocolate, and ®Coca Cola Ltd. available. The test subjects are also advised to complete the test well nourished in order to minimise the risk of hypoglycaemia . A fully functional and always accessible defibrillator (Lifepak Express Defibrillator, reaplus.ch) is located in our institution. This information is communicated to the test persons (subject information).

Furthermore, any injuries to the musculoskeletal system were defined as an exclusion criterion in order not to provoke a relapse of a past injury.

More information on securing the test subjects can be found in chap. 8.1.1.

The test subject does not derive direct benefits from participating in the study.

SUPSI

3.8 Justification of the choice of population (ClinO, Art 25d4, Art. 15-17; I/IT 6.2.6; AGEK 11.2) ICH: Description of the population to be studied.

This series of studies will be conducted with healthy young volunteers. The risk of injury is considered low in this test subject population.

Inclusion Criteria

Young healthy adults aged 18 - 30 years

- No surgical interventions on the musculoskeletal system in the trunk area and lower extremities
- Injuries to the trunk and/or lower extremities that occurred more than 1 year ago and no longer cause symptoms

Anti-conception drugs

Exclusion criteria

Current injuries of any kind affecting the trunk and/or lower extremities

Injuries to the torso and/or lower extremities that occurred less than 1 year ago

Injuries to the trunk and/or lower extremities that occurred more than 1 year ago and are still causing symptoms.

Fear of cooling intervention

Taking medication of any kind (even self-purchased)

Pacemakers & cardiac arrhythmias

Known circulatory problems Pregnancy

Diagnosed skeletal static deviations

Appendectomy less than 2 years ago

Raynaud's syndrome

The subjects will be comprehensively informed about the process and possible risks before they are confirmed to participate in the study. You will also receive the information in written form and have the opportunity to ask questions. The declaration of consent explains to them what their rights and obligations are when participating. In addition, they are informed that they may withdraw from participation at any time without giving reasons, without incurring any disadvantages as a result.

SUPSI

4 STUDY OBJECTIVES

(ICH/E6 6.3; AGEK 3; SPIRIT #7)
ICH: A detailed description of the objectives and the purpose of the trial.

4.1 General objectives

In this study, the effect of a 20-minute cooling application (+8 °C) on the subjective muscle soreness and general fatigue data, as well as the objectively measurable MVIC of the ventral thigh muscles and the maximum vertical jump height after exhausting physical exertion will be investigated. The effects of the cooling application are compared with a thermoneutral application (+32 °C) and compared directly, 24 hours, 48 hours and 72 hours after the interventions. The aim is to substantiate the state of scientific knowledge and to provide therapists and physicians with further insights. This study can benefit all those who work with amateur and elite athletes.

4.2 Primary objectives

Post-Activity Cooling - Subproject A

In this subproject, the effect of post-activity cooling on objective and subjective recovery parameters is measured and compared to thermoneutral post-activity application.

Objective recovery parameters:

Maximum vertical jump height (cm)

Subjective recovery parameters:

Muscle soreness in the legs (VAS) General fatigue

data (BORG)

Preactivity Cooling - Subproject B

In this subproject, the Effect the Pre-activity cooling measured on objective and subjective performance parameters and compared with the thermoneutral preactivity application.

Objective Performance Parameters:

Maximum Arbitrary Isometric Muscle contraction (MVIC) the ventral thigh muscles (mV)

Subjective performance parameters: Muscle

soreness in the legs (VAS)

Information on general exhaustion (BORG)

SUPSI

4.3 Secondary Objectives

None

4.4 Other safety aspects (long-term)

No short-term risks are to be expected from this study other than those discussed so far.

SUPSI

5 STUDIES OUTCOMES

(I/E6 6.4.1; AGEK 4.1; SPIRIT #12)
ICH: A specific statement of the primary endpoints and the secondary endpoints, if any, tobe measured during the trial.

5.1 Primary outcomes

Muscle activity (EMG)

Increased activation of a muscle can be measured in a larger amplitude of the EMG signal. For this purpose, the absolute amplitude [mV] and the Intergral over a certain time interval [mVs] are used in this study.

MVIC is measured on the Cor 1 - ergometer chair [Nm] in 90° knee flexion. The measurement moments take place directly, 24 hours, 48 hours and 72 hours after the intervention.

Jump height measurement

The jump height after the load is determined with a jump height measuring plate designed for this purpose (Just Jump Plate, Probotics Inc., Huntsville, USA). The test subjects assume a standing position on the plate. The test subjects are allowed to start from the squatting position (countermovement jump) to measure the jump height.The arms must be placed at the hips during the entire movement . The measurement moments take place directly, 24 hours, 48 hours and 72 hours after the intervention.

Subjective muscle soreness

The subjects reported their subjective feeling of muscle soreness on a visual analogue scale (VAS). The scale will be scaled from "0" (no muscle soreness present) to "10" (largest imaginable muscle soreness) in cm increments. The measurement moments take place directly, 24 hours, 48 hours and 72 hours after the intervention.

Subjective information on general exhaustion

The subjects indicate their subjective general feeling of exhaustion on a visual analogue scale (VAS). The scale is from "O" (no general exhaustion present) to
"10" (greatest conceivable general exhaustion) can be scaled in cm steps. The measurement moments take place directly, 24 hours, 48 hours and 72 hours after the intervention.

5.2 Secondary outcomes

None available

SUPSI

5.3 Safety Aspects

No further outcomes are included that serve safety.

Stopping the experiment is up to the testers' assessment of the subject's resilience. If this is estimated to be too low, the examiner can discontinue the experiment.

If, according to his or her own subjective assessment, the subject does not feel well, he or she can discontinue the experiment and withdraw his or her participation in the study. There are no disadvantages for him as a result.

SUPSI

6 DESIGN STUDIES

(I/EG6.4; AGEK 4; SPIRIT #8)

6.1 Study design and justification

Randomized, controlled, double-blind study

It is an observational study of Cat. A (ClinO, Art. 20), whereby a tested medical device is tested on young, healthy volunteers during the intervention.
The group size of the participants is 40 people each (sub-protocols A & B) and 20 respectively

people (subproject A) and 20 people (subproject B).
The group classification of the test setup to be tested (cooling vs. thermoneutral application) will be randomized.

Blinding of the participants is ensured by the use of a thermoneutral application. The examiners are blinded by the addition of another examiner who operates the apparatus. The responsible statistician is blinded and has no access to the personal data of the test persons and is not present when the data is collected.

6.2 Method for minimizing influence

(ICH/EG6.4.3; AGEK 4.3; SPIRIT #16, 17)
ICH: A description of the measures taken to minimize/avoid bias, including: Randomization, Blinding.

6.2.1 Randomization

The allocation to the various groups and persons of the sub-protocols takes place randomly with the drawing of a lot.

6.2.2 Blinding

Blinding of the participants is prevented by the use of a thermoneutral application warranted. The examiners are blinded by the addition of another examiner who operates the apparatus. The responsible statistician is blinded and has no access to the personal data of the test persons and is not present when the data is collected.

6.2.3 Other Methods

No other methods are used.

6.3 Code break procedure (ICH/EG6.4.8; AGEK 4.2; SPIRIT #17b)

ICH: Maintenance of trial treatment randomization codes and procedures for breaking codes.

There is no provision for the statistician to be blinded.

SUPSI

7 POPULATION STUDIES

7.1 Inclusion Criteria

Inclusion Criteria

Young healthy adults aged 18 - 30 years

No surgical interventions on the musculoskeletal system in the Torso area and the lower extremities

Injuries to the trunk and/or lower extremities that occurred more than 1 year ago and no longer cause symptoms

Anti-conception drugs

Exclusion criteria

- Current injuries of any kind affecting the trunk and/or lower extremities

  Injuries to the torso and/or lower extremities that occurred less than 1 year ago

  Injuries to the trunk and/or lower extremities that occurred more than 1 year ago and are still causing symptoms.

  Fear of cooling intervention Taking

  medication of any kind Type (also self-bought)

  Pacemakers & cardiac arrhythmias

  Known circulatory problems
- Pregnancy
- Diagnosed skeletal static deviations

  Appendectomy less than 2 years ago

  Raynaud's syndrome

7.2 Recruitment and screening
(ClinO, Art 25, Appendix 3, 1.4 & 1.6; AGEK 5.1; SPIRIT #15)

Recruitment

It is advertised on the website (homepage & Facebook) of Thim van der laan University College Physiotherapy. The exact texts have been prepared according to the checklist of the Cantonal Ethics Commission Zurich.

SUPSI

As an advertisement (text visible):

- Homepage of the Thim van der Laan Physio School: www.Physio school.Ch
- Facebook page of the Thim van der Laan Physiotherapy School: https:www.facebook.com/physioschule

Screening

At the first contact, the pot. Participant informs about the course and risks of the study, as well as the conditions for and the amount of compensation. A checklist for contacting them by telephone serves as a basis.

During the first telephone contact, a questionnaire is filled out by the study management to determine the suitability as a study participant.

Advertisements, questionnaires and checklists for the first contact can be found in the supplement.

7.3 Group assignment

The group assignment in the sub-protocols is hidden with the help of a lot in a sealed envelope, which is drawn under the supervision of the study director. The test subject number and the application are noted on this lot. The examiner sets the exam condition and only announces the subject number to the other examiners. At the end of the entire data collection , this auditor pools the data sets according to the application.

7.4 Discontinuation of study participation

If a participant withdraws from the study, the lot of the allocation is returned to the pot in a new envelope and a new participant is sought.

The already completed consent form is stored in the locked filing cabinet, data that has already been collected is stored, but is not included in the further data analysis of the study.

The conditions for the termination of the experiment were described in chap. 5.4.

There is no disadvantage for the participants if they discontinue their participation in

the study.

SUPSI

8 STUDY INTERVENTION

8.1 Medical Product ID

8.1.1 Intervention Product

Zamar Therapy

Zamar Therapy is a certified medical device according to European directives (Directive 93/42/EEC and ISO 13485:2012). The temperature can be reduced from -5.0 °C to +45 °C. The maximum adjustable treatment time is 1 hour. Cuffs ensure optimal contact with the corresponding body region. A coolant circulates continuously between the device and the man's boot.

This device is used for the following indications: treatment of the musculoskeletal system (contusions, contractures, lesions of the second degree, hematomas), tendinogenic system (tendinitis, tenosynovitis, tendinopathy), bone-cartilage system (contusions, post fractures), capsuloligamentary system (sprains, torsions, Contusions of ligaments), postoperative, aesthetic surgery and to accelerate recovery time.

In this study, temperatures of + 8 °C and thermoneutral temperatures (+ 32 °C) are used for 20 minutes.

Fig. 1: Zamar Therapy (www.zamarmedical.com)

Securing the test subjects

The temperature of 8 °C is a cooling temperature commonly used in the literature (cf. Ascensao et al., 2011; Bailey et al., 2007; Crystal et al., 2013; Delextrat et al., 2013; Ingram et al., 2009; Jakeman et al., 2009; King et al., 2009; Leal et al., 2011; Paddon-Jones et al., 1997; Pointon et al., 2011; Pointen et al., 2012; Sellwood et al., 2007; Yanagisawa et al., 2003). Nevertheless, the test subjects can stop the cold treatment at any time if they feel unwell.

During the maximum and submaximal loads, the test subjects go to their personal performance limit. Although they are motivated by the examiners, they are not encouraged to exceed the personal performance threshold. The test subjects can be used in case of discomfort or If the personal performance limit is reached, stop the loads at any time. Furthermore, 2 examiners will be next to the subject during the jumping load in order to be able to catch him/her in case of circulatory problems. An emergency kit is available at all times during the examinations (see Chapter 3.7. Risk/ Benefits).

SUPSI

8.1.2 Packaging, labeling and use

ICH: Also include a description of the dosage form, packaging, and labelling of the investigational product(s).

Zamar Therapy is a certified medical device according to European directives (Directive 93/42/EEC and ISO 13485:2012).

8.1.3 Storage

The Zamar Therapy must be stored inside a room. The equipment is therefore located all year round in a closed room with room temperature and low humidity.

8.2 Administration of the experiment (ICH/IT 6.4.4)

Since no medication or invasive methods are used, chap. 8.2 - 8.4 and referred exclusively to chapter 9.

8.2.1 Experimental intervention

ICH: Description of and justification of the treatment(s) tobe administered, including the name(s) of all the product(s), the dose(s), the dosing schedule(s), the route/mode(s) of administration, and the treatment period(s), including the follow-up period(s) for subjects for each investigational product treatment/trial treatment group/arm of the trial.

Cooling application of + 8°C for 20 min on the right thigh

8.2.2 Control Intervention

ICH: Description of and justification of the treatment(s) to be administered, including the name(s) of all the product(s), the dose(s), the dosing schedule(s), the route/mode(s) of administration, and the treatment period(s), including the follow-up period(s) for subjects for each investigational product treatment/trial treatment group/arm of the trial.

Thermoneutral application of + 32 °C for 20 min on the right thigh

8.3 Can / Device modifications

(SPIRIT #11b)

8.4 Compliance with study Intervention

(ICH/E6 6.6.3; AGEK Checklist 2, item 2; SPIRIT #11c)
ICH: Procedures for monitoring subject compliance.

Subjects are motivated to perform MVIC and jump height measurement to keep their compliance high.

8.5 Follow-up treatment of eliminated participants

(ICH/E6 6.5.3; AGEK 9.2; SPIRIT #18b)
I: ..... b) The type and timing of the data to be collected for withdrawn subjects. d) The follow-up for subjects withdrawn from investigational product treatment/trial treatment.

Data already collected from eliminated participants will be stored, but will not be included in the further data analysis of the study.

There is no disadvantage for the participants if they discontinue their participation in the study and no further interventions are necessary for follow-up treatment.

If study participation was due to an injury occurred during study participation,

SUPSI

is aborted, it will be recorded and reported to the sponsor investigator (head of the research laboratory), study leader and supervisor of the study. Furthermore, the report is sent to the public liability insurance of Thim van der Laan University College Physiotherapy in Landquart.

8.6 Preventive measurements

(I/IT 6.6.2; AGEK 9; SPIRIT #11d)
ICH: Medication(s)/treatment(s) permitted (including rescue medication) and not permitted before and/or during the trial.

The questionnaire asks about the intake of medication and excludes pregnancy. If static deviations are visible but not medically diagnosed, the study leader reserves the right to measure them (leg length difference, scoliosis angle) and thus exclude a test subject from participation.

8.7 Accompaniments

(I/IT 6.6.2; AGEK 9; SPIRIT #11d)
ICH: Medication(s)/treatment(s) permitted (including rescue medication) and not permitted before and/or during the trial.

No further side effects are expected from study participation.

8.8 Liability

(I/IT 6.4.7; AGEK Checklist 2, item 1; SPIRIT 11c)
ICH: Accountability procedures for the investigational product(s), including the placebo(s) and comparator(s), if any.

The experiments take place exclusively in the research laboratory of the Thim van der Laan University College Physiotherapy in Landquart. This means that there is no transport of the products and measuring instruments used. There is therefore no liability for the transport of the product .

8.9 Return of the medical device

(AGEK Checklist 2, item 1; SPIRIT 11c)

The Zamar Therapy is returned to the manufacturer after the end of the measurement period.

SUPSI

9 STUDIES STRUCTURE &

EVALUATION (ICH/E6 6.7, 6.8; AGEK 6,

7; SPIRIT #18a)

9.1 Schedules of the various protocols

1. ***Subject Information*** & ***Informed Consent***
   10 Min
2. ***Preparation of the subject***
   15 min
3. ***Running the Test Log (Sublogs)***

Partial Protocol A: first measurement + intervention - approx. 60 min	
Cooling vs. Thermoneutral application on recovery	
Cooling panel	**Thermoneutral Application Group**
1. 3 x **Vertical Leaps VAS & BARROW** (max 5 min)	1. **3 x Vertical Jumps, VAS** & **BARROW** (max 5 min)
Max 1 min break	Max 1 min break
2. **1 minute max vertical Leaps, VAS & BARROW** (1 min)	2. **1 minute max vertical jumps, VAS** & **BARROW** (1 min)
Max 5 min break	Max 5 min break
3. **Cooling** (20 min)	3. **Thermoneutral application** (20 min)
Max 1 min break	Max 1 min break
4. 3 **x Vertical Leaps, VAS & BARROW** (max 5 min)	4. **3 x Vertical JumpeVAS** & **BARROW** (max 5 min)
Directly after the Intervention	immediately after the intervention
5. 3 **x Vertical Leaps, VAS & BARROW** (max 5 min)	5. **3 x Vertical Jumps, VAS** & **BARROW** (max 5 min)
24 hours after the intervention	24 hours after the intervention
6. 3 **x Vertical Jumps, VAS & BARROW** (max 5 min)	6. **3 x Vertical Jumps, VAS** & **BARROW** (max 5 min)
48 hours after the intervention	48 hours after the intervention
7. 3 **x Vertical Leaps, VAS & BARROW** (max 5 min)	7. **3 x Vertical jumps, VAS** & **BARROW** (max 5 min)
72 hours after the intervention	72 hours after the intervention

SUPSI

Partial Protocol B: first measurement + intervention - approx. 60 min	
Cooling vs. Thermoneutral application on performance	
Cooling panel 1. **1 x MVIC, VAS** & **BORG** (max 3 min) Max 1 min break 2. **Cooling** (20 min) Max 1 min break 3. **60** % **MVIC for 1 min** (2 min) Max 5 min break 4. **1 x MVIC, VAS** & **BORG** (max 5 min) immediately after the intervention 5. **1 x MVIC, VAS** & **BORG** (max 5 min) 24 hours after the intervention 6. **1 x MVIC, VAS** & **BORG** (max 5 min) 48 hours after the intervention 7. **1 x MVIC, VAS** & **BORG** (max 5 min) 72 hours after the intervention	**Thermoneutral Application Group** 1. **1 x MVIC, VAS** & **BORG** (max 3 min) Max 1 min break 2. **Thermoneutral application** (20 min) Max 1 min break 3. **60** % **MVIC for 1 min** (2 min) Max 5 min break 4. **1 x MVIC, VAS** & **BORG** (max 5 min) immediately after the intervention 5. **1 x MVIC, VAS** & **BORG** (max 5 min) 24 hours after the intervention 6. **1 x MVIC, VAS** & **BORG** (max 5 min) 48 hours after the intervention 7. **1 x MVIC, VAS** & **BORG** (max 5 min) 72 hours after the intervention

Sub-protocol	**Total time commitment**	**Visits**
A	Approx. 80 min	Once 60 min and 3 times for max 5 min
B	Approx. 80 min	Once 60 min and 3 times for max 5 min

9.2 Measuring outcomes

ICH: Specification of the efficacy parameters. Specification of safety parameters.

Sub-protocol	**Total time commitment**	**Measurement parameters**
A	Approx. 80 min (1x 60 min & 3 x 5 min)	Measurements: Jump height VAS & BORG
B	Approx. 80 min (1x 60 min & 3 x 5min)	Measurements: MVIC VAS & BORG

SUPSI

9.2.1 Measurement of primary outcomes

ICH: Methods and timing for assessing, recording, and analysing of efficacy & safety parameters.

The following data are collected during all tests described in the sub-protocols in chap. 9.1.

MVIC of the ventral thigh muscles

Muscle activity is measured using the sEMG active sensors wireless surface electromyogram system from PLUXwireless bodysignals S.A. {physioplux.com). Skin-friendly electrodes from Synmedig AG are used: Ambu® Blue Sensor N. MVIC is measured on the Cor 1 - ergometer chair [Nm] in 90° knee flexion.

The electrodes are placed according to the internationally recognized SENIAM guidelines (SENIAM project 2013).

Jump height (cm)

The jump height after the load is determined with a jump height measuring plate designed for this purpose (Just Jump Plate, Probotics Inc. , Huntsville, USA). The test subjects assume a standing position on the plate. The test subjects are allowed to start from the squatting position (countermovement jump) to measure the jump height. The arms must be placed at the hips during the entire movement (countermovement jump).

Subjective muscle soreness (VAS)

The subjects reported their subjective feeling of muscle soreness on a visual analogue scale (VAS). The scale will be scaled from "0" (no muscle soreness present) to "10" (greatest conceivable muscle soreness) in cm steps.

Subjective data on general exhaustion (BORG)

The subjects indicate their subjective general feeling of exhaustion on a visual analogue scale (VAS). The scale is from "O" (no general exhaustion present) to
.,10" (greatest conceivable general exhaustion) can be scaled in cm steps.

9.2.2 Measurement of secondary outcome

None available

9.2.3 Survey of further outcomes

None available

9.2.4 Collection of safety parameters

No corresponding parameters are collected.

SUPSI

9.2.4.1

9.2.4.2 *Adverse events*

9.2.4.3 *Laboratory parameters*

9.2.4.4 *Vital signs*

SUPSI

9.2.5 Collection of data in case of early termination of studies

For the participants, there is no disadvantage in the event of an (arbitrary) discontinuation of study participation and no further interventions are necessary for follow-up treatment.

However, if the study participation was discontinued due to an injury occurred during the study participation, this will be recorded and reported to the sponsor investigator (head of the research laboratory), study leader and supervisor of the study. The report is then sent to the business liability insurance of Thim van der Laan University College Physiotherapy in Landquart.

The following are recorded:

- Date and time of the incident
- Course of the incident
- Persons involved & witnesses Study
- ID (assigned by the CEC)
- Personal details of the person concerned (if not previously recorded)

SUPSI

9.3 Procedure at each visit of the test subjects

9.3.1 Single measurement

Sub-protocols A and B are multiple visits.

9.3.2 Multiple visits

(e.g. Visit 1, Baseline (Day e.g., 1): List all exams/tests, actions to be performed according to flow chart (9.1) including also e.g., Dispense of trial medication, Scheduling of next visit)

The sub-protocols A and B are multiple visits with a duration of about 80 minutes each, which takes place as follows:

1. Subject Information & Informed Consent

The subjects will be comprehensively informed about the process and possible risks before they are confirmed to participate in the study . You will also receive the information in written form and have the opportunity to ask questions. The declaration of consent explains to them what their rights and obligations are when participating and that they may withdraw from participation at any time without giving reasons , without incurring any disadvantages as a result. More detailed information can be found in Chapter 2.7.

2. Preparing the subject

If the subject is willing to participate, he or she is instructed to change in a separate room:

- Subproject A

  this sub-project does not require any further preparations.

- Subproject B

  First, the areas for the placement of the electrodes are marked with a skin-friendly pen (eyeliner) and, if necessary, individual areas of the EMG electrode placements are shaved (both were explained to the test subject beforehand according to a checklist). Then the electrodes are attached.

3. Measurements

The tests are carried out in the same way as in the corresponding sub-protocol and the data is collected.

4. Indemnification and Conclusion

After completion of the individual measurement, the test subject is asked to change again in a separate room. Afterwards, they are asked how they are feeling. The subject is asked to report back to the laboratory at intervals of 24 hours (up to a maximum of 72 hours) to carry out the follow-up measurement. After completion of the measurements, the test subject will be happy to receive the compensation to which he/she is entitled. Information in the advertisement:

Flat rate Str. 200 .- after completion of the 72-hour measurement.

SUPSI

Subproject A

In subproject A, the subjects return to the laboratory 24 hours, 48 hours and 72 hours after the intervention. Here, the maximum jump height during 3 attempts is determined again. Furthermore, the subjective muscle soreness and general states of exhaustion are recorded.

Subproject B

In subproject B , the subjects return to the laboratory 24 hours, 48 hours and 72 hours after the intervention. Here the MVIC is determined again. Furthermore, the subjective muscle soreness and general states of exhaustion are recorded.

SUPSI

10 SAFETY

Observational study Cat. ***A*** ClinV (Art. 20)

The test subjects are not given medication or tissue samples are taken. No invasive methods are used.

No further security aspects are expected.

10.1 Drug studies

10.1.1 Definition and assessment of (serious) adverse events and other safety related events

ICH: Procedures for eliciting reports of and for recording ... adverse event and intercurrent illnesses.

10.1.2 Reporting of serious adverse events (SAIE) and other safety related events

(KlinV Art. 37)
ICH: Procedures for ... reporting adverse event and intercurrent illnesses.

10.1.3 Follow up of (Serious) Adverse Events

(ICH/EG 6.8.4; SPIRIT #30)
ICH: The type and duration of the follow-up of subjects after adverse events.

10.2 Medical Device Category C studies

10.2.1 Definition and Assessment of (Serious) Adverse Events and other safety related events

(MD: ISO 14155)

10.2.2 Reporting of (Serious) Adverse Events and other safety related events

10.2.3 Follow up of (Serious) Adverse Events

(SPIRIT #30)

10.3 Medical Device Category A studies

This study is a category A clinical observational study in accordance with the Swiss Ordinance on Clinical Trials in Human Research (ClinO: Art. 20).

It is a study with 20 or fewer subjects in each experimental group. The study also focuses on basic findings. The monitoring for quality assurance is carried out by Prof. Dr. Peter Clarys.

SUPSI

10.3.1 Definition and Assessment of safety related events

If a circulatory problem leads to an injury, this is assessed as a safety-relevant event and must be investigated in more detail before continuing the study.

The following are recorded:

Date and time of the incident

Incident Procedure

Persons involved & witnesses Study

ID (assigned by the CEC)

Personal details of the person concerned (if not previously recorded)

10.3.2 Reporting of Safety related events

A safety-related event is logged and immediately reported to the sponsor investigator (head of the research laboratory), study leader and supervisor of the study.

The CEC will be informed as soon as possible and appropriate changes to the protocol of the study will be requested. Further explanations on this can be found in chap. 2.10. Safety and protective measures must be reimbursed to the responsible CEC within 2 days .

The report continues to the public liability insurance of Thim van der Laan University College Physiotherapy in Landquart.

SUPSI

11 STATISTICAL METHODS

11.1 Hypotheses & Factors

Subprojects A and B

Subproject A

Jump height (cm)

HO The jump height is not increased by the post-activity cooling compared to thermoneuapplication during a 72-hour recovery period.

H1 The Jump height becomes through the Post-activity cooling increased opposite thermoneutral application during a 72-hour recovery period.

Subjective muscle soreness (VAS)

HO The subjective muscle soreness information is not reduced by post-activity cooling compared to thermoneutral application for 72 hours.

H1 The subjective muscle soreness information is lower due to post-activity cooling compared to thermoneutral application for 72 hours.

Subjective data on general exhaustion (BORG)

HO The subjective information on general fatigue is not reduced by post-activity cooling compared to thermoneutral application for 72 hours.

H1 The subjective data on general fatigue are lower with post-activity cooling compared to thermoneutral application for 72 hours.

Subproject B

MVIC (mV)

HO MVIC performance is measured by the pre-activity cownot increased compared to thermoneutral application for 72 hours

H1 The MVIC Efficiency is increased by pre-activity cooling compared to thermoneutral application for 72 hours

Subjective muscle soreness (VAS)

HO The subjective muscle soreness data is not reduced by the pre-activity cooling compared to thermoneutral application for 72 hours.

H1 The subjective muscle soreness is lower due to the pre-activity cooling compared to thermoneutral application for 72 hours.

SUPSI

Subjective data on general exhaustion (BORG)

HO The subjective information on general fatigue is not reduced by pre-activity cooling compared to thermoneutral application for 72 hours.

H1 The subjective information on general fatigue is lower due to pre-activity cooling compared to thermoneutral application for 72 hours.

A 2-factor analysis will be carried out in both projects. Factor 1:

Intervention (cooling vs. Thermoneutral application) Factor 2:

Time (0, 24 hours, 48 hours, 72 hours)

11.2 Sample Size

The planned number of 20 subjects (project A), or 20 subjects (Project B) correspond to the group sizes of the comparable cooling studies (cf. (Ascensao et al. 2011, Bailey et al. 2007, Crystal et al. 2013, Rowsell et al. 2009, De Pauw et al. 2014, Guilhem et al. 2013, Jakeman et al. 2009, Howatson et al. 2009, Montgomery et al. 2008, Rupp et al. 2012, Yanagisawa et al. 2003, e.g.).

11.3 Criteria for the statistical use of data in the case of incomplete data sets

Only data from subjects who have completed the entire protocol will be included in the study. This ensures that all data has been collected under the same conditions and thus remains homogeneous and comparable.

11.4 Scheduled analyses

11.4.1 Datasets and Data Populations

Only complete data sets will be included in the study. Data that contains only part of the protocol is not included in the analysis. No new subject is being sought to replace the missing data.

11.4.2 Primary Analysis

The statistical data analysis is carried out by Prof. Dr. Jean-Pierre Baeyens after completion of the data collection of the respective sub-protocol.

Repeated measures analysis of variance (MANOVA)

2 factors:

Protocol A

Intervention (cooling vs. Thermoneutral application) Time

(0, 24 hours, 48 hours, 72 hours)

SUPSI

Protocol B

Intervention (cooling vs. Thermoneutral application) Time

(0, 24 hours, 48 hours, 72 hours)

The significance level is set at $P < 0.05$, and the statistical data analysis is carried out with SPSS 22.

11.4.3 Secondary Analysis

Non-existent.

11.4.4 Interim analyses

The data is processed after the end of the respective subprotocol. Thus, the data analysis for the entire study takes place in stages.No additional interim analyses are planned.

11.4.5 Security analyses

11.4.6 Deviations

Deviations from the planned statistical analysis are recorded and justified and reported to the CEC in the annual report.

11.5 Drop-outs and missing data

If a test subject does not complete the experiment, any data that has already been collected is retained, but is not further incorporated into the final data analysis.

SUPSI

12 QUALITY ASSURANCE AND CONTROL

12.1 Data Archiving

12.1.1 Forms

Data protection and confidentiality are guaranteed and no personal data is presented or published. The signed declaration of consent, as well as the completed questionnaire with the other personal details, are kept in the original as a study document in a locked filing cabinet.

Direct access to the personal data is only allowed to authorized persons of the CEC.

12.1.2 Specification of forms

The data collected in writing is recorded in the form listed in the enclosures and is marked as study data. They are therefore subject to data protection and are regulated in accordance with the provisions set out in chap. 12.1.1.

12.1.3 Archiving of data

The data collected will be kept for 1O years. Data protection will continue to be guaranteed.

12.2 Data management

12.2.1 Data Management System

The raw data of muscle activity is processed with the software of PLUXwireless biosignals S.A. The jump height is determined with a jump height measuring plate designed for this purpose (Just Jump Plate, Probotics Inc., Huntsville, USA). Excel is used for further data analysis. For the be used.

12.2.2 Data security and backup

The digital data will be anonymized and treated confidentially, and access to the personal data will not be allowed to third parties. The digital data collected is stored solely on the institute's own computers and is not passed on to any external persons or transferred to other computers. Access to the computers of the research laboratory at Thim van der Laan University College Physiotherapy is only available to the principal investigator and the investigators (listed in Chapter 1), as well as persons authorised by the CEC.

12.2.3 Analysis and archiving

After collection, the digital data is anonymized and stored on another external hard drive for further backup. This is stored in a locked filing cabinet to ensure data protection.

SUPSI

12.2.4 Validation of electronic data

Immediately after data collection , the quality of the data is checked and verified with the help of the visual representation.

12.3 Monitoring

The data collected can be viewed by authorised persons at any time (see section 12.2.2)

12.4 Audits and inspections

A report on the current status of the study is sent monthly to the study supervisor (Prof. Dr. Peter Clarys). This also includes demonstrating and presenting results and statistical analyses.

An annual report on the course of the study will be submitted to the CEC. Authorized persons of the CEC can view the data forms and the digital data on the institute's own computers at any time.

Data protection is guaranteed at all times.

12.5 Confidentiality and data protection

The examiners ensure that the privacy of the participant is maintained. In particular, data protection and confidentiality are guaranteed and no personal data is presented or published. The signed declaration of consent, as well as the completed questionnaire with the other personal details, are kept in the original as a study document in a locked filing cabinet.

The digital data will be anonymized and treated confidentially, and access to the personal data will not be allowed to third parties. The digital data collected is stored solely on the institute's own computers and is not passed on to any external persons or transferred to other computers.

Direct access to the personal data is only allowed to authorized persons of the CEC.

12.6 Storage of biological material and related health Data

SUPSI

13 PUBLICATION AND DISCLOSURE OF DATA

The examiners ensure that the privacy of the participant is maintained. In particular, the data protection and confidentiality of the data are guaranteed and no personal data is presented or published nor passed on to outsiders and unauthorized persons.

SUPSI

14 FINANCING AND SUPPORT

14.1 Financing
(ClinO, Art. 25i)

The study is funded by the University of Applied Sciences and Arts Southern Switzerland, Physiotherapy Graubünden. The auditors are employed by this institute and are remunerated for their work on this study in accordance with their employment contracts.

14.2 Further support

The Zamar Therapy thermotherapy device is kindly provided by the manufacturer during the entire duration of study. There is no written agreement.

Zamar Medical d.o.o. Zamar Medical

Mate Vlasica 26
52440 Porec,

Croatia

T +385 52 496 111

info@zamarmedical.com
www.zamarmedical.com

15 INSURANCE

(ClinV Art 12, 13; I/ES 6.14, AGEK 10.3; SPIRIT#30)
I:..... and insurance if not addressed in a separate agreement.

There is a public liability insurance for the Thim van der Laan Univesity College Physiotherapy in Landquart, where the study is being conducted.

SUPSI

16 REFERENCES

(ME/EG6.2.7)
ICH: References to literature and data that are relevant to the trial, and that provide background for the trial.

Ascensao, A., et al., *Effects of cold water immersion on the recovery of physical performance and muscle damage following a one-off soccer match.* J Sports Sei, 2011. **29(3):** p. 217-25.

Bacon, N.T., et al., *Effect of two recovery methods on repeated closed-handed and open-handed weight-assisted pull-ups.* J Strength Cond Res, 2012. **26(5):** p. 1348-52.

Bailey, D.M., et al., *Influence of cold-water immersion on indices of muscle damage following prolonged intermittent shuttle running.* J Sports Sei, 2007. **25(**11): p. 1163-70.

Banfi, G., et al., *Whole-body cryotherapy in athletes.* Sports Med, 2010. **40(6):** p. Letter 509-17.

Barnett, A., *Using recovery modalities between training* sessions *in elite athletes: does it help?* Sports Med, 2006. **36(9):** p. 781-96.

Bastos, F.N., et al., *Effects of cold water immersion and active recovery on post-exercise heart rate variability.* Int J Sports Med, 2012. 33(11): p. 873-9.

Bleakley, C., et al., *Cold-water immersion (cryotherapy) for preventing and treating muscle* soreness *after exercise.* Cochrane Database Syst Rev, 2012. **2:** p. CD008262.

Bogerd, N., Perret, C., Bogerd C.P., Rossi, R.M., Daanen, A.M., *The effect of pre-cooling on cooling efficiency and exercise performance.* J Sports sei. 2010 May; 28(7):771-9

Broatch, J.R., A. Petersen, and D.J. Bishop, *Postexercise cold water immersion benefits are not greater than the placebo effect.* Med Sei Sports Exerc, 2014. 46(11): p. 2139-47.

Crowe, M.J., D. O'Connor, and D. Rudd, *Cold water recovery reduces anaerobic performance.* Int J Sports Med, 2007. 28(12): p. 994-8.

Crystal, N.J., et al., *Effect of cryotherapy on musc/e recovery and inflammation fol/owing* a *baut of damaging exercise.* Eur J Appl Physiol, 2013. **113(1O):** p. 2577-86.

Delextrat, A., et al., *Effects of sports* massage *and intermittent co/d-water immersion on recovery from matches by basketba/1 players.* J Sports Sei, 2013. **31**(1): p. 11-9.

De Pauw, K., et al., *Effect of recovery inteNentions on cycling performance and pacing strategy in the heat.* Int J Sports Physiol Perform, 2014. **9(2):** p. 240-8.

SUPSI

Elias, G.P., et al., *Effects of water immersion on posttraining recovery in Australian footballers.* Int J Sports Physiol Perform, 2012. **7(4):** pp. 357-66.

Eston, R. and D. Peters, *Effects of cold water immersion on the symptoms of exercise-induced muscle damage.* J Sports Sei, 1999. **17(3):** p. 231-8.

Goodall, S. and G. Howatson, *The effects of murtiple cold water immersions on indices of muscle damage.* J Sports Sei Med, 2008. **7(2):** p. 235-41.

Grahn, D.A., J.L. Dillon, and H.C. Heller, *Heat lass through the glabrous skin surfaces of heavily insulated, heat-stressed individuals.* J Biomeeh Eng, 2009. **131(7):** p. 071005.

Gregson, W., et al., *Inf/uence of cold water immersion on limb and cutaneous blood flow at rest.* Am J Sports Med, 2011. **39(6):** p. 1316-23.

Guilhem, G., et al., *Effects of air-pulsed cryotherapy on neuromuscular recovery subsequent to exercise-induced muscle damage.* Am J Sports Med, 2013. **41(8):** p. 1942-51.

Hausswirth, C., et al., *Effects of whole-body cryotherapy vs. far-infrared vs. passive modalities on recovery from exercise-induced muscle damage in highly-trained runners.* PLoS One, 2011. 6(12): p. e27749.

Heyman, E., et al., *Effects of four recovery methods on repeated maximal rock climbing performance.* Med Sei Sports Exere, 2009. **41(6):** p. 1303-10.

Howatson, G., S. Goodall, and K.A. van Someren, *The influence of cold water immersions on adaptation following a single bout of damaging exercise.* Eur J Appl Physiol, 2009. **105(4):** p. 615-21.

Ingram, J., et al., *Effect of water immersion methods on post-exercise recovery from simulated team sport exercise.* J Sei Med Sport, 2009. **12(3):** p. 417-21.

Jakeman, J.R., R. Macrae, and R. Eston, *A single 10-min bout of cold-water immersion therapy after strenuous plyometric exercise has no beneficial effect on recovery from the symptoms of exercise induced muscle damage.* Ergonomics, 2009. **52(4):** p. 456-60.

King, **M.** and R. Duffield, *The effects of recovery interventions on consecutive days of intermittent sprint exercise.* J Strength Cond Res, 2009. **23(6):** p. 1795-802.

Kuligowski, L.A., et al., *Effect of whirlpool therapy on the signs and symptoms of delayed-onset muscle soreness.* J Athl Train, 1998. **33(3):** p. 222-8.

Leal Junior, E.C., et al., *Comparison between cold water immersion therapy (CWIT) and light emitting diode therapy (LEDT) in short-term ske/etal muscle recovery after high-intensity exercise in athletes-preliminary results.* Lasers Med Sei, 2011. **26(4):** pp. 493-501.

Leeder, J., et al., *Cold water immersion and recovery from strenuous exercise:* a *meta-analysis.* Br J Sports Med, 2012. **46(4):** p. 233-40.

SUPSI

Myrer, W.J., et al., *Muscle Temperature Is Affected by Overlying Adipose When Cryotherapy Is Administered.* J Athl Train, 2001. **36(1**): p. 32-36.

Paddon-Jones, D.J. and B.M. Quigley, *Effect of cryotherapy on muscle soreness and strength folfowing eccentric exercise.* Int J Sports Med, 1997" **18(8):** p. 588-93.

Pointen, M. and R. Duffield, *Cold water immersion recovery after simulated collision spart exercise.* Med Sei Sports Exerc, 2012. **44(2):** p. 206-16.

Pointon, **M.,** et al., *Cold water immersion recovery following intermittent-sprint exercise in the heat.* Eur J Appl Physiol, 2012. **112(7):** p. 2483-94.

Pointon, M., et al., *Cold application for neuromuscular recovery following intense lower-body exercise.* Eur **J** Appl Physiol, 2011. **111(12):** p. 2977-86.

Pournot, H., et al., *Short term effects of various water immersions on recovery from exhaustive intermittent exercise.* Eur J Appl Physiol, 2011. **111** (7): p. 1287-95.

Rowsell, G.J., et al., *Effects of cofd-water immersion on physicaf performance between successive Matches in High-Performance Junior Male Soccer Players.* J Sports Sei, 2009. **27(6):** p. 565-73.

Rupp, K.A., et al., *The effect of cold water immersion on 48-hour pertormance testing in collegiate soccer players.* J Strength Cond Res, 2012. **26(8):** p. 2043-50.

Sellwood, K.L., et al., */ce-water immersion and delayed-onset muscle soreness: a randomised controlled trial.* Br J Sports Med, 2007. **41(6):** p. 392-7.

Stanley, J., J.M. Peake, and **M.** Buchheit, *Consecutive days of cold water immersion: effects on cycling performance and heart rate variability.* Eur J Appl Physiol, 2013. **113(2):** p. 371-84.

Tseng, C.Y., et al., *Topical cooling (icing) delays recovery from eccentric exercise-induced muscle damage.* J Strength Cond Res, 2013. **27(5):** p. 1354-61.

Tucker, T.J., et al., *Effect of local cold application on glycogen recovery.* J Sports Med Phys Fitness, 2012. **52(2):** p. 158-64

Vaile, J., et al., *Effect of hydrotherapy on the signs and symptoms of de/ayed onset muscle soreness.* Eur J Appl Physiol, 2008. **102(4):** p. 447-55.

Wegmann, **M.,** Faude, 0., Poppedieck, W., Hecksteden, A., Fröhlich, **M.** Meyer, T. *Pre-cooling and* Sports *Performance - A Meta-Analytical Review.* Sports Med. 2012 Jul 1;42(7):545-64.

White, G.E. and G.D. Wells, *Co/d-water immersion and other forms of cryotherapy: physiological changes potentially affecting recovery from high-intensity exercise.* Extrem Physiol Med, 2013. **2(1):** p.

SUPSI

26.

Yanagisawa, 0., et al., *Evaluations of cooling exercised muscle with MR imaging and 31P MR spectroscopy.* Med Sei Sports Exerc, 2003. **35(9):** p. 1517-23.

-*COO/ING and Sports Performance* - a *meta-analytical review.* Sports Med. 2012 Jul 1;42(7):545-64.

17 APPENDIX

ICH: (NOTE: Since the protocol and the clinical trial/study report are closely related, further relevant information can be found in the ICH Guideline for Structure and Content of Clinical Study Reports.)

1. Subject Information, Informed Consent & Demographic Data (Questionnaire)
    a. Sub-protocols A
    b. Sub-protocols B

2. Subject recruitment (advertisements, checklist for initial contact)

3. Data collection form for subject data
    a. Sub-protocols A
    b. Sub-protocols B

4. Data Collection Form Measurements

5. Protocol Synopsis

6. Employee

7. CVs of all examiners

8. CE Certification Zamar Therapy