Originally in German, translated into English

Clinical Investigation Plan (CIP)

EFFECTS OF IMMEDIATE AND DELAYED REPEATED COLD EXPOSURE AFTER PHYSICAL EXERTION: A RANDOMISED CONTROLLED TRIAL

Type of investigation:	Clinical investigation concerning medical devices (MD).
Categorisation:	Category according to Art 6 ClinO-MD A1
Registration:	NCT06813690
Identifier:	2024-D0116
Principal Investigator and Sponsor, or Sponsor-Investigator:	Dr. Ron Clijsen University of Applied Sciences of Southern Switzerland SUPSI Rehabilitation and Exercise Science Laboratory (RES Lab) Weststrasse 8 7302 Landquart +41 81 300 01 75 Thim van der Laan jr Thim van der Laan AG Weststrasse 8 7302 Landquart +41 81 300 01 70
Sponsor representative (if the Sponsor is not located in Switzerland)	-
Medical Device:	Axanova Cold Hot Pearls Compresses Basic UDI-DI (GMN): 764011364_PG_04_RF
CIP Version and Date:	Version 06 (03.03.2025)

CONFIDENTIALITY STATEMENT

Signature Page

ID number of the investigation:	2024-D0116
Title:	Effects of immediate and delayed repeated cold exposure after physical exertion: a randomised controlled trial

The Sponsor, the Principal Investigator and the Statistician have approved the CIP version [6 (dated 03.03.2025),], and confirm hereby to conduct the investigation according to the CIP, the current version of the World Medical Association Declaration of Helsinki, ISO14155 norm [2024], ICH-GCP as far as applicable, and the local legally applicable requirements.
The Investigator has received the ICF and consider it appropriate for use.

Sponsor:

Name: *Thim van der Laan jr.*

Place/Date	Signature

Principal Investigator:

Name: *Dr. Ron Clijsen*

Place/Date	Signature

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SYNOPSIS

Sponsor / Sponsor-Investigator	Thim van der Laan AG Thim van der Laan jr. Weststrasse 8, CH-7302 Landquart University of Applied Sciences and Arts Southern Switzerland (SUPSI) Dr. Ron Clijsen Weststrasse 8, CH-7302 Landquart
Title	Effects of immediate and delayed repeated cold exposure after physical exertion: a randomised controlled trial
Short Title / Invesigation ID	Local cooling and recovery
Protocol Version and Date	Version *06 of date (03/03/2025)*
Registration	ClinicalTrials.gov PRS
Category and Rationale	Category according to Art 6 ClinO-MD A1. This study investigates whether repeated, local cold applications have a positive effect on recovery after a muscle soreness protocol. The test subjects are not given medication, nor are tissue samples taken. Minimally invasive techniques are used (venous blood sampling).
Name of the MD, Unique Device Identification (UDI), name of the manufacturer	Axanova Cold Hot Pearls Compresses Basic UDI-DI (GMN): 764011364_PG_04_RF Axanova AG
Stage of development:	Post-market stage
Background and Rationale	Sporting activity is characterized by factors of the type, duration and intensity of exertion. Depending on the extent of these factors and the associated recovery time, damage to the muscles, inflammation and fatigue in the nervous system occur. Energy substrate degradation and local swelling also occur. Therefore, rapid regeneration after intensive sports has become all the more important. According to the meta-analysis by Bleakley et al. 2012, cold is considered to be one of the most effective recovery methods after sporting activity to delay delayed onset muscle soreness (DOMS). The DOMS are microscopic tears in muscle tissue called exercise-induced muscle damage, which can lead to delayed onset muscle soreness. DOMS usually peak between 24 and 48 hours – sometimes up to 72 hours – after exercise and are characterized by muscle shortening, increased passive stiffness, swelling, decrease in strength and power, localized muscle soreness, and altered proprioception. The physiological background of cryotherapy is based on the removal of body heat by reducing tissue temperature. This is reflected in a reduced perception of muscle pain, so that the body feels more "awake" after training and causes less fatigue. In addition, due to the cold, the body lowers the heart rate and cardiac output, inducing vasoconstriction. The results are smaller vessel diameters, a reduced incidence of oedema and an improved oxygen supply to the cells. In order to maintain the body's core temperature, the central metabolism also increases, which promotes the transport of waste products. All these effects, in composition, could reduce the inflammation caused by exercise by reducing the death or damage of hypoxic cells and minimizing damage to secondary tissues by reducing the infiltration of leukocytes and monocytes. (Bleakley et al. 2012, Hohenauer E et al. 2015, Hubbard et al. 2004, Ostrowski et al 2018). Postoperative cryotherapy utilizing ice packs, cooling, or continuous cryotherapy devices reduced visual analogue scale pain scores and analgesic consumption in approximately half of the prior art research studies comparing these outcomes to the control group (no cryotherapy) (11 [44%] out of 25 studies on pain and 11 [48%] out of 23 studies on opioids). However, an effect in increasing range of motion (3 [19 %] out of 16) or reducing swelling (2 [22 %] out of 9) was reported less frequently. (Kunkle et al. 2021). The study induced here is intended to provide new data, especially in the reduction of swelling and inflammation values, and to confirm the reduction of pain by cold application of the Axanova Cold Hot Pearls Maxi Pack.

Risk / Benefit Assessment	In this examination, the risk can be classified as minimal. The product is applied as recommended by the manufacturer. The muscle soreness protocol is a protocol that has already been described in the scientific literature.
Objective(s)	The aim of this study is to investigate how local, repeated cold applications affect the recovery phase according to a muscle soreness protocol. Recovery is assessed at 24-hour intervals (up to 72 hours according to the muscle soreness protocol).
Endpoint(s)	Primary endpoints: • Sore muscles (VAS 0 – 10 cm) • Inflammation levels (blood sinking velocity, C-reactive protein, creatine kinase) • Muscle swelling (in cm using ultrasound) • Maximum Voluntary Isometric Thigh Contraction (in kg) Secondary endpoints • Surface temperature of the skin (in °C) • Thermal Perception
Investigation Design	Randomized, controlled trial
Statistical Considerations	Repeated measure ANOVA, n=45 subjects
Inclusion- / Exclusion Criteria	Inclusion Criteria • Young, healthy adults between the ages of 18 and 30 • No surgical interventions on the musculoskeletal system in the trunk area and lower extremities Exclusion criteria • Current pain conditions: Subjects with acute pain conditions. • Current inflammatory conditions: Presence of inflammation or known inflammatory diseases. • Medication intake (excl. anticonception drugs): Subjects taking medication, with the exception of anticonception drugs. • Pregnant subjects: Women who are pregnant or breastfeeding. • Competitive athletes: Test subjects who practice competitive sports. • Children/adolescents: Subjects under 18 years of age or over 30 years of age. • Non-intact skin conditions (e.g. psoriasis): Skin conditions, such as psoriasis or other non-intact skin conditions. • Known circulatory disorders: Presence of known circulatory disorders. • Cold allergy (Raynaud's syndrome): Allergy to cold or the presence of Raynaud's syndrome. • Previous operations on the upper body or legs: Operations in the areas of the body examined. • Disturbed perception in the thigh: Disturbances in the sensation of temperature or touch in the thigh area. • Diagnosed diseases: Presence of diagnosed diseases that could affect participation in the examination. • Smoking: When the subject smokes.
Number of Participants with Rationale	The study is planned with 3 study arms. The average initial VAS score after pain induction varies depending on the examination and is an approximation of an initial value of 4.73 ± 2.39. A reduction in the VAS score after cold application was assumed to be an average of 47%. After taking into account the dropout rate of 10% and splitting into 3 randomized groups, a patient count of 15 subjects per study arm is considered statistically appropriate. The results were determined using the PASS 2023 version 23.0.1 software.
Intervention	The product "Cold Hot Pearls Maxi Pack" of the company Axanova AG is used for testing. This product has a gel bead filling that can be used for cold and heat applications. According to the manufacturer, the cold compresses must be stored in a freezer for at least 1 hour before they can be used. The cold pack can then be placed on the affected area on the fleece side, but for a maximum of 20 minutes in the same place.

	In our study, there are 2 cooling groups: The first group starts cooling immediately after the muscle soreness protocol. The second group starts cooling 24 hours after the muscle soreness protocol.
Control Intervention	The control group will not receive an intervention according to the muscle soreness protocol.
Investigation procedures	**Subject recruitment** The test subjects are recruited at the University of Physiotherapy THIM in Landquart and the University of Applied Sciences and Arts Southern Switzerland in Landquart. The subject information and the declaration of consent are given to the test subjects. In the subject information there is a checklist that the test persons must fill out in order to check the inclusion and exclusion criteria. If the subjects agree with the information, have completed the checklist and signed the consent, they will be summoned to make an appointment for the measurement. **Investigation Protocol** As soon as the signed consent of the test person has been received and an appointment has been made, the following procedure is followed. The anthropometric data of the subjects will be collected and group randomization will be carried out. Afterwards, the baseline measurements are carried out and the measurement of the skin temperature is started. Then the muscle soreness protocol (6 x 12 to 15 one-legged knee extensor protocol with a 2-minute break between sets) is completed. Cooling group A then receives the first 20-minute cooling of the thigh group. Cooling group B and the control group leave the laboratory 20 minutes after the muscle soreness protocol. The measurement of skin temperature is stopped within all groups 20 minutes after the muscle soreness protocol. Cooling group B begins cooling the thigh muscles 24 hours after the muscle soreness protocol. Both cooling groups perform cooling of the thigh muscles 3 x / day for the duration of the data collection (up to 72 hours according to the muscle soreness protocol). At intervals of 24, 48 and 72 hours, the primary endpoints of the study are collected again. These include: muscle soreness, inflammation levels, muscle swelling, maximum voluntary isometric contraction of the thigh muscles. **Measurement of sore muscles** The level of ventrally felt muscle soreness symptoms is measured using a VAS scale (0 – 10 cm). The test subjects rate the perceived muscle soreness in both ventral thighs in a 90° knee flexion position. The measurement takes place during the baseline measurement and, 24 hours, 48 hours and 72 hours after the intervention. **Inflammation levels** The inflammation values (blood sedimentation rate, C-reactive protein, creatine kinase) are determined by venous blood sampling. The measurement takes place during the baseline measurement and, 24 hours, 48 hours and 72 hours after the intervention. **Muscle swelling** The measurement of muscle swelling of the quadriceps femoris muscle is carried out by ultrasound measurement. The measurement is carried out in a resting position and a cross-section of the muscle is recorded. The measurement takes place during the baseline measurement and, 24 hours, 48 hours and 72 hours after the intervention. **Measurement of maximum voluntary isometric thigh muscle contraction (MVIC)** MVIC is performed on an ergometer chair for biomechanical measurements and is given in kg. The measurement takes place during the baseline measurement and, 24 hours, 48 hours and 72 hours after the intervention. **Surface temperature** The surface temperature is measured using the iButton system. These sensors are glued to the skin for the duration of the measurement. The button sensors store the current temperature information and can then be read out via a computer. These measurements are started during the baseline survey, for the duration of the muscle soreness protocol, and up to 20 minutes after the muscle soreness protocol. **Detection of thermal perception** The thermal perception of the participants is recorded immediately after exposure to cold using the standardized Thermal Perception Scale (-4 to +4). This scale ranges from -4 ("extremely cold") to +4 ("extremely warm"), with 0 being considered neutral. The evaluation is carried out

	as a subjective self-assessment in order to document the individual temperature perception. Participants will be informed about the scale in advance. Since this is a purely subjective assessment, there is no risk for the participants.
Investigation Duration and Schedule	Planned start of measurements: 01/2025 Planned end of measurements: 07/2025
Investigator(s)	Dr. Ron Clijsen Dr. Erich Hohenauer Vanessa Wellauer Contact / Director of Studies Clijsen Ron, PhD University of Applied Sciences Southern Switzerland Physiotherapy Graubünden Weststrasse 8, 7302 Landquart +41 81 300 01 75 ron.clijsen@supsi.ch
Investigation Center(s)	University of Applied Sciences Southern Switzerland Physiotherapy Graubünden Rehabilitation Research Laboratory RESlab SUPSI Landquart, Weststrasse 8 7302 Landquart
Data privacy	The data are only accessible to the head of the study (Ron Clijsen) and persons of the KEK (in case of any quality controls on site). The data is coded numerically (001, 002, 003, etc.). Only the study leader can solve the code. All data is stored in the laboratory of the University of Applied Sciences and Arts Southern Switzerland in Landquart.
Ethical consideration	Athletes could benefit from these results as they receive information on how they could increase their athletic performance or recovery by applying cold after exercise. The methodological approach is based on the current scientific literature. The risk can be considered minimal because the product is used as prescribed.
Compliance statement	This investigation will be conducted in full compliance with the CIP, the current version of the Declaration of Helsinki, ISO 14155 [2024], ICH-GCP (as far as applicable) as well as all national legal and regulatory requirements.

ABBREVATIONS

AE	*Adverse Event*
AIP	*Azure Information Protection*
ASR	*Annual Safety Repot*
BASEC	*Business Administration System for Ethical Committees*
ClinO	*Ordinance on Clinical Trials in Human Research (in German: KlinV, in French: OClin, in Italian: OSRUm)*
CRF	*Case Report Form*
CTCAE	*Common Terminology Criteria for Adverse Events*
CATHEDRAL	*Delayed Onset Muscle Soreness*
eCRF	*electronic Case Report Form*
FADP	*Federal Act on Data Protection (in German: DSG, in French: LPD, in Italian: LPD)*
FOPH	*Federal Office of Public Health*
GCP	*Good Clinical Practice*
HRA	*Human Research Act (in German: HFG, in French: LRH, in Italian: LRUm)*
I	*International Conference on Harmonisation*
ISO	*International Organisation for Standardisation*
MDR	*Medical Device Regulation*
MVIC	*Maximum Voluntary Isometric Contraction*
SAE	*Serious Adverse Event*
SDV	*Source Data Verification*
SSL	*Secure Sockets Layer*
SUE	*Serious Unexpected Events*
TLS	*Transport Layer Security*
UAE	*Unanticipated Adverse Event*

1 BACKGROUND AND RATIONALE

Sporting activity is characterized by factors of the type, duration and intensity of exertion. Depending on the extent of these factors and the associated recovery time, damage to the muscles, signs of inflammation and fatigue in the nervous system occur, as well as energy substrate degradation and local swelling. Therefore, rapid regeneration after intensive sports has become all the more important. According to the meta-analysis by Bleakley et al. 2012, cold is considered one of the most effective recovery methods after sporting activity to delay delayed onset muscle soreness (DOMS). The DOMS are microscopic tears in muscle tissue called exercise-induced muscle damage, which can lead to delayed onset muscle soreness. DOMS usually peak between 24 and 48 hours – sometimes up to 72 hours – after exercise and are characterized by muscle shortening, increased passive stiffness, swelling, decrease in strength and power, localized muscle soreness, and altered proprioception.
The physiological background of cryotherapy is based on the removal of body heat by reducing tissue temperature. This is reflected in a reduced perception of muscle pain, so that the body feels more "awake" after training and causes less fatigue. In addition, due to the cold, the body lowers the heart rate and cardiac output, inducing vasoconstriction. The results are smaller vessel diameters, a reduced incidence of oedema and an improved oxygen supply to the cells. In order to maintain the body's core temperature, the central metabolism also increases, which promotes the transport of waste products. All these effects, in composition, could reduce the inflammation caused by exercise by reducing the death or damage of hypoxic cells and minimizing damage to secondary tissues by reducing the infiltration of leukocytes and monocytes. (Bleakley et al. 2012, Hohenauer E et al. 2015, Hubbard et al. 2004, Ostrowski et al 2018). Postoperative cryotherapy using ice packs, cooling, or continuous cryotherapy devices reduced pain scores on the visual analogue scale and analgesic consumption in about half of the prior art research studies comparing these outcomes to the control group (no cryotherapy) (11 [44%] of 25 studies on pain and 11 [48%] of 23 studies on opioids). However, an effect in increasing range of motion (3 [19 %] out of 16) or reducing swelling (2 [22 %] out of 9) was reported less frequently. (Kunkle et al. 2021). The study induced here is intended to provide new data, especially in the reduction of swelling and inflammation values, and to confirm the reduction of pain by cold application of the Axanova Cold Hot Pearls Maxi Pack.

2 INVESTIGATION OBJECTIVES AND DESIGN

2.1 Hypothesis and primary objective

The aim of this study is to investigate whether repeated, local cold applications have a positive impact on recovery compared to no intervention. Furthermore, it will be investigated whether cooling, which has been carried out immediately after the exertion, has a more positive influence on the recovery compared to refrigeration that has started 24 hours after the exertion. The ability to recover is determined on the basis of the following parameters: muscle soreness, inflammation levels, muscle swelling and maximum voluntary isometric contraction of the thigh muscles.

- H0A: There is no significant difference between repeated cold applications and the control intervention in terms of recovery capacity.
- H1A: There is a significant difference between repeated cold applications and the control intervention in terms of recovery capacity.

- H0A: There is no significant difference between immediate cold applications and delayed cold applications in terms of recovery.

H1A: There is a significant difference between immediate cold applications and delayed cold applications in terms of recovery.

2.2 Primary and secondary endpoints

Primary endpoints

Muscle soreness

A visual analogue scale (VAS) is used for the quantitative assessment of muscle soreness. The muscle soreness of the test subjects is measured on both ventral thighs in 90° knee flexion within 2 seconds. Participants perform a squat at a 90-degree angle and use a hand-held glide scale to choose a value between 0 and 10 cm, with 0 representing no discomfort and 10 representing maximum discomfort. This scale is designed without visible numerical indicators to minimize repetition bias. Measurements are taken during baseline and 24, 48, and 72 hours after the intervention. The examination staff records the selected value in centimetres to document the level of the muscle soreness symptoms felt ventrally.

Inflammation levels

The general inflammation values are determined on the basis of 3 parameters. These are the blood sinking rate, the C-reactive protein and the creatine kinase. These parameters are determined by means of a venous blood draw.

Muscle swelling

Swelling of the ventral thigh muscles is performed using ultrasound diagnostics. The test subjects are in a lying position. Subsequently, a cross-sectional image of the thigh muscles is created and the distance from the femur to the outer boundary of the muscles is measured.

Maximum voluntary isometric muscle contraction

This parameter is measured using an ergometer chair in 90° hip flexion. The test subjects have to try to stretch their thigh as much as possible for 3 seconds. The maximum deflection is used to determine the maximum voluntary isometric muscle contraction.

Secondary endpoints

Superficial skin temperature

The skin temperature of the thighs is measured using the iButton system. The sensors are glued to the skin and continuously measure the temperature of the skin. The data from the sensors is then read out on a computer. This measurement is started during the baseline survey, for the duration of the muscle soreness protocol, and up to 20 minutes after the muscle soreness protocol.

Thermal Perception

The thermal perception of the participants is recorded immediately after exposure to cold using the standardized Thermal Perception Scale (-4 to +4). This scale ranges from -4 ("extremely cold") to +4 ("extremely warm"), with 0 being considered neutral. The evaluation is carried out as a subjective self-assessment in order to document the individual temperature perception. Participants will be informed about the scale in advance. Since this is a purely subjective assessment, there is no risk for the participants.

2.3 Investigation design

This study is a single-center, randomized, controlled trial with 3 arms. After the subjects have been admitted to the examination, they are divided into one of the 3 groups.
Group A: immediate cooling according to the muscle soreness protocol
Group B: delayed cooling according to the muscle soreness protocol (24 hours delayed)
Group C: Control group
This examination is a clinical trial of risk category A.

Group A: immediate cooling

In this group, cooling starts immediately after the muscle soreness protocol. Cooling is carried out 3 x /day both on the day of the muscle soreness protocol and for a period of 72 hours. Both thighs are cooled with the "Cold Hot Maxi Pack" from Axanova for a period of 20 minutes.
Cooling log: Day 1 (muscle soreness log, 3x/day), Day 2 (3x/day), Day 3 (3x/day), Day 4 (3x/day)

Group B: delayed cooling

In this group, cooling starts 24 hours after the sore muscle protocol. Cooling is also carried out 3 x /day for a period of 72 hours. Both thighs are cooled with the "Cold Hot Maxi Pack" from Axanova for a period of 20 minutes.
Cooling log: Day 1 (muscle soreness log, no cooling), Day 2 (3x/day), Day 3 (3x/day), Day 4 (3x/day).

Group C: Control group

This group receives according to the muscle soreness protocol and will not receive any cooling intervention, nor any other intervention, for the entire period thereafter.

Sore muscle protocol

To induce sore muscles, the test subjects perform one-sided, 6 times 12 – 15 knee extensors on an ergometer chair. They straighten and bend the knee as far as possible. They repeated the exercise 5 more times after a 2-minute break (a total of 6 rounds). The movement sequences are observed and corrected if necessary. A similar protocol has already been successfully used in the literature to induce muscle soreness (Ruas et al 2022, Comparison between eccentric-only and coupled concentric-eccentric contractions for neuromuscular fatigue and muscle damage). In order to increase the significance of the investigation, the subjects are asked not to carry out any additional interventions during the experimental phase.

2.4 Investigation intervention

The repeated cold applications are carried out by the test subjects independently at home. For this, the test subjects receive 6 cold packs to take home (CE-certified Axanova Cold Hot Pearls Maxi Pack, Axanova AG). One cold pack is applied per thigh (2 packs per cooling session). The packs must be placed in a chest freezer/freezer compartment 1 hour before use. After the minimum cooling time has expired, the cold packs are placed on the thighs for 20 minutes with the fleece side down (skin contact). There must be at least 4 hours between cold applications. The cold packs may only be applied to the skin, without any additional pressure. The 20-minute cold application is described by the manufacturer Axanova as follows. We do not change the duration of application or form. The applications 24, 48 and 72 hours after the muscle soreness protocol are carried out by the test subjects at home and documented with a photo. Study participants upload these images directly to a designated cloud-based folder via a secure, password-protected link. This ensures that the data is transmitted and stored in encrypted form. When the intervention is carried out in the laboratory by the study staff, the temperature of each

cold pack is checked using an infrared thermometer to document possible temperature differences. This measurement is used to record potential variations in the cooling effect without influencing the application of the cold pack.

3 INVESTIGATION POPULATION AND INVESTIGATION PROCEDURES

3.1 Inclusion and exclusion criteria, justification of Investigation population

For this study, n=45 subjects will be recruited

Inclusion Criteria

- Young, healthy adults between the ages of 18 and 30
- No surgical interventions on the musculoskeletal system in the trunk area and lower extremities

Exclusion criteria

- Current pain conditions
- Current inflammatory states
- Medication intake (excl. anticonception drugs)
- Pregnant subjects
- Athletes
- Children/adolescents
- Non-intact skin conditions (e.g. psoriasis)
- Known circulatory disorders
- Cold allergy (Raynaud's syndrome)

3.2 Recruitment, screening and informed consent procedure

Participants are recruited via the websites of the Thim van der Laan (www.physioschule.ch) physiotherapy schools and the SUPSI (www.supsi-landquart.ch University of Applied Sciences and Arts Southern Switzerland). An electronic advertisement is placed on the screens in the building of Thim van der Laan AG in Landquart. The ad texts are based on the guidelines of the Ethics Committee of the Canton of Zurich.

The initial contact is made directly with the head of studies in the research laboratory of the University of Applied Sciences and Arts Southern Switzerland. At the first contact, the potential participants are informed about the procedure and the risks of the examination, as well as about the conditions and the amount of compensation.

The principal investigator will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the possible risks and benefits, and any inconveniences it may entail. Each participant is informed that participation in the examination is voluntary and that he or she can withdraw from the examination at any time and that withdrawing consent will not affect his or her subsequent supervision.

The study leader then hands over the subject information and the declaration of consent. In the subject information there is a checklist that the potential test persons must fill out. This checklist checks whether the potential test subjects are suitable for the examination. The test subjects are told that they can contact us at any time if questions arise.

The completed checklist and the signed consent form will be handed over to the Director of Studies and checked by him. If all exclusion criteria are checked and the consent form is signed

and the subject has no more questions, he/she is admitted to the examination. A copy of the signed informed consent form will be given to the study participant. The declaration of consent is kept as part of the study documents. The original of the signed consent form is kept as a study document in a locked filing cabinet.

After successful completion of the intervention, the participants receive a remuneration of 50 Swiss francs.

3.3 Investigation procedures

Investigation duration:
Subject to EC approval in 2024.
Recruitment period: January 2025
Conduct of the study: February - March 2025
Statistics and writing of the article: April – July 2025

Individual Investigation duration for each participant

Phase	Content	Time
Recruitment	Handing out patient information & consent, explanation of the experiment	30 min
Experiment Day 1	Anthropometry, baseline measurement, muscle soreness protocol (cooling: group A)	60 min
Experiment Day 2	Follow-up measurement 24 hours (Cooling: Group A and Group B)	30 min
Experiment Day 3	Follow-up measurement 48 hours (Cooling: Group A and Group B)	30 min
Experiment Day 4	Follow-up measurement 72 hours (Cooling: Group A and Group B)	30 min
Total time required per subject		**180 min**

Recruitment
As soon as the potential test subjects show interest in the study and arrive at the laboratory in Landquart, they are sent to the study director. If he is not present or available at that time, interested parties are asked to appear on another day.
The head of the study explains the procedure, the tests carried out, the risks and possible inconveniences. In addition, it is explained that participation is voluntary and that withdrawal is possible at any time, without giving reasons, without this having a negative impact on future care. The test subjects then receive the test subject information, which describes the examination in detail and in an understandable way. The document also contains a checklist that must be completed. The declaration of consent is also provided and must be signed and returned by the Director of Studies before the start of the programme. The doctor checks the completed checklist and confirms the consent.
Baseline measurements
The room temperature and relative humidity are determined at the beginning of each measurement day (Voltcraft MT52 digital multimeter, Hirschau, Germany). Baseline measurements include demographic data (age, gender, height). Anthropometric features at the beginning of the study included height (GPM Stadiometer, Zurich, Switzerland), body mass, and body fat percentage estimation. Body mass and lower body fat percentage were measured using

a TANITA-TBF 611 scale (Tokyo, Japan). The estimate of the percentage of lower body fat was chosen because the cold pack was applied to the thigh, which mainly affects the lower extremities. Recovery parameters (see Exact Measurement Method and Primary and Secondary Endpoints) are assessed in the same order for each measurement: (1) Inflammation Scores, (2) Muscle Swelling, (3) Soreness, (4) Maximum Voluntary Isosomic Muscle Concontraction. The measurement method and the equipment used for each specific result are described in Chapter 3.2.

Randomization

The randomization of the subjects will take place on the day of the baseline measurement by drawing a lot. It is ensured that the same number of tickets are available for each intervention group (5 tickets per group) and test subjects. If the number of subjects in a group exceeds one third of the total number of subjects, the lots of this group will be removed. In order to ensure the equality of the groups in terms of covariates and gender, the composition of the groups is monitored. As soon as half of a group consists of male or female subjects, the tickets for this group are removed for future subjects of the same sex.

The tickets are made of folded paper with the numbers 1, 2 or 3 on them and are placed together in a container. The drawing of the lots is carried out by the test persons themselves. After the draw, the subjects hand over the unread ticket to the study director, who assigns them to a group.

Blinding

Since cold applications are tested in this study, blinding is not possible.

Sore muscle protocol

To induce muscle soreness, a one-legged knee extensor protocol is used, which has already been used (Ruas et al., 2022). Participants will be instructed to straighten and bend their knee for 6 sets for 12 – 15 reps, with 2 minutes rest between sets. During the 2-minute break, the participants are allowed to sit on the chair. The execution of the knee extensor protocol is visually observed by an examiner. Verbal corrections of the execution can be given by the examiner. However, no verbal encouragement is given. All training sessions are carried out in the morning. If a participant is unable to perform all the repetitions of a set due to extreme fatigue or maximum effort, the set can be stopped and a new set can be started after the rest period. However, at least 12 repetitions per set must be performed. Otherwise, this will be considered an abandonment.

Intervention

The subjects in group A (immediate cooling) receive the first cooling on the two thighs immediately after the end of the muscle soreness protocol. This cooling is carried out in the supine position. After this application, you can leave the laboratory and the measurement of the skin temperature is stopped.

Group B (delayed cooling) and group C (control group) do not receive any cooling (group B) or any intervention (group C) immediately after the muscle soreness protocol. However, you will also need to spend 20 minutes lying on your back in the lab before the skin temperature measurement is completed.

Follow-up measurements

Follow-up measurements will be taken for each measurement in the same order as for the baseline measurement ((1) Inflammatory markers, (2) Muscle swelling, (3) Soreness of muscles, (4) Maximum voluntary isometric contraction). The follow-up measurements take place 24, 48, and 72 hours after the muscle soreness protocol in the laboratory in Landquart.

The measurement method and the equipment used for each specific result are described in Chapter 3.2.

3.4 Withdrawal and discontinuation

Participants can withdraw from the investigation at any time and without giving reasons. Participants will be excluded from the examination if an imminent injury or illness contraindications exercise or cold application.

4 STATISTICS AND METHODOLOGY

4.1. Statistical analysis plan and sample size calculation

Sample calculation

Before any data collection is carried out, case number planning and sample size calculation is necessary in order to obtain valid results. In clinical trials and animal experiments, this is even mandatory and is carefully reviewed before approval is granted (MDR, Annex XV, Chapter 1, Section 2.1, DIN EN ISO 14155:2021-05). Postoperative cryotherapy utilizing ice packs, cooling, or continuous cryotherapy devices reduced pain scores on the visual analogue scale and analgesic consumption in about half of the research studies comparing these outcomes against their control (no cryotherapy). The average initial VAS score after pain induction varies depending on the study and is an approximation of a baseline value of 4.73 ± 2.39. A reduction in the VAS score after cold application was assumed to be an average of 47%. After taking into account the dropout rate of 10% and splitting into 3 randomized groups, a patient count of 15 subjects per study arm is considered statistically appropriate. The results were determined using the PASS 2023 version 23.0.1 software.

Numerical Results for Multiarm Tests for the Difference Between Treatment and Control Agents Assuming Equal Variance

Solve for:	Number of cases
Group distribution:	equal to (Nc = N1 = N2 = ...)
Test Type:	T-Test
Hypothesis:	H0: $\delta = 0$ vs. H1: $\delta \neq 0$
Number of groups:	3
Bonferroni Correction:	none (divisor = 1)

Comparison	Target Strength	Real	Number of cases	Mean μi	Difference δi	Standard deviation σ	Alpha
Control			13	4,72		2,1	
vs. A	0,8	0,83233	13	2,21	-2,51	2,1	0,05
vs. B	0,8	0,91287	13	1,87	-2,85	2,1	0,05
Total	n/a	n/a	39	n/a	n/a	n/a	n/a

Comparison	The group involved in the comparison between the treatment and control shown in this report row. The comparison is made on the basis of the difference.
Test Strength	The desired test strength. Test strength is the probability of rejecting a false null hypothesis for this comparison. This test strength refers only to the comparison shown in this line.
Really. TS	The test strength that is really achieved.
Ni	The number of elements in the ith group. The total sample size is displayed in the last row of the column.
μ i	The mean value of the ith group at which the test strength is calculated. The first row contains μ c, the mean value of the control group.
δi	The difference between the ith treatment agent and the control agent (μ i - μ c) for which the test strength is calculated.
σ	The standard deviation of the responses within each group.
Alpha	The probability of rejecting the null hypothesis that the control mean is equal to the treatment mean described in this row.

Summary statements

A parallel 3-group design (with a control group and 2 treatment groups) is used to test whether the mean for each treatment group is different from the mean of the control group (H0: δ = 0 versus H1: δ ≠ 0, δ = μi – μc). The hypotheses are evaluated using two two-sided, two-sample methods. Bonferroni-adjusted t-tests with the same variance and an overall (experimental) Type I error rate (α) of 0.05. The common standard deviation for all groups is assumed to be 2.1. The mean value of the control group is assumed to be 4.72. The treatment means 2.21 and 1.87 with at least 80% test strength to be detected in each case. For the test, the (same) group sample size needed for each of the three groups (control and treatments) is 13 (39 subjects in total). The group sample sizes were calculated using PASS 2023, version 23.0.1.

Sample size taking into account the dropout rate

Group	Dropouts Abandonment Rate	Number of cases/group	Number of cases with drop-out rate	Number of abandoned participants
1 - 3	10%	13	15	2
Total	n/a	39	45	6

Taking into account the drop-out rate

Taking into account a dropout rate of 10%, the group sizes in each study arm should be 15 subjects.

Statistical approach to examine the primary endpoints

The statistical analysis is performed using IBM SPSS Statistics (version 27, IBM Corp., Armonk, NY, USA) and the significance level is set to p <0.05.

In order to be able to determine the differences between groups, a repeated ANOVA analysis is carried out with a factor of 1: time (baseline, 24 hours, 48 hours, 72 hours) and a factor of 2:

intervention (group A, group B, group C) for the recovery parameters (inflammation values, muscle swelling, muscle soreness data, maximum voluntary isometric muscle contraction).
The examination is discontinued if the participants are intolerant to the local cold application. Participants in the IC and DC groups are instructed to cancel a cooling session and contact their contact immediately if they experience symptoms such as severe pain/discomfort as a result of the cooling.

4.2. Handling of missing data and drop-outs

Missing data, missed training sessions or abandonments are noted in the CRF. Data from patients who discontinue the examination is collected and stored. Drop-outs are not replaced by the recruitment of new participants. If consent is withdrawn, the data already collected will be included in the analysis for scientific and safety reasons. These are only anonymised after the analysis (Art. 32 ClinO-MD).

5 SAFETY

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

Adverse Event (AE) (Art. 2 para. 57 MDR)
Any adverse medical event, unintentional illness or injury, or adverse clinical sign (including abnormal laboratory findings) in subjects, users, or others, whether or not related to the medical device (MP).
These include events related to the MP or comparator product being studied, as well as the associated procedures. For users or other persons, this is limited to events that are related to the MP.

Serious Adverse Event (SAE) (Art. 2 para. 58 MDR)
An adverse event that resulted in one of the following:

(a) death;
(b) serious deterioration in the subject's health resulting in any of the following:
    (i) life-threatening illness or injury,
    (ii) permanent impairment of a body structure or function,
    (iii) hospitalization or extension of an existing hospitalization,
    (iv) medical or surgical intervention to prevent a life-threatening illness or injury or permanent impairment of a body structure or function;
    (v) chronic illness,
(c) fetal distress, fetal death, or a congenital physical or mental impairment or birth defect.

Note: Planned hospitalizations due to a pre-existing condition or a procedure specified in the protocol (CIP) without a serious deterioration in the subject's health are not considered SAE.

Device Deficiency (Art. 2 para. 59 MDR)
Shortcomings of a medical device related to the identity, quality, durability, reliability, safety, or performance of an investigational device, including malfunctions, user error, and insufficient information from the manufacturer.
The definition includes defects in both the test product and the comparator product.

Malfunction (ISO14155)
The failure of an investigational product to perform its intended function in accordance with the instructions for use or protocol (CIP).

Defects of the medical device with SAE potential (Device Deficiency with Serious Adverse Event Potential)

(Art. 80 para. 1 letter c MDR; ISO14155)
Any product defect that could have resulted in a serious adverse event if appropriate measures had not been taken, no intervention had been made, or if the circumstances had been less favourable.

Adverse Device Effect (ADE) (ISO14155)
An adverse event that maybe, probably, or causally related to the use of an investigational product or associated procedures.
This includes events resulting from inadequate or incorrect instructions for use, from use, implantation, installation or operation, or from malfunctions of the test product. This also includes events caused by operating errors or intentional misuse.

Serious Adverse Device Effect (SADE) (ISO14155)
An adverse reaction of the medical device (ADE) that has led to one of the consequences of a serious adverse event.

Unanticipated Serious Adverse Device Effect (USADE) (ISO14155)
A serious adverse reaction of the medical device (SADE) that has not been identified in its nature, frequency, severity or outcome in the current version of the risk report.
Note: An anticipated serious adverse reaction of the medical device (Anticipated SADE, ASADE) is an effect that has already been identified in the risk report in terms of type, frequency, severity or outcome.

Causal relationship of SAEs (MDCG 2020-10/1)
The causal relationship with the medical device or the methods of investigation is assessed by the principal investigator and the sponsor as follows:

- Unrelated: The association with the product or process can be excluded.

- Possible: The connection with the use of the test product is weak, but cannot be completely ruled out. Alternative causes are also possible. Cases where assessment is not possible or no information is available are also considered possible.

- Probable: The association with the use of the investigational product appears relevant and/or the event cannot be meaningfully explained by another cause.

- Causal relationship: The serious event is unequivocally related to the investigational product or procedures.

5.2 Documentation and reporting in Medical Device Category A clinical investigations

Device defects (DD) and all adverse events (AE), including all serious adverse events (SAE), are recorded, fully investigated, and documented in the source document as well as in the corresponding Case Report Forms (CRF) throughout the study period, i.e. from the time of patient consent to the last CIP-specific procedure, including a safety follow-up phase.

- **Documentation of AEs (including SAEs)** by the investigator includes diagnosis or symptoms, start and end dates of the event, treatment of the event, resolution of the event, assessment of the severity and causal relationship with the medical device and/or the examination procedure (Art. 32 ClinO-MD, ISO14155).

- **Documentation of DDs** by the auditor includes the description of the event, the start date, information about the examination device, actions taken with respect to the examination device and the determination of whether the DD has resulted in an AE. The sponsor examines all DDs and decides and documents in writing whether they could have led to an SAE (DD with SADE potential) (Art. 32 ClinO-MD, ISO14155).

Reporting of SAEs (Art. 32 ClinO-MD)
All SUEs are documented and reported immediately (within a maximum of 24 hours) to the sponsor/investigator of the investigation.
If it cannot be ruled out that the SUE that occurred in Switzerland is due to the intervention under investigation, the investigator reports it to the Ethics Committee via BASEC within 15 days.

Follow up of (Serious) Adverse Events
An aftercare plan is created for the affected subjects, ensuring that they receive appropriate medical care and support. Communication with the affected subjects is maintained to monitor their recovery .

Notification of safety and protective measures (Art. 32 ClinO-MD)
If immediate safety and protective measures must be taken during the conduct of the investigation, the investigator shall inform the Ethics Committee within 7 days of these measures and the circumstances that require them.

5.2.1 Foreseeable adverse events and anticipated adverse device effects

Predictable AEs during the application of the MD.

Type of event	Description	Mitigation or Treatment Measures	Probable incidence
Skin irritation or redness	Localized erythema or irritation at the application site due to pressure or cold.	use protective materials; apply soothing creams or gels if necessary.	Medium
Pain or discomfort	Temporary pain or discomfort during or after application due to refrigeration.	Ask participants to report pain.	Small
Decreased local sensitivity	Temporary numbness or decreased sensitivity due to exposure to cold.	inform participants about this possibility; Avoid prolonged use to prevent complications.	Medium
Slight swelling or edema	Localized swelling due to prolonged cooling or improper use.	Monitor application time; elevate the affected area; Reduce application time for subsequent applications.	Small

No SAEs are expected.

5.2.2 Reporting of Safety related events

Reporting to the Sponsor:
All Serious Adverse Events (SAEs), Equipment Defects (DDs) with potential for SAEs, and health hazards requiring action will be reported by the investigator (or authorized representative) to the

sponsor immediately upon becoming aware of the event. DDs are evaluated as to whether they could lead to an SAE.

Pregnancies
Pregnant or breastfeeding women are excluded from participating in the examination. Participants who become pregnant during the study must notify the study leader immediately and are not allowed to continue participating in the study. However, the examination does not pose a risk to pregnant women.

Reporting to the Competent Ethics Committee:
The sponsor shall immediately notify the Ethics Committee of any SAE in which a causal relationship between the event and the clinical trial test procedure has been established (Art. 33 ClinO-MD).
To ensure prompt notification, the sponsor may first submit an incomplete notification.
In the event that safety or health risks arise during the conduct of the investigation that require immediate action, the sponsor shall inform the Ethics Committee within 2 days of these measures and the circumstances that made them necessary (Art. 34 ClinO-MD).

Periodic safety reporting (Art. 35 ClinO-MD):
Once a year, the sponsor submits a list of SAEs and DDs to the Ethics Committee and submits a report on their severity, the causal relationship with the device and the intervention, and the safety of the participants. The sponsor informs the Ethics Committee annually about the general progress of the clinical trial.

Materiovigilance reporting to Swissmedic:
The sponsor is responsible for ensuring that Swissmedic is informed of serious incidents in accordance with Art. 66 MedDO.
Mate vigilance reports are not sent to the Ethics Committee.
If the sponsor is not the manufacturer of the device under investigation or the Swiss representative of the manufacturer:

- In the event of incidents, verify that the event has occurred in accordance with Art. 66 para. 4 MedDO is subject to the reporting obligation (using the instructions MU680_20_008e_WL).
- The sponsor must ensure that reportable incidents are sent to Swissmedic using Form MU680_20_015d_FO (materiovigilance@swissmedic.ch).
- Users are required by law to inform the suppliers of the equipment (Art. 66 para. 4 MedDO).

5.3 Radiation

No radiation is included in this examination.

5.4 Amendments (Art. 15 ClinO-MD)

Significant changes to the study structure, organisation, protocol and associated study documents will be submitted to the Ethics Committee for approval in accordance with Art. 15 ClinO-MD before implementation. In addition, a list of non-material changes is reported annually to the EC, together with the CSA or the *general study progress report*.

5.5 Notification and reporting upon completion, discontinuation or interruption of the

Investigation

After the regular conclusion of the investigation, the Ethics Committee will be informed about BASEC within 30 days in accordance with Article 38 of the ClinO-MD.
The sponsor/investigator may terminate the investigation early in certain circumstances, such as:

- ethical concerns,
- Insufficient participant recruitment,
- doubts about the safety of the participants or if the participants are at risk (e.g. if the benefit-risk assessment is no longer positive),
- Changes in accepted clinical practice that make it no longer make sense to continue the investigation,
- Early indications of harm or benefit of experimental intervention.

In the event of an early termination or interruption of studies, the Ethics Committee will be informed of BASEC within 15 days in accordance with Article 38 of the ClinO-MD.

Within 12 months of completion or termination of the investigation, a final report is submitted to the Ethics Committee via BAREC, unless a longer period is provided for in the protocol (Article 38 ClinO-MD).

5.6 Insurance

There is a business liability insurance with Baloise Insurance for Thim van der Laan AG in Landquart, where the investigation is carried out.

6 FURTHER ASPECTS

6.1 Overall ethical considerations

The study is carried out on healthy volunteers. Participants can opt out of the investigation at any time without consequences, as indicated in the informed consent form. The sample of healthy participants between the ages of 18 and 30 reflects a population that engages in regular physical activity, so the results are transferable to the general population. To prioritize the safety of participants, vulnerable populations such as pregnant women and those with certain health conditions were excluded to minimize possible adverse reactions. The results of this research can provide valuable insights into the mechanisms and application of recovery strategies after exercise. The use of a randomized controlled trial design is scientifically rigorous and minimizes bias. By considering covariates (e.g., gender) in randomization, the study increases internal validity and reduces the potential for confounding variables. The informed consent form explicitly states that participants will be informed of personal findings and random results relevant to their health (e.g. abnormal blood values).

6.2 Risk-benefit assessment

There are no health risks associated with participation in this examination. The CE-certified cold packs used in this study (Axanova Cold Hot Pearls Maxi Pack, Axanova AG) have been specially developed to exert a safe cooling effect on the affected muscle tissue. The product is used according to the instructions for use. As stated in the declaration of consent, the instructions for use of the CE-certified cold packs (Axanova Cold Hot Pearls Maxi Pack, Axanova AG) refer to the possibility of overcooling and the subsequent development of local frostbite due to local cold application. Through explicit instructions on use, duration, and frequency, the protocol helps

ensure that participants use the ice packs correctly, minimizing the risk of adverse effects and optimizing the intended appropriate cooling effect on muscle tissue.

As stated in the informed consent form, participants must expect to fatigue and become sore muscles due to the physical exertion from the knee extensor protocol and the measurement of the maximum isometric contraction level associated with a medium to high intensity. However, these complaints are not considered a significant health risk. The study design aims to ensure a safe and controlled practice environment with minimal potential for injury.

Venous blood samples were taken from a cubital vein by a trained specialist. However, as stated in the consent form for blood collection, there is a possibility that bruising, bleeding or swelling may occur at the injection site.

Participation in the study does not bring any direct benefit to the participants. While there is no direct personal benefit, participation in this research has the potential to provide valuable data for the scientific community. The results could benefit future athletes, people who engage in physical activity, and patients by shedding light on the best practices for post-exercise recovery.

7. QUALITY CONTROL AND DATA PROTECTION

7.1 Quality measures

Monitoring is carried out by Dr. Biomed. Ing. Ursula Hohenauer-Küng from the institution THIM - The International University of Physiotherapy, Weststrasse 8, 73002 Landquart.

In her function as a monitor of the examination, Dr. Ursula Hohenauer-Küng ensures that the examination is carried out and documented correctly. A detailed description of her activities as a monitor is listed below in point 9. This ensures that Dr. Biomed. Ing. Ursula Hohenauer-Küng is independent of the study team and is not employed by the examiner of the study.

For quality assurance, the sponsor, the ethics committee or an independent study monitor can visit the research facilities. On such occasions, direct access to the source data and all study-related files is granted. All parties involved treat the subject data in the strictest confidence.

7.2 Data recording and source data

The answers to the checklist, demographic and anthropometric data (gender, age, height, weight, estimated lower body fat percentage), room conditions (temperature, relative humidity), muscle soreness, and maximum isometric contraction force are manually recorded in the CRF. The data is then transferred in Microsoft Excel to the research computer of the research laboratory. Activation of the "Track Changes" option is ensured in the Excel file to ensure data protection and security. In addition, the Excel file is password-protected and backups are carried out regularly after measurements.
The study participants who run and document the application at home upload the images directly to a dedicated folder on Microsoft OneDrive for Business via a secure, password-protected link. It uses Azure Information Protection (AIP) to ensure secure transmission (with TLS/SSL encryption) and secure storage of data. The uploaded images are only accessible to authorized members of the study team, and storage is done in accordance with applicable data protection regulations. After the scan is completed or after the retention period has expired, the images are securely and completely deleted.
The blood sinking velocity is determined on site in the research laboratory using the Westergren method. The blood samples are then destroyed. The remaining parameters are determined by an external laboratory (Dr. Riesch, Buchs). After the blood is taken, the samples are picked up by a blood courier during the same day and taken to the medical laboratory, where the standardized analyses are performed. The creatine kinase values are determined by the ultraviolet method and the C-reactive protein values by the turbidimetric method. The blood samples are destroyed by the external laboratory and the reports are sent to us. We keep these reports confidential for a period of 10 years. The external laboratory only receives the numerical code of the participants (e.g. 001, 002, 003, etc.). No conclusions can be drawn from the external laboratory about the test subjects.
The images for the examination of muscle swelling are stored on an ultrasound machine. Only the numerical code is stored in the ultrasound machine and no personal data. It is not possible to draw conclusions about the test persons on the basis of the numerical code. The ultrasound machine is the property of the research laboratory and is operated only by research staff.
The key to the code is only with the principal investigator of this study. Only the study leader can decipher the code.

7.3 Confidentiality and coding

Study and participant data will be treated with the utmost discretion and will only be accessible to authorized personnel who need the data to perform their duties as part of the investigation. On the CRFs and other study-specific documents, participants are identified only by a numerical participant number. No personal data is presented or published. The signed declaration of consent and the completed checklist are kept in the original as a study document in a locked filing cabinet.

7.4 Retention and destruction of Investigation data and biological material

All study data will be archived for 20 years after the end of the study or premature termination of the examination. After this period, all data will be securely destroyed in accordance with the applicable data protection regulations and the guidelines of the study protocol. This applies to both the electronic data and all biological samples collected during the examination. The destruction is carried out in a secure way, for example by encrypting and completely deleting the electronic data and by physically destroying biological materials in order to exclude any possibility of reconstruction or identification of test subjects. If longer storage is necessary, for example for regulatory or scientific reasons, this will be coordinated in advance with the responsible ethics committees and authorities.

8 MONITORING AND REGISTRATION

The monitoring is carried out by Dr. Ursula Hohenauer-Küng from the institution THIM - The International University of Physiotherapy, Weststrasse 8, 73002 Landquart. Dr. biomed. Ing. Ursula Hohenauer-Küng ensures that the investigation is properly conducted and documented by performing the following activities, provided that they are relevant and necessary for the investigation:

- Verify that the investigator has and maintains adequate qualifications and resources throughout the duration of the study, that the facilities, including laboratories, equipment, and personnel, are suitable for the safe and proper conduct of the study and are maintained throughout the duration of the study.
- Review of study interventions
- Ensuring that the conditions for the interventions are acceptable.
- Verify that the study interventions are only made available to those subjects who are eligible.
- Ensuring that examinees are given the necessary instructions on the study interventions.
- Verify that the auditor is complying with the approved protocol and, if applicable, any approved changes.
- Verify that written informed consent has been obtained prior to each examinee's participation in the investigation.
- Ensuring that the auditor and the auditor's audit staff are adequately informed about the audit.
- Verify that the examiner is recruiting only eligible examinees.
- Reporting on the subject recruitment rate.
- Verify that the auditor provides all required reports, notifications, applications, and submissions, and that these documents are accurate, complete, timely, legible, dated, and identify the review.
- Verify that adverse events, concomitant medications and intercurrent diseases are reported according to the protocol on the CRFs.
- Ensuring that missed visits by the examinees, tests and examinations that were not carried out are clearly identified as such in the examination forms.
- All withdrawals and discontinuations of enrolled subjects from the study are reported and explained on the CRFs.

Source Data Verification (SDV) is also carried out as part of the monitoring to ensure the accuracy and integrity of the study data. This includes the following specific activities:

- Verification of sources and original data: It verifies that all data collected in the CRFs matches the original, primary data sources, such as participant data, lab reports, and notes from investigations.
- Control of study participants' documentation: Verify that all relevant demographic and medical data (e.g., age, gender, medical history, test results) are correctly captured and consistent with the original records.
- Verification of informed consent: Ensuring that each participant has written and informed consent prior to the start of the study.
- Adverse Event (AE) Review: Confirmation that all adverse events and concomitant medications are documented correctly and in accordance with the protocol.
- Control of study interventions: Verification that only eligible participants have received the study interventions and that all intervention data has been properly recorded.
- Examination of dropouts and non-perceptions: Verification that all abandonments from study visits and non-attendances have been properly noted and declared in the CRFs.

These measures ensure that the investigation meets the highest standards of data integrity and security, and that all recorded data is accurate and reliable.

9. FUNDING / PUBLICATION / DECLARATION OF INTEREST

This study is financed by Thim van der Laan AG in Landquart. The auditors are employed by this institute and are remunerated for their work on this study in accordance with their employment contracts. No further financial support is needed.
The sponsor shall enter and publish the summary of the study results in a public register in accordance with ClinO Art. 65a within one year of completion or termination of the investigation. An interruption of more than two years is considered to be an interruption of the investigation. For publication in the public register, the sponsor shall also ensure that an easy-to-understand summary of the study results is entered in BASEC within one year of completion or termination of the study. The entry is made at least in the national language of Switzerland in which the study participants were recruited.

10. REFERENCES

Bleakley, C., McDonough, S., Gardner, E., Baxter, G.D., Hopkins, J.T., & Davison, G.W. (2012). Cold-water immersion (cryotherapy) for preventing and treating muscle soreness after exercise. *Cochrane Database Syst Rev, 2*, CD008262. doi:10.1002/14651858.CD008262.pub2

Hohenauer, E., Taeymans, J., Baeyens, J.P., Clarys, P., & Clijsen, R. (2015). The Effect of Post-Exercise Cryotherapy on Recovery Characteristics: A Systematic Review and Meta-Analysis. *PLoS One, 10*(9), e0139028. doi:10.1371/journal.pone.0139028

Hubbard, T. J., & Denegar, C. R. (2004). Does Cryotherapy Improve Outcomes With Soft Tissue Injury? *J Athl Train, 39*(3), 278-279.

Ostrowski, J., Purchio, A., Beck, M., Leisinger, J., Tucker, M., & Hurst, S. (2018). Examination of Intramuscular and Skin Temperature Decreases Produced by the PowerPlay Intermittent Compression Cryotherapy. *J Sport Rehabil, 27*(3), 244-248. doi:10.1123/jsr.2016-0244

Ruas, C., Latella, C., Taylor, J., Gregory Haff, G., Nosaka, K. (2022). Comparison between eccentric-only and coupled concentric-eccentric contractions for neuromuscular fatigue and muscle damage. Med Sci Sports Exerc, 1; 54(10):1635-1646. Doi: 10.1249/MSS.0000000000002959

Chow, S.C., Shao, J., Wang, H., and Lokhnygina, Y. 2018. Sample Size Calculations in Clinical Research, 3rd Edition. Chapman & Hall/CRC. Boca
Raton, FL. Pages 86-88.

Machin, D., Campbell, M.J., Tan, S.B., and Tan, S.H. 2018. Sample Sizes for Clinical, Laboratory, and Epidemiology Studies, 4th Edition. Wiley Blackwell.

Julious, Steven A. 2004. 'Tutorial in Biostatistics. Sample sizes for clinical trials with Normal data.' Statistics in Medicine, 23:1921-1986.

Zar, Jerrold H. 1984. Biostatistical Analysis (Second Edition). Prentice Hall. Englewood Cliffs, New Jersey.