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A randomised controlled trial to investigate the effectiveness of repeated cold and hot water baths on performance and recovery

Clinical Study Protocol

Effectiveness of cold and hot water baths on performance and recovery

Study Type:	Other Clinical Trial according to ClinO, Chapter 4
Study Categorisation:	**Risk category A according to ClinO, Art. 61**
Study Registration:	**KEK-ZH-Nr. 2021-00546** ClinicalTrials.gov (NCT04902924)
Sponsor	Thim van der Laan AG Thim van der Laan jr. Weststrasse 8 / CH- 7302 Landquart
Sponsor-Investigator or Principal Investigator:	**University of Applied Sciences Southern Switzerland** **Department of Business Economics, Health and Social Care** **Physiotherapy Graubünden** Weststrasse 8 / 7302 Landquart +41 (0)81 300 0170 **Contact / Director of Studies** **Clijsen Ron**, PhD University of Applied Sciences and Arts Southern Switzerland Physiotherapy Graubünden Weststrasse 8, 7302 Landquart Phone: +41 (0)81 300 01 75 ron.clijsen@supsi.ch
Investigational Product:	Whirlpool Aspen (NetSpa) Certified according to European directives, regulations and standards (2011/65/EU, 2006/95/EC, 2004/108/EC, 2009/125/EC)

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Protocol Version and Date: Study protocol – Version 06 – 28.09.2021

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PROTOCOL SIGNATURE FORM

Study Title	Eine randomisierte, kontrollierte Studie zur Untersuchung der Effektivität von wiederholten Kalt- und Warmwasserbäder auf die Leistungsfähigkeit und Erholung
Study ID	2021-00546

The Sponsor-Investigator and trial statistician have approved the protocol version 03 (04.06.2021), and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm if applicable and the local legally applicable requirements.

Fachhochschule Südschweiz, Department of Business Economics, Health and Social Care, Physiotherapie Graubünden

Sponsor (Thim van der Laan AG):

Thim van der Laan jr.

Landquart, 23.09.2021 _____

Place/Date Signature

Local Principal Investigator at study site*:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Fachhochschule Südschweiz, Department of Business Economics, Health and Social Care, Physiotherapie Graubünden

Investigator:

Ron Clijsen, PhD

Landquart, 23.09.2021 _____

Place/Date Signature

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STUDY SYNOPSIS

Sponsor/ Sponsor-Investigator	**Sponsor** **Thim van der Laan AG** **Thim van der Laan jr.** Weststrasse 8 / CH- 7302 Landquart **Investigator** **University of Applied Sciences and Arts Southern Switzerland (SUPSI)** **Department of Business Economics, Health and Social Care** Physiotherapy Graubünden Attn: Ron Clijsen, PhD Weststrasse 8 CH-7302 Landquart
Study Title:	A randomised controlled trial to investigate the effectiveness of repeated cold and hot water baths on performance and recovery
Short Title / Study ID:	Effectiveness of repeated cold- and hot water baths on performance and recovery Ref. 2021-00546
Protocol Version and Date:	Version 06 of 28.09.2021
Study registration:	FOPH Register SNCTP ClinicalTrials.gov (NCT04902924)
Study category and Rationale	Other clinical trial of risk category A according to ClinO (Chapter 4) This study is concerned with basic physiological research on the effects of hot and cold water baths on the function of the body and not about the medical application of the devices used. No medication is administered to the test subjects and no tissue samples are taken. Only non-invasive or minimally invasive methods are used.

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Background and Rationale:	External cooling applications have been known and used in elite sports for years. Hot water baths have also been used for several years, both in sports and in the medical field. These non-invasive interventions are becoming increasingly popular, as hydrotherapy is said to have positive effects before and after sporting activity. For example, hydrotherapy is said to contribute to improved regeneration after sporting activity. Literature on this topic is already available. However, the results are inconclusive and more randomised controlled trials need to be conducted to clarify the results. In addition to the investigations between hot and cold water, recent research in the field of sports shows that exposure to extreme environments (e.g. stays at altitude) can impair not only cognitive function but also physical performance and the perception of pain. To the authors' knowledge, there is limited literature on how physical performance affects recovery under hypoxia conditions. Numerous studies have already looked at cold water baths and training under hypoxia conditions to investigate the effects on recovery. The scientific results do not yet allow a clear recommendation for hydrotherapy as an accelerator of recovery. The extent to which training under hypoxia influences regeneration has only been minimally investigated. Further, randomised studies are needed to investigate the effects of hydrotherapy and hypoxia on the ability to regenerate.
Objective(s):	The aim of this study is to investigate the effectiveness of repeated cold and hot hot baths and provide new insights into the appropriate use of this form of application. The study investigates the effect of repeated cold and hot water baths on the maximum voluntary isometric muscle contraction of the thigh muscles, vertical jump height, inflammation parameter from venous blood, muscle swelling and the evaluation of subjective muscle soreness in the legs and perceived general exhaustion during 72 hours of recovery. As an exhaustive measure, the subjects must complete a total of 5 x 20 maximum vertical jumps.

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Outcome(s):	**Primary outcomes** Physiological measurements • Jump height measurement (cm) • Maximum Voluntary Isometric thigh Contraction (kg) • Muscle swelling (mm) • Blood oxygen saturation (%) • Oxygen saturation of the muscles (%) • Heart rate (bpm) • Skin temperature (°C) • Core Body Temperature (°C) • Inflammation parameters: IL6 (pg/ml), CRP (mg/dl), creatine kinase (U/L) Psychological measurements • Dyspnea questionnaire (mod. Borg scale) • Exhaustion questionnaire (Borg scale) • Subjective muscle soreness (VAS scale) • Thermal comfort and thermal sensation **Secondary outcomes:** none
Study design:	Randomised, controlled trial

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Inclusion / Exclusion criteria:	**Inclusion Criteria** • Young, healthy women aged 18 – 35 years • No surgical interventions on the musculoskeletal system in the trunk area and lower extremities • Anticonceptives **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 one year ago • Injuries to the trunk and/or lower extremities that occurred more than one year ago and are still causing symptoms • Fear of cold and/or hot water intervention • Fear of hypoxia • Taking medication of any kind (even self-purchased) • Pacemakers & cardiac arrhythmias • Known circulatory problems • Positive pregnancy test • Diagnosed skeletal static deviations • Appendectomy less than 2 years ago • Raynaud's syndrome

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Measurements and procedures:	***Subject recruitment*** The subject information and consent form are given to the test subjects. Questions may be asked. If the test subject agrees with the information, she will be asked to attend the first contact day. This includes the explanations on site, signing of the consent form, and the completion of the health questionnaire. The pregnancy test must be negative. Once all tests have been passed, an appointment is made for the intervention. ***Measurement the maximum arbitrary isometric thigh muscle contraction (MVIC)*** MVIC is performed on the Cor 1 – ergometer chair for biomechanical measurements V1.0 and is expressed in kg (www.otbioelettronica.it). The conduction velocity is measured using the surface electromyogram system EMG-USB2 from OT Bioelettronica (otbioelettronica.it), band-pass filtered between 10-750Hz and rectified (full-wave rectify). MVIC is measured on the Cor 1 ergometer chair in 90° knee flexion. The raw data of muscle activity to measure conduction velocity is processed using OT Bioelettronica's software (MISO II). The electrodes are placed according to the guidelines of Barbero et al. (2012) "Atlas of Muscle Innervation Zones". The measurement takes place directly, 24 hours, 48 hours and 72 hours after the intervention. ***Jump height measurement*** The jump height after exercise is determined using 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. To measure the jump height, the test subjects are allowed to start from the squatting position (squat jump). The arms must be positioned 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 intervention takes place. ***Skin temperature*** The skin temperature is measured using the iButton System (http://www.ibuttonlink.com). The self-adhesive sensors transmit the information about the skin temperature wirelessly to a computer. The skin temperature is measured immediately after the intervention. The iButtons are attached with a strip of tape to the following locations: center of the shoulder blade, neck (6th cervical vertebra), back of the hand, center of the shin, center of the thigh. The iButtons are removed from the affected areas during the cold or hot water bath and then reattached. Skin temperature is also recorded using the FLIR A655 sc series infrared camera (InfrarotTec Systems, Ranstadt, Germany). A total of seven recordings are taken; baseline, before, during, and after the intervention. ***Core Body Temperature*** The core body temperature is determined via the e-Celsius® Performance (https://www.bodycap-medical.com). This non-invasive, disposable, ingestible capsule continuously monitors and records core body temperature and transmits the data wirelessly to a monitor (BodyCAP medical, Hérouville Saint-Clair, France). Core body temperature is measured before, during, and after the intervention.

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Muscle swelling

Muscle swelling is measured using ultrasound imaging (MyLabClassC, Esaote, Genoa, Italy). The images are analysed using the OsiriX DICOM viewer software (OsiriX, Pixmeo SARL, Switzerland). The measurements take place before, immediately after, 24, 48, and 72 hours after the intervention.

Heart rate

The heart rate is determined by means of a heart rate monitor with a chest strap (Polar T31, Polar Inc. Kempele, Finland). Heart rate is measured before, during, and immediately after the intervention.

Oxygen saturation of the blood

The oxygen saturation of the blood is measured with a portable pulse oximeter with finger clip probe (Nonin 7500, Nonin medical B.V., Plymouth, USA). This measurement takes place during the baseline measurements as well as during the jump protocol.

Oxygen saturation of the thigh muscle

The oxygen saturation of the muscles is measured non-invasively with a deep tissue oxygenation monitor (moorVMS-NIRS, moor instruments, www.moor.co.uk). For this purpose, adhesive electrodes are placed over the muscle. The measurement is carried out during and immediately after the intervention.

Inflammation parameters

The measurement of inflammatory parameters such as creatine kinase (CK), interleukin 6 (IL6) or C-reactive protein (CRP) is carried out via venous blood sample (approx. 6 ml) (Accutrend, Roche Diagnostic, Rotkreuz, Switzerland & Reflotron, Roche Diagnostic, Rotkreuz, Switzerland). The measurement is carried out at baseline, 24 hours, 48 hours and 72 hours after the intervention by trained specialists.

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 conceivable muscle soreness) in cm increments. The Measurement takes place before, 24 hours, 48 hours and 72 hours after the intervention.

Subjective information on general exhaustion

The subjects reported their subjective general feeling of exhaustion on a BORG scale (6 – 20). The scale will be scaled from "6" (very, very light load) to "20" (very, very strenuous load). The measurement takes place before the intervention.

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	Subjective information on shortness of breath The subjects reported their subjective shortness of breath on a BORG scale (0 – 10). The scale will be scaled from "0" (no shortness of breath at all) to "10" (maximum shortness of breath). The measurement takes place before the intervention. ***Thermal comfort and temperature sensation*** These parameters are measured using a scale. This measurement is carried out before and after the intervention. ***Further processing of the data*** For further data analysis, the software MATLAB® will be used, while Excel from Windows® will be used for data pooling and for the graphical representation of the end data. All digital documents are password-protected. Paper data is stored in a separate, lockable filing cabinet. The data collected with Excel is given version numbers and stored in read-only mode. The corresponding versions are printed out, signed by the director of studies and filed in the above-mentioned filing cabinet. Only the director of studies has access to this filing cabinet.
Study Product / Intervention:	The Aspen inflatable whirlpool (NETSPA) is a certified product that complies with European regulations, directives and standards. 2006/95/EC Low Voltage Directive, 2004/108/EC (Electromagnetic compatibility), 2011/65/EU (restriction of the use of certain Substances in appliances), 2009/125/EC (ecodesign of energy-related products). All guidelines are in writing in the Operations Manual Chapter 1 (page 40). In the inflatable whirlpool, temperatures of 40°C are generated with the help of a heating motor. The whirlpool is already commercially available and is used in the private wellness area. Simulated altitude training is quickly becoming the most promising development in the world in terms of athletic performance and peak fitness. With the Cloud 9, a simulated altitude of up to 4000 m above sea level can be achieved. This corresponds to an oxygen partial pressure of 12.7 %. The Cloud 9 altitude training device is designed for all levels of athletes (including amateurs) who want to improve their performance. The Cloud 9 is a solid, certified product that complies with the European Directives on Electromagnetic Compatibility, Machinery Directive, Air Pressure Equipment and Low Voltage Equipment (89/336/EEC, 91/368/CEE, 93/68/CEE, 97/23/EC, EN61010-1).

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Control Intervention (if applicable):	In order to obtain high-quality study results, the subjects were exposed to only one experimental condition at a time. There are four experimental groups. To keep the quality of the study high, a randomised controlled trial design was chosen. The allocation into the groups is randomised. The data collected in this way is pooled by outcome or group. The following interventions are carried out: group rCWI with repeated cold water baths (1 x 10 min at 10°C to the sternum and again after 2 hours), group rHWI with repeated hot water baths (1 x 10 min at 40°C to the sternum and again after 2 hours), a hypoxia group (performs the jump protocol under hypoxia and then lies on the back for 10 minutes) and a passive control group (lying on the back for 10 minutes).
Number of Participants with Rationale:	The power analysis with the G*Power App (Düsseldorf, North Rhine-Westphalia, Germany) showed that a minimum number of 33 subjects was necessary to ensure the statistical power (0.80) to prove a parameter difference at the 5% significance level. Taking into account a possible loss of 20% of the datasets (due to drop-out or incomplete datasets), the minimum sample size is 40 participants. The number of subjects in this study is comparable to other studies in the field of thermotherapy (Kuligowski et al. 1998, Pournot et al. 2011, Vaile et al. 2008).
Study Duration:	May 2021-December 2023
Study Schedule:	May-December 2021: Subject information, screening, planning, data collection January-August 2022: Statistics September-May 2023: Writing the article June-December 2023: Time for publication

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Investigator(s):	Examiner - Ron Clijsen, PhD[1,2,3] - Hohenauer Erich, PhD[1,2,3] - Deflorin Carlina[2] - Friday Livia2 - Herten Miriam2 [1] *University College Physiotherapy Thim van der Laan, Weststrasse 8, 7302 Landquart* [2] *University of Applied Sciences and Arts Southern Switzerland, Rehabilitation Research Laboratory 2rLab, Rehabilitation and Exercise Science Group, Department of Business Economics, Health and Social Care, Physiotherapy Graubünden, Weststrasse 8, 7302 Landuart* [3] *Vrije Universiteit Brussels, Faculty of Physical Education and Physical Therapy, Pleinlann 2, 1050 Brussels* ***Contact / Director of Studies*** **Clijsen Ron**, PhD University of Applied Sciences and Arts Southern Switzerland Physiotherapy Graubünden Weststrasse 8, 7302 Landquart Phone: +41 (0)81 300 01 75 ron.clijsen@supsi.ch
Study Centre(s):	University of Applied Sciences and Arts Southern Switzerland Physiotherapy Graubünden Rehabilitation Research Laboratory 2rLab Rehabilitation and Exercise Science Group Weststrasse 8 7302 Landquart
Statistical Considerations:	The effectiveness of repeated cold and hot water baths on recovery and performance is tested by: Repeated measures analysis of variance (MANOVA) Factors: • Protocol • Task (repeated cold water immersion vs. repeated hot water immersion, repeated cold water immersion vs. control group, repeated hot water immersion vs. control group, repeated cold water immersion vs. passive group with hypoxia conditions, repeated hot water immersion vs. passive group with hypoxia conditions) • Time (these factors are measured immediately after cooling/warming and 24 hours, 48 hours and 72 hours after the interventions). The significance level is set at $P < 0.05$, and the statistical data analysis is performed with SPSS 27.

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GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP or ISO EN 14155 (as far as applicable) as well as all national legal and regulatory requirements.

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ABBREVIATIONS

AE	Adverse Event
BASEC	Business Administration System for Ethical Committees, (https://submissions.swissethics.ch/en/)
CA	Competent Authority
CEC	Competent Ethics Committee
CK	Creatine Kinase
CRF	Case Report Form
CRP	C-reactive protein
ClinO	Ordinance on Clinical Trials in Human Research *(in German: KlinV, in French: OClin, in Italian: OSRUm)*
eCRF	Electronic Case Report Form
CTCAE	Common terminology criteria for adverse events
DSUR	Development safety update report
GCP	Good Clinical Practice
IB	Investigator's Brochure
Ho	Null hypothesis
H1	Alternative hypothesis
HRA	Federal Act on Research involving Human Beings *(in German: HFG, in French: LRH, in Italian: LRUm)*
IL6	Interleukin 6
IMP	Investigational Medicinal Product
IIT	Investigator-initiated Trial
ISO	International Organisation for Standardisation
ITT	Intention to treat
MD	Medical Device
MedDO	Medical Device Ordinance *(in German: MepV, in French: ODim)*
MVIC	Maximum voluntary isometric muscle contraction
PI	Principal Investigator
SDV	Source Data Verification
SOP	Standard Operating Procedure
SPC	Summary of product characteristics
SS Test	Pregnancy test
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File

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US	Ultrasound
VAS	Visual Analog Scale

STUDY SCHEDULE

Table 1: Schedule

The completion of the measurement series is planned for December 2021, and the completion of the entire project at the end of 2023.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Head
2021					Screening, data collection							
2022	Statistics								Writing the article			
2023	Writing the article					Publication						

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1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor, Sponsor-Investigator

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1.3 Statistician ("Biostatistician")

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1.4 Laboratory

University of Applied Sciences and Arts of Southern Switzerland
Physiotherapy Graubünden
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Rehabilitation and Exercise Science Group
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1.5 Monitoring institution

Vrije Universiteit Brussels
Faculty of Physical Therapy
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1.6 Data Safety Monitoring Committee

All data obtained from the study participants will be encoded 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 concurrent study director have access, whereby the administration of the documents is the responsibility of the head of the research laboratory.

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All digital data is encoded. This means that no conclusions can be drawn about individuals. The digital data is stored and archived on the institute's internal computers and is not released to third parties.

Employees involved in data processing have no access to the personal data and the coding of it.

1.7 Any other relevant committee, person, organisation, institution

The study will be conducted at the University of Applied Sciences and Arts of Southern Switzerland, Physiotherapy Graubünden, Weststrasse 8, CH7302 Landquart, in the research laboratory of the research group officially known as the Rehabilitation Research Laboratory 2rLab, Rehabilitation and Exercise Science Group.

The mentioned sponsor, Thim van der Laan AG, is responsible for the management according to KlinV §2c.

The test products and measuring equipment are all located in this laboratory (4th floor of the building), and therefore all measurements can be carried out at the same location. The room offers sufficient space to easily fulfil all the safety aspects described below. The room in which the whirlpools are located has the following dimensions: 7m x 14m x 2.5m (98 square meters or 245 cubic meters) and can be ventilated.

No further studies with this arrangement are currently taking place in this experimental laboratory. No further studies are currently being conducted at our institute.

No other institutes and committees are involved in this study or the authorisation or monitoring of the study.

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2. ETHICAL AND REGULATORY ASPECTS

2.1 Study registration

The present study was primarily registered in the register ClinicalTrials.gov (NCT04902924).

The conduct of the present study lies within the remit of the Zurich Cantonal Ethics Committee. An application for authorisation will be submitted. Any further conditions of the responsible ethics committee must be implemented in the study protocol.

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

2.2 Categorisation of study

The present study is categorized as Other Clinical Trial of Risk Category A (chapter 4). The present study is concerned with basic physiological research on the effects of hot and cold water baths on the function of the body. No medication is administered to the subjects and no tissue samples are taken. Only minimally invasive methods are used. Experiments are carried out with CE-certified equipment in accordance with the instructions for use.

2.3 Competent Ethics Committee (CEC)

Human research in the Canton of Graubünden falls under the remit of the Cantonal Ethics Committee Zurich. Changes to the study protocol are not permitted without the prior approval of the CEC, except for the immediate elimination of obvious risks to the participants. These must be reported to the CEC in accordance with section 2.10.

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 study. Corrections must be reported in accordance with section 2.10.

2.4 Competent Authorities (CA)

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

2.5 Ethical Conduct of the Study

The study will be conducted in accordance with the present protocol and the current version of the Helsinki Declaration, the ICH-GCP.

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

2.6 Declaration of Interest

The sponsor/investigator (principal investigator) has no conflict of interest of a financial nature, nor is there a corresponding relationship of dependency.

2.7 Patient Information and Informed Consent

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

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Each participant will receive written subject information and the consent form with a description of the study with all the necessary information to be able to decide whether to participate in the study. She will be given enough time to read this written information at home and ask questions to the responsible investigator.

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

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

2.8 Participant privacy and confidentiality

The examiners ensure that the privacy of the participant is guaranteed. 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 is encrypted and treated confidentially in accordance with the encryption (coding) accepted by Swissethics for a person participating in a research project; third parties are not permitted access to the personal data. The digital data collected will only be stored on the institute's own computers and will not be passed on to any external persons or transferred to other computers.

Direct access to the personal data is only allowed to the head of the research laboratories (study director), the study supervisor, as well as authorised persons of the CEC.

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3. BACKGROUND AND RATIONALE

3.1 Background and Rationale

Thermotherapies

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 whose effect 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 therapies are used to positively influence objective parameters such as 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, cold therapy is not only used to optimise 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 following 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, little evidence was found that favoured whole-body cooling over "non-cooling". In contrast, Leeder et al. (2012) were able to show that cold water immersion is 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 pre-activity cooling can have an effective positive effect on endurance performance.

Hot water immersions have been used in medicine for a wide variety of diseases for some time. Hydrotherapy has thermal, mechanical and chemical effects and has a vasodilatory and circulation-promoting effect (An et al. 2019). The effect of hot water immersion on recovery after intense physical exertion was first studied in 1995 (Viitasalo et al. 1995). At that time, it was hypothesised that hot water immersions increased the release of proteins from muscle tissue into the blood, thus promoting the maintenance of neuromuscular performance capacity. Three years later, a research team investigated the effects of hot water immersions compared to cold water and contrast therapy and found that cold thermotherapies showed statistically significant differences on the subjects' pain reports compared to warm thermotherapies (Kuligowski et al. 1998). However, especially in activities that require excessive force production, they recommend muscle strength rather than pain perception, which should be considered as the decisive factor when returning to training or competition. More recent studies comparing hot water therapy with cold water therapy found that while cold water therapy is more effective, hot water therapy also showed effects on various objective recovery parameters and tests measured after intense exercise (Ascensão et al. 2011, Vaile et al. 2008). Different protocols, for example in relation to water temperature, duration and immersion depth, as well as a lack of answers regarding the effect of hot water immersion on recovery, still do not allow any clear conclusion to be drawn (Versey et al. 2013).

At present, there are still too few studies in the literature that have investigated the effect of cold water immersion compared to hot water immersion on recovery.

Hypoxia

The best example of multiple stressors occuring in combination is when climbing to high altitudes. Although the percentage of oxygen remains constant at different altitudes (20.93%), the air pressure

decreases exponentially as the altitude increases. As a result, the partial pressure of oxygen in the arterial blood and tissues is reduced (hypoxia), leading to a deterioration in both physical and cognitive performance. However, in addition to the stressful effects of hypoxia, other stressors, such as temperature and fatigue, can also be added, which are known to significantly affect human function (McMorris et al. 2017).

To the knowledge of the authors, however, there are only limited studies investigating physiological parameters of recovery in laboratory conditions. In addition, various patient groups also suffer from lung dysfunction (such as COPD) and are therefore at least partially under hypoxic conditions. With this study, we can contribute to understanding the psychophysiological responses of recovery under hypoxia.

Aim of the study

In this project, the effect of repeated cold water immersions (10 °C for 10 min and again after 2 hours), repeated hot water immersions (40°C for 10 min and again after 2 hours), a hypoxia group (jump protocol under hypoxia and subsequent supine position for 10 min), and a passive control group (lying still in a supine position for 10 min) will be compared. The influence of these interventions and their effects will be investigated after a maximum jump load (5 x 20 jumps). The objective recovery parameters are vertical jump height, MVIC of the ventral thigh muscle, heart rate, muscle swelling, skin and core body temperature, measurement of inflammatory parameters, oxygen saturation of the blood and thigh muscles. Subjective recovery parameters are the local muscle soreness data, the general exhaustion and shortness of breath as well as information on thermal comfort and temperature sensation. The parameters are measured directly and 24, 48 and 72 hours after exercise. The aim is to determine the effects of thermotherapy in order to be able to make recommendations. The current state of scientific knowledge calls for further studies to substantiate or refute effects that have already been established.

3.2 Investigational Product (treatment, device) and Indication

The Aspen whirlpool from NetSpa is an inflatable pool with four seats and a capacity of 700 litres. The heating of the 168cm x 168cm hot tub can be adjusted up to 42°C. The water filtration runs automatically. The CE marking indicates that the product fulfils the essential requirements of the European Directives 2006/95/ EC (Low Voltage): 2004/108/ EC (electromagnetic compatibility) 2011/ 65 EU (restriction of the use of certain substances in equipment e) 2009/125/EC (Ecodesign of Energy-Related Products).

Image of the Aspen whirlpool: https://www.netspa.eu/en/produit/2020-spa-netspa-aspen

Simulated altitude training is rapidly becoming the most promising development in the world in terms of athletic performance and peak fitness. With the Cloud 9, a simulated altitude of up to 4000 m above sea

level can be achieved. This corresponds to an oxygen partial pressure of 12.7 %. The Cloud 9 altitude training device is designed for all levels of athletes (including amateurs) who want to improve their performance. The Cloud 9 is a solid, certified product that complies with the European Directives on electromagnetic compatibility, machinery directive, air pressure equipment and low voltage equipment (89/336/EEC, 91/368/CEE, 93/68/CEE, 97/23/EC, EN61010-1).

Image of the Cloud 9: https://chasingtargets.eu/altitude-tent-rental-service/

An already approved ethics application with Cloud9 has already been carried out by the research staff in the laboratory premises in Landquart (BASEC ID 2019-00504). The research staff also have experience in the field of cold water baths based on an already approved ethics application, which investigated the effect of cold water baths following to a muscle damage protocol (BASEC ID PB_2016-01125).

All those present are able to carry out "first aid" measures. A fully functional and annually serviced defibrillator is located in the research laboratory and can be put into operation by all investigators present. The room in which the study takes place has the following dimensions: 7m x 14m x 2.5m (98 square meters or 245 cubic meters) and can be ventilated.

3.3 Preclinical Evidence

Various studies have already shown that cooling has a positive influence on performance and recovery capacity compared to non-cooling measures. These positive effects have been demonstrated 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; Pointon, 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).

To date, only a few studies have investigated the effect of hot water immersion on recovery (Bieuzen 2013, Kuligowski 1998, Pournot 2010, Vaille 2007, Viitasalo 1995). In one study, isometric force recovery

was found to be more effective after the hot water bath compared to other thermotherapies such as a cold water bath and a passive control intervention (Vaile, 2007). Pournot (2010) also found significant changes in subjective symptom assessment, the number of leukocytes and creatine kinase. Cold water baths are already used in the scientific literature for a duration of 10 minutes at a water temperature of 10°C (Hohenauer et al 2018, Hohenauer et al 2020). However, cold water baths have also been carried out for up to 20 minutes at a water temperature of 10°C (Kositsky et al 2020). In the field of hot water baths, studies have already been carried out using water temperatures of 40°C for a duration of 40 minutes (McIntyre et al 2021, Zurawlew et al 2015). No side effects were reported with these water baths. Repeated cold and hot water interventions after 2 hours could help to reduce the intracellular volume increase that occurs within this period (White et al 2013) after exercise.

Studies under hypoxic conditions have already been conducted in the medical field with a wide variety of pathologies, such as obesity under stress (Girard, Malatesta, and Millet 2017, Park et al. 2019) and also with senior citizens (Pramsohler et al. 2017). In addition to physiological changes, hypoxic conditions can lead to psychological changes (Taylor et al. 2015). Chronic altitude exposure can further lead to physiological changes (Smith et al. 2014)

It is known that cognitive and physical performance in normoxia improves with light moderate exercise (Chang et al. 2012). In addition, there is growing evidence that exercise can even reverse the negative effects of hypoxia on cognitive performance (Ando et al. 2013, Komiyama et al. 2017).

3.4 Clinical Evidence to Date

Current research results regarding the effects of thermotherapy and training under hypoxia conditions are listed in chapter 3.3.

According to the authors' knowledge, there are currently hardly any clinical studies that contrast the psychophysiological effects of thermotherapy with simulated altitude training and investigate its recovery.

3.5 Dose Rationale / Medical Device: Rationale for the intended purpose in study (pre-market MD)

Doctors and physiotherapists can opt for cold treatments in their treatment, for example, to relieve pain. However, these professional groups not only work with patients but also with amateur and elite athletes and also provide them with individualised care. The project uses the Aspen hot tub from NetSpa. This is used within the scope of the intended application and in accordance with the operating manual. The applied temperatures do not pose a risk to the test subject at any time. The safety instructions are strictly followed.

The temperature for hot water immersion of 40°C is generated for a maximum of 10 minutes (and again after 2 hours). With this setting, we are within the manufacturer's recommendations from the operating manual. For cold water immersion, a temperature of 10°C is chosen for 10 minutes (and again after 2 hours). Physiotherapists, sports physicians and coaches can benefit from these findings. The question of whether the recovery phase can be shortened or whether performance can be increased by means of cooling applications is legitimate. These two parameters include not only objective values but also subjective information from the athlete.

During the normobaric, hypoxic situation, an altitude of up to 4000 m.a.s.l. is simulated, which corresponds to an oxygen partial pressure of 12.7%. This situation is realized with the help of Cloud 9.

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3.6 Explanation for choice of comparator (or placebo)

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 assignment is carried out randomly. Randomization is guaranteed by drawing a lot.

Cold water bath vs. hot water bath vs. hypoxia group vs. passive control group

Four groups are compared to each other in order to compare the effect of cold water immersion versus hot water immersion, as well as the hypoxia group and the passive control group. The aim is to analyse the efficiency of thermotherapy on the ability to recover. All groups undergo the jump protocol. Both water bath groups are then immersed up to the sternum in water at a temperature of 10°C or 40°C (1 x 10 min and after 2 hours). The hypoxia group will perform the jumping protocol under hypoxic conditions and will then recover for 10 minutes at room temperature in a supine position like the passive control group.

3.7 Risks / Benefits

For the test subjects, there is a minimal risk of overloading during the maximum jumping exercise (5 x 20) of the ventral thigh muscles until exhaustion. The test subjects are expressly informed that they can stop the maximum load at any time. Furthermore, two examiners will be next to the test subject during the 1 min jumping exercise in order to be able to catch her in case of circulatory problems. A fully equipped emergency kit is in the examination room at all times during the examinations. In the event of circulatory problems due to hypoglycaemia, dextrose, chocolate, and Coca-Cola® Ltd. are also available in the examination room. The test subjects are also advised to complete the test well nourished in order to minimise the risk of hypoglycaemia. A fully functional defibrillator (Lifepak Express Defibrillator, reaplus.ch) which is accessible at all times, is available at our institution. This information is communicated to the test persons (subject information).

During body cooling or heating, there is only a minimal risk for the test subjects due to the short application times. The test subjects enter the water baths in swimwear and without body jewellery (piercings, rings, necklaces, etc.). Furthermore, the test subjects should not apply skin creams or other cosmetics to the skin. The investigators of this study are able to operate a fully functional annually maintained defibrillator. An examiner is at eye level with the test subject during the entire application period and monitors her. The investigators in this study can competently perform "first aid" measures. A "first aid" kit is in the research laboratory at all times. Furthermore, any injury to the musculoskeletal system was defined as an exclusion criterion in order not to provoke a relapse of a past injury.

Possible risks in the situation of hypoxic conditions are the occurrence of headaches, loss of consciousness, disorientation and nausea. Potentially at-risk subjects are identified by the screening procedure and the health questionnaire and are not included in the study. However, the risk is to be classified as low, as studies have already been conducted with obese patients and seniors under hypoxic conditions, which did not lead to any complications.

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

The test subjects do not gain any direct benefits from participating in the study.

3.8 Justification of choice of study population

This remaining category A clinical trial (Chapter 4) will be conducted with healthy young female volunteers. The risk of injury is considered low in this test population.

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Inclusion criteria	Exclusion criteria
- Young healthy women aged 18 - 35 years	- Current injuries of any kind affecting the trunk and/ or lower extremities
- No surgical interventions on the musculoskeletal system in the trunk area and on the lower extremities	- Injuries to the trunk and/ or lower extremities that occurred less than 1 year ago
- Anticonceptives	- Injuries to the trunk and/ or lower extremities that occurred more than 1 year ago and are still causing symptoms
	- Fear of cold and/or hot water intervention
	- Fear of hypoxia
	- Taking medication of any kind (including self-purchased medication)
	- Pacemakers & cardiac arrhythmias
	- Known circulatory problems
	- Positive pregnancy test
	- Diagnosed skeletal static deviations
	- Appendectomy less than 2 years ago
	- Raynaud's syndrome

Before agreeing to participate in the study, the test subjects are given comprehensive information about the procedure and possible risks. They will also receive the information in written form and have the opportunity to ask questions. The informed consent form explains to them what their rights and obligations are if they participate. The subject information states that female subjects agree to take a pregnancy test (urine test) if they have decided to participate in the study. This test will be carried out once the test subjects have signed the consent form. The urine test will be given, received and interpreted by the study director. The test subjects do not have to expect any costs for this test. A positive urine test will result in the exclusion of this study. Only the principal investigator knows the result of the urine tests of the female subjects. Nobody except the head of the research laboratories (study director), the study supervisor, as well as authorised persons of the CEC can view this test result (see point: 2.8. Confidentiality and privacy). In addition, all test subjects are informed that they may withdraw from participation at any time without giving reasons, without any disadvantages for them. The current regulations of the Canton of Graubünden and those of the FOPH for protection against the coronavirus will be taken into account and implemented when conducting the study.

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4. STUDY OBJECTIVES

4.1 Overall Objective

In this study, the effect of body cooling for a maximum of 10 minutes and renewed cooling or warming after 2 hours on the subjective muscle soreness and general exhaustion 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. In addition to the parameters already mentioned, the heart rate, skin temperature, core body temperature, muscle swelling and oxygen saturation of the thigh muscles as well as inflammation parameters of the blood are to be determined. The effects of thermal application will be contrasted with a hypoxia group and a passive control group 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 Objective

In this project, the effect of post-activity cooling or warming on physiological and psychological recovery parameters is measured and compared to the hypoxia intervention and the passive control group.

Physiological measurements

- Jump height measurement (cm)
- Maximum voluntary isometric thigh contraction (kg)
- Muscle swelling (mm)
- Blood oxygen saturation (%)
- Oxygen saturation of the muscles (%)
- Heart rate (bpm)
- Skin temperature (°C)
- Core Body Temperature (°C)
- Inflammation parameters; IL6 (pg/ml), CRP (mg/dl), creatine kinase (U/L)

Psychological measurements

- Dyspnea questionnaire (mod. Borg scale)
- Exhaustion questionnaire (Borg scale)
- Subjective muscle soreness (VAS scale)
- Thermal comfort and thermal sensation

4.3 Secondary Objectives

none

4.4 Safety Objectives

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

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5. STUDY OUTCOMES

5.1 Primary Outcome

Jump height measurement

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

Muscle activity (MVIC)

MVIC is performed on the Cor 1 – ergometer chair for biomechanical measurements V1.0 and is expressed in kg (www.otbioelettronica.it). The conduction velocity is measured using the surface electromyogram system EMG-USB2 from OT Bioelettronica (otbioelettronica.it), bandpass filtered between 10-750Hz and rectified (full-wave rectify). MVIC is measured on the Cor 1 ergometer chair in 90° knee flexion. The raw data of muscle activity to measure conduction velocity is processed using OT Bioelettronica software (MISO II). The electrodes are placed according to the guidelines of Barbero et al. (2012) "Atlas of Muscle Innervation Zones". The measurement takes place immediately, 24 hours, 48 hours and 72 hours after the intervention.

Muscle swelling

Muscle swelling of the thigh is measured by ultrasound imaging (MyLabClassC, Esaote, Genoa, Italy). The images were evaluated using the OsiriX DICOM viewer software (OsiriX, Pixmeo SARL, Switzerland). The measurement takes place before, directly, 24 hours, 48 hours and 72 hours after the intervention.

Oxygen saturation of the blood

The oxygen saturation of the blood is measured with a portable pulse oximeter with a finger clip probe (Nonin 7500, Nonin medical B.V., Plymouth, USA). This measurement takes place during the baseline measurements as well as during the jump protocol.

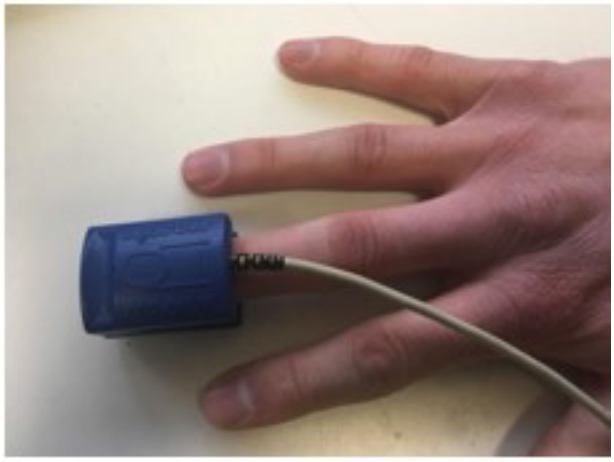

Figure: Measurement of the oxygen content of the blood

Oxygen saturation of the muscle

The oxygen saturation (%) of the thigh muscles is measured with the tissue oxygenation monitor using near-infrared spectroscopy (moorVMS-NIRS, moor instruments, www.moor.co.uk). The measurement is non-invasive and takes place via near-infrared light. The sensors are fixed around the corresponding

muscles. The muscle oxygen saturation measurement is taken directly and 24, 48 and 72 hours after the intervention.

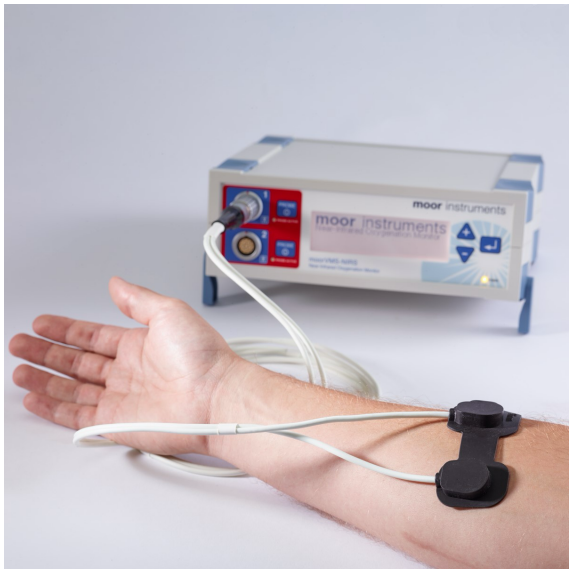

Figure: Measurement of the oxygen saturation of the muscle

Heart rate

The heart rate is determined by means of a heart rate monitor with a chest strap (Polar T31, Polar Inc. Kempele, Finland). Blood pressure and heart rate are measured directly and 24, 48 and 72 hours after the intervention.

Skin temperature

Skin temperature is measured via the iButton system (www.ibuttonlink.com). The self-adhesive sensors transmit the information about the skin temperature wirelessly to a computer. Furthermore, the skin temperature is recorded using a thermal imaging camera (A655 sc series, FLIR, InfrarotTec Systems, Ranstadt, Germany). The Skin temperature is measured directly and 24, 48 and 72 hours after the intervention. The iButtons are attached to the following locations using a tape strip: centre of the shoulder blade, neck (6th cervical vertebra), back of the hand, centre of the shin and centre of the thigh. The iButtons are removed from the affected areas during the cold water bath or hot water bath and then reattached.

Core Body Temperature

The core body temperature is determined via the e-Celsius® performance (https://www.bodycapmedical.com). With the help of this ingestible capsule, the core body temperature is continuously monitored, recorded and transmits the data wirelessly to a monitor (BodyCAP medical, Hérouville Saint-Clair, France). Core body temperature is measured directly and 24, 48 and 72 hours after the intervention.

Inflammation parameters

The measurement of inflammatory parameters such as creatine kinase (CK), interleukin 6 (IL6) or C-reactive protein (CRP) is carried out via a venous blood sample of approx. 6ml (Accutrend, Roche Diagnostic, Rotkreuz, Switzerland & Reflotron, Roche Diagnostic, Rotkreuz, Switzerland). The measurement takes place directly, 24 hours, 48 hours and 72 hours after the intervention by trained specialists.

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Subjective information on breathlessness

The subjects indicate their subjective feeling of breathlessness on a modified BORG scale (0 – 10). The scale will be scaled from "0" (no shortness of breath at all) to "10" (maximum breathlessness). The measurement takes place before the intervention.

Subjective information on general exhaustion

The subjects indicate their subjective general feeling of exhaustion on a BORG scale (6 – 20). The scale will be scaled from "6" (very, very light load) to "20" (very, very strenuous load). The measurement takes place before 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" (greatest imaginable muscle soreness) in cm increments. The measurements are take immediately, 24 hours, 48 hours and 72 hours after the intervention.

Thermal comfort and temperature sensation

This parameter is measured using a scale. The test subjects indicate their subjective temperature perception on a 9-point scale from -4 (very cold) to +4 (very hot). They then rate the thermal comfort between 0 (pleasant) and 4 (extremely unpleasant) on a 5-point scale. These measurements are taken before and after the intervention.

5.2 Secondary outcomes

None available.

5.3 Other Outcomes of Interest

None available.

5.4 Safety Outcomes

No further outcomes are included that serve the purpose of safety.

Discontinuation of the experiment is the responsibility of the examiners with regard to the subject's resilience. If this is judged to be too low, the examiner can stop the experiment.

If the test subject does not feel well according to her own subjective assessment, she can stop the experiment and withdraw participation in the study. If the cold application is unacceptable for the test subject, she can discontinue the application. She will not suffer any disadvantages as a result.

The current regulations of the Canton of Graubünden and those of the FOPH for protection against the coronavirus will be taken into account and implemented when conducting the study.

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6. STUDY DESIGN

6.1 General study design and justification of design

Randomized, controlled trial

This is a remaining clinical trial in risk category A (Chapter 4).

The group size of the participants is N = 60 people. Group allocation is randomised.

6.2 Methods of minimising bias

All measurements are carried out and recorded in a standardized manner by the same tester. The laboratory conditions are always the same.

6.2.1 Randomsation

The allocation to the different groups (cold water bath, hot water bath, hypoxia, control group) takes place randomly by drawing of a lot.

6.2.2 Blinding procedures

Blinding the participants is not possible in this study. The investigators are blinded by adding another investigator who operates the apparatus. The responsible statistician is blinded and has no access to the personal data of the test subjects and is not present when the data is collected.

6.2.3 Other methods of minimising bias

The content of the dialogue between the test subjects and the examiner is kept to a minimum.

6.3 Unblinding Procedures (Code break)

There is no provision for cancelling the statistician's blindness.

Unblinding of the investigators and/or the study participants is only permitted if an unforeseen event or medical problems occur during or after completion of the study participation in a subject which may be attributable to participation in the study.

In the event of adverse events (AEs) occurring during or shortly after study participation that may be attributable to the intervention, the principal investigator may, at his or her discretion, immediately unblind the study participant and the attending investigators to ensure adequate treatment of the study participant.

In the case of incidents (AE) that occur for longer than 24 hours, clarification by a doctor is mandatory and a medical certificate is required for the cancellation of the blindness. Data may then also be passed on to the attending physician with the consent of the study participant concerned. This approach is justified, as no life-threatening incidents are to be expected as a result of the interventions used here.

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7. STUDY POPULATION

7.1 Eligibility criteria

Inclusion criteria

- Young healthy women aged 18 - 35 years
- No surgical interventions on the musculoskeletal system in the trunk area and on the lower extremities
- Anticonceptives

Exclusion criteria

- Current injuries of any kind affecting the trunk and/ or lower extremities
- Injuries to the trunk 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 cold and/or hot water intervention
- Fear of hypoxia
- Taking medication of any kind (including self-purchased medication)
- Pacemakers & cardiac arrhythmias
- Known circulatory problems
- Positive pregnancy test
- Diagnosed skeletal static deviations
- Appendectomy less than 2 years ago
- Raynaud's syndrome

To our knowledge, only a few studies have so far investigated the physiological response or recovery after a hot/ cold water bath, hypoxia and control treatment in women. This is consistent with the significantly underrepresented female participation in the wider sports and exercise medicine literature (Costello et al. 2014). In addition, a recent Cochrane review on whole-body cryotherapy concluded that the existing literature on cryotherapy may not be applicable to women and warrants future research on female participants in this area (Costello et al. 2015). In addition, the altered hormone status during the female cycle (e.g., estrogen) and anthropometric differences (e.g., fat distribution and total body fat) could lead to differences according to a muscle-damaging protocol in women.

7.2 Recruitment and screening

Recruitment

It is published on the website (homepage & Facebook) of Thim van der Laan University College Physiotherapy and the University of Applied Sciences and Arts of Southern Switzerland (SUPSI). The exact texts have been prepared according to the checklist of the Cantonal Ethics Commission Zurich.

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As advertisements (text visible):

- Homepage of the Thim van der Laan School of Physiotherapy: www.physioschule.ch and SUPSI: www.supsi.ch
- Facebook page of the Thim van der Laan Physiotherapy School: https://www.facebook.com/physioschule and SUPSI:

  https://www.facebook.com/supsi.physiotherapie/

Screening

During the initial contact, the potential participants are informed about the procedures and risks of the study, as well as its conditions and the amount of compensation. A checklist for contacting them by telephone serves as a basis.

During the first telephone contact, a questionnaire to determine the suitability as a study participant is completed by the study management. In advance, it is explained to the subject that answering the questions is voluntary and can be interrupted at any time and that all registered information will be deleted immediately if they decide not to participate in the study.

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

7.3 Assignment to study groups

Group allocation is carried out secretly with the help of a ticket in a sealed envelope, which is drawn under the supervision of the study director. This lot indicates whether the test subject is undergoing the cold water bath, hot water bath, hypoxia or control intervention.

7.4 Criteria for withdrawal / discontinuation of participants

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

The already completed consent form is kept in the locked filing cabinet. If consent is withdrawn, the data and samples already collected must be included in the analysis for scientific and safety reasons. These will only be anonymised after the analysis (Art. 9 ClinO).

The conditions for discontinuing the trial were described in chap. 5.4. There is no disadvantage for the participants if they discontinue their participation in the study.

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8. STUDY INTERVENTION

8.1 Identity of Investigational Products (treatment / medical device)

8.1.1 Experimental Intervention (treatment / medical device)

Both application devices (Cloud9 and Whirlpool Aspen) are certified products.

Whirlpool Aspen

The Aspen Whirlpool is an inflatable water pool with four seats and a capacity of 700l. The heating of the 168cm x 168cm whirlpool can be adjusted up to 42°C The water filtration runs automatically. The CE marking indicates that the product meets the essential requirements of the European Directives 2006/95/EC (Low Voltage), 2004/108/EC (electromagnetic compatibility) 2011/65/EU (restriction of the use of certain substances in appliances e) 2009/125/EC (ecodesign of energy-related products).

Securing the test subjects

Only subjects who have completed the health questionnaire without risk factors will be admitted to the study. In this way, risk factors are recognised at an early stage and the subjects for the study are not recruited.

The examiner is in direct contact with the subject at all times. The examiner is at eye level with the test subject and monitors the procedure. With their signed consent form, the test subjects declare that they have already taken a pregnancy test (urine test). This test is handed out, collected and interpreted by the head of the study. Only the principal investigator, the study supervisor and authorized employees of the CEC can view this test result. The thermo- or hypoxia application can be discontinued by the investigator at any time. If the thermal or hypoxia application is perceived as unpleasant by the test subjects, they can cancel the application at any time. Therefore, there is only a minimal risk for the test subjects. Furthermore, a fully functional and annually maintained defibrillator is available at the time of the study. All research staff and investigators can operate this defibrillator and have the competence of "first aid" measures. For these reasons, the presence of a doctor is not required. The test subjects are only allowed to enter the interventions without metal body jewellery (no piercings, rings, necklaces, etc.). Furthermore, the test subjects are advised to refrain from skin creams or other skin cosmetics. This is also evident in the subject information.

8.1.2 Control Intervention (standard/routine/comparator treatment / medical device)

Hypoxia group

With the Cloud 9, a simulated altitude of 2700 m above sea level and 4000 m above sea level can be achieved. This corresponds to an oxygen partial pressure of 15.0% and 12.7% respectively. The Cloud 9 altitude training device is intended for anyone (including amateurs) who wants to inprove their performance. The Cloud 9 is a solid, certified product that complies with the European Directives on electromagnetic compatibility, Machinery Directive, air pressure equipment and low voltage equipment (89/336/EEC, 91/368/CEE, 93/68/CEE, 97/23/EC, EN61010-1).

Control group

The control group will lie on their backs for 10 minutes after the jump protocol.

8.1.3 Packaging, Labelling and Supply (re-supply)

The Whirlpool Aspen from NetSpa complies with European directives (2006/95/EC, 2004/108/EC, 2011/65/EU, 2009/125/EC). The Cloud 9 is a certified product according to European directives (89/336/EEC, 91/368/CEE, 93/68/CEE, 97/23/EC, EN61010-1).

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8.1.4 Storage Conditions

The whirlpools are kept in a room at room temperature and low humidity all year round. The room in which the whirlpools are located is fully ventilated and has the following dimensions: 7m x 14m x 2.5m and thus complies with the specifications in the operating manual.

The Cloud 9 can be stored both outside in the dry and inside a room. The equipment is located inside our laboratory for the duration of the measurements and is only accessible to laboratory staff.

8.2 Administration of experimental and control interventions

As no prescription drugs and only minimally invasive methods are used, the experiment is not discussed further in chapters 8.2-8.4 and reference is made to cChapter 9.

8.2.1 Experimental Intervention

Cold water immersion

Test subjects in this group are asked to take a cold water bath after carrying out the jump protocol. The application lasts 10 minutes (and is repeated after 2 hours) at a water temperature of 10°C. In a sitting position, the water level must reach the sternum.

Hot water immersion

Test subjects in this group are asked to take a hot water bath after carrying out the jump protocol. The application lasts 10 minutes (and is repeated after 2 hours) at a water temperature of 40°C. In a sitting position, the water level must reach the sternum.

In all experimental interventions, an examiner is located directly next to the test person for monitoring at all times.

8.2.2 Control Intervention

Hypoxia group

The subjects of the hypoxia group will perform the jump protocol under hypoxia, which is generated with the Cloud 9. This intervention is used to determine the physiological effect under hypoxic conditions.

Control group

The test subjects of the control group are in our laboratory and are asked to lie on their back for 10 minutes after completing the jump protocol.

During all control interventions, an examiner is located directly next to the test person for monitoring at all times.

8.3 Dose / Device modifications

All participants will be treated with the same duration or dosage within their group.

8.4 Compliance with study intervention

The test subjects are only motivated to perform MVIC and the jump height measurement in order to keep their compliance high. The rest of the communication is kept to a minimum. Only study-related conversations are conducted in order not to influence the psychophysiological effects and to ensure homogeneity.

8.5 Data Collection and Follow-up for Withdrawn Participants

If consent is withdrawn, the data and samples already collected must be included in the analysis for scientific and safety reasons. These are only anonymised after the analysis (Art. 9 ClinO).

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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 discontinued due to a reaction occurring during study participation, this will be recorded and reported to the sponsor investigator (head of the research laboratory), study director and supervisor of the study. If medical treatment is required, the report will be forwarded to the business liability insurance of Thim van der Laan AG (Basler Versicherung) to ensure the appropriate follow-up treatment.

8.6 Trial specific preventive measures

The questionnaire asks about the intake of medication. In addition, the test subjects agree to undergo a pregnancy test (urine test) by signing the consent form. This test is handed over, explained, received and interpreted by the head of the study (Ron Clijsen, PhD). They do not have to pay any costs for it – the test is provided to them free of charge. A positive pregnancy test leads to the exclusion of this study. Only the principal investigator, the study supervisor and authorised persons of the ethics committee can view this test result. No one else will know the result of this test. If deviations in the statics are visible but not medically diagnosed, the study director reserves the right to measure them (leg length difference, scoliosis angle) and to exclude test subjects from participation.

8.7 Concomitant Interventions (treatments)

No further side effects are expected as a result of participation in the study.

8.8 Study Drug / Medical Device Accountability

The experiments take place exclusively in the research laboratory of the University of Applied Sciences and Arts of Southern Switzerland, Physiotherapy Graubünden, in Landquart. This means that there is no transport of the products and measuring devices used. There is therefore no liability for the transport of the product.

8.9 Return or Destruction of Study Drug / Medical Device

The Cloud 9, as well as the two Aspen whirlpools, were acquired by the research department of the "University of Applied Sciences and Arts of Southern Switzerland" and will remain in the research laboratory after completion of the measurements.

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9. STUDY ASSESSMENTS

9.1 Study flow chart(s) / table of study procedures and assessments

The test subject information is sent to the test subjects' homes in advance or given to them personally

1. **Subject Information & Informed Consent**

   30 min (questions and ambiguities will be discussed individually)

2. **Preparation of the test person**

   15 min

3. **Executing the test protocol**

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Test protocol: Time required – maximum 255 min			
Cold Water Immersion vs. Hot Water Immersion vs. Hypoxia Group vs. Passive Control Group			
Cold water immersion **1.) Baseline** Informed consent, written consent, review of inclusion/exclusion criteria, health questionnaire, pregnancy test Heart rate Skin/ core body temperature Oxygen saturation (blood, muscle) Inflammation parameters VAS & BORG thermal comfort and thermal sensation US 3 x Vertical Jumps 3 x MVIC *=30 min* **2.) Jump protocol** 5 x 20 max vertical jumps *after 30sec* Heart rate Skin/ core body temperature	Hot water immersion **1.) Baseline** Informed consent, written consent, review of inclusion/exclusion criteria, health questionnaire, pregnancy test Heart rate Skin/core body temperature Oxygen saturation (blood, muscle) Inflammation parameters VAS & BORG thermal comfort and thermal sensation US 3 x Vertical Jumps 3 x MVIC *=30 min* **2.) Jump protocol** 5 x 20 max vertical jumps *after 30sec* Heart rate Skin/ core body temperature	Hypoxia Intervention **1.) Baseline** Informed consent, written consent, review of inclusion/exclusion criteria, health questionnaire, pregnancy test Heart rate Skin/core body temperature Oxygen saturation (blood, muscle) Inflammation parameters VAS & BORG thermal comfort and thermal sensation US 3 x Vertical Jumps 3 x MVIC *=30 min* **2.) Jump protocol under hypoxia** 5 x 20 max vertical jumps *after 30sec* Heart rate Skin/ core body temperature	Passive Control Intervention **1.) Baseline** Informed consent, written consent, review of inclusion/exclusion criteria, health questionnaire, pregnancy test Heart rate Skin/core body temperature Oxygen saturation (blood, muscle) Inflammation parameters VAS & BORG thermal comfort and thermal sensation US 3 x Vertical Jumps 3 x MVIC *=30 min* **2.) Jump protocol** 5 x 20 max vertical jumps *after 30 sec* Heart rate Skin/ core body temperature

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Oxygen saturation (blood, muscle) Mod. BORG & BORG *=30 min* **3.) Cold water immersion** 10 min at 10°C Heart rate Skin/core body temperature Oxygen saturation (muscle) Thermal comfort and Temperature sensation *=10 min* **4.) Follow-up** immediately after the intervention Heart rate Skin/core body temperature Oxygen saturation (blood, muscle) Inflammation parameters US VAS & BORG 3 x Vertical Jumps 3 x MVIC *=30 min*	Oxygen saturation (blood, muscle) Mod. BORG & BORG *=30 min* **3.) Hot water immersion** 10 min at 40°C Heart rate Skin/core body temperature Oxygen saturation (muscle) Thermal comfort and Temperature sensation *=10 min* **4.) Follow-up** immediately after the intervention Heart rate Skin/core body temperature Oxygen saturation (blood, muscle) Inflammation parameters US VAS & BORG 3 x Vertical Jumps 3 x MVIC *=30 min*	Oxygen saturation (blood, muscle) Mod. BORG & BORG *=30 min* **3.) Passive supine position** 10 min at room temperature Heart rate Skin/core body temperature Oxygen saturation (muscle) Thermal comfort and Temperature sensation *=10 min* **4.) Follow-up** immediately after the intervention Heart rate Skin/core body temperature Oxygen saturation (blood, muscle) Inflammation parameters US VAS & BORG 3 x Vertical Jumps 3 x MVIC *=30 min*	Oxygen saturation (blood, muscle) Mod. BORG & BORG *=30 min* **3.) Passive supine position** 10 min at room temperature Heart rate Skin/core body temperature Oxygen saturation (muscle) Thermal comfort and Temperature sensation *=10 min* **4.) Follow-up** immediately after the intervention Heart rate Skin/core body temperature Oxygen saturation (blood, muscle) Inflammation parameters US VAS & BORG 3 x Vertical Jumps 3 x MVIC *=30 min*

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5.) Cold water immersion 10 min at 10°C Heart rate Skin/core body temperature Oxygen saturation (muscle) Thermal comfort and thermal sensation *=10 min*	**5.) Hot water immersion** 10 min at 40°C Heart rate Skin/core body temperature Oxygen saturation (muscle) Thermal comfort and thermal sensation *=10 min*		
6.-8.) Follow up 24, 48 and 72 hours after the intervention Heart rate Skin/ core body temperature Oxygen saturation (blood, muscle) Inflammation parameters US VAS & BORG 3 x Vertical Jumps 3 x MVIC *=30 min per follow-up*	**6.-8.) Follow up** 24, 48 and 72 hours after the intervention Heart rate Skin/ core body temperature Oxygen saturation (blood, muscle) Inflammation parameters US VAS & BORG 3 x Vertical Jumps 3 x MVIC *=30 min per follow-up*	**5.-7.) Follow up** 24, 48 and 72 hours after the intervention Heart rate Skin/ core body temperature Oxygen saturation (blood, muscle) Inflammation parameters US VAS & BORG 3 x Vertical Jumps 3 x MVIC *=30 min per follow-up*	**5.-7.) Follow up** 24, 48 and 72 hours after the intervention Heart rate Skin/core body temperature Oxygen saturation (blood, muscle) Inflammation parameters US VAS & BORG 3 x Vertical Jumps 3 x MVIC *=30 min per follow-up*

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Test protocol	Time exposure	Visits
Information, written consent, examination of inclusion/ exclusion criteria, health questionnaires, pregnancy test, detection of adverse events	30min + 15min	Unique
Baseline,recording of adverse events	30min	Unique
Jump log, recording of adverse events	30min	Unique
Cold water immersion, recording of adverse events	20min + 130min (3x30min) = 150min	Unique
Hot water immersion, recording of adverse events	20min + 130min (3x30min) = 150min	Unique
Hypoxia group, recording of adverse events	20min + 120min (3x30min) = 140min	Unique
Control group, recording of adverse events	20min + 120min (3x30min) = 140min	Unique
Total time spent	245min or 255min	

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9.2 Assessments of outcomes

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

9.2.1 Assessment of primary outcome

Jump height measurement

The jump height after exercise is determined using 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. They are allowed to start from the squatting position to measure the jump height (countermovement jump). 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.

Muscle activity (MVIC)

MVIC is performed on the Cor 1 – ergometer chair for biomechanical measurements V1.0 and is given in kg (www.otbioelettronica.it). The conduction velocity is measured with the help of the surface electromyogram system EMG-USB2 from OT Bioelettronica (otbioelettronica.it), bandpass filtered between 10-750Hz and rectified (full-wave rectify). MVIC is measured on the Cor 1 ergometer chair in 90° knee flexion. The raw data of muscle activity to measure conduction velocity is processed using OT Bioelettronica's (MISO II) software. The electrodes are placed according to the guidelines of Barbero et al. (2012) "Atlas of Muscle Innervation Zones". The EMG electrodes are placed on the front of the thigh and on the tibia. These areas must be shaved if there is too much hair. The measurement takes place immediately, 24 hours, 48 hours and 72 hours after the intervention.

Muscle swelling

Muscle swelling of the thigh is measured by ultrasound imaging (MyLabClassC, Esaote, Genoa, Italy). The images are analysed with the OsiriX DICOM viewer software (OsiriX, Pixmeo SARL, Switzerland). The measurements are taken before, immediately, 24 hours, 48 hours and 72 hours after the intervention.

Oxygen saturation of the blood

The oxygen saturation of the blood is measured with a portable pulse oximeter with a finger clip probe (Nonin 7500, Nonin medical B.V., Plymouth, USA). This measurement takes place during the baseline measurements as well as during the jump protocol.

Oxygen saturation of the muscle

The oxygen saturation (%) of the thigh muscles is measured using the tissue oxygenation monitor using near-infrared spectroscopy (moorVMS-NIRS, moor instruments, www.moor.co.uk). The measurement is non-invasive and takes place using near-infrared light. The sensors are fixed around the corresponding muscles. The oxygen saturation measurement is taken directly and 24, 48 and 72 hours after the intervention.

Heart rate

The heart rate is determined using a heart rate monitor with a chest strap (Polar T31, Polar Inc. Kempele, Finland). Blood pressure and heart rate are measured directly and 24, 48 and 72 hours after the intervention.

Skin temperature

Skin temperature is measured via the iButton system (www.ibuttonlink.com). The self-adhesive sensors transmit the information about the skin temperature wirelessly to a computer. Furthermore, the skin temperature is recorded using a thermal imaging camera (A655 sc series, FLIR, InfrarotTec Systems, Ranstadt, Germany). The skin temperature is measured directly and 24, 48 and 72 hours after the intervention. The iButtons are applied with a tape strip to the following areas: centre of the shoulder blade, neck (6th cervical vertebra), back of the hand, centre of the shin, and centre of the thigh. The iButtons are removed from the affected areas during the cold water bath or hot water bath and then reattached.

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Core Body Temperature

The core body temperature is determined via the e-Celsius® performance (https://www.bodycapmedical.com). With the help of this ingestible capsule, the core body temperature is continuously monitored, recorded and transmits the data wirelessly to a monitor (BodyCAP medical, Hérouville Saint-Clair, France). Core body temperature is measured directly and 24, 48 and 72 hours after the intervention.

Inflammation parameters

The measurement of inflammatory parameters such as creatine kinase, interleukin 6 or C-reactive protein (CRP) is carried out via a venous blood sample of approx. 6ml (Accutrend, Roche Diagnostic, Rotkreuz, Switzerland & Reflotron, Roche Diagnostic, Rotkreuz, Switzerland). The measurement is carried out immediately, 24 hours, 48 hours and 72 hours after the intervention by trained specialists.

Subjective information on shortness of breath

The subjects reported their subjective feeling of shortness of breath on a modified BORG scale (0 – 10). The scale will be scaled from "0" (no shortness of breath at all) to "10" (maximum breathlessness). The measurement takes place before the intervention.

Subjective information on general exhaustion

The subjects reported their subjective general feeling of exhaustion on a BORG scale (6 – 20). The scale will be scaled from "6" (very, very light load) to "20" (very, very strenuous load). The measurement takes place before the intervention.

Subjective muscle soreness

The test 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.

Thermal comfort and temperature sensation

This parameter is measured using a scale. The test subjects indicate their subjective temperature perception on a 9-point scale from -4 (very cold) to +4 (very hot). They then rate the thermal comfort from 0 (pleasant) and 4 (extremely unpleasant) on a 5-point scale. These measurements are taken before and after the intervention.

9.2.2 Assessment of secondary outcomes

None available.

9.2.3 Assessment of other outcomes of interest

None available.

9.2.4 Assessment of safety outcomes

No corresponding parameters are collected.

9.2.4.1 Adverse events

-

9.2.4.2 Laboratory parameters

-

9.2.4.3 Vital signs

-

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9.2.5 Assessments in participants who prematurely stop the study

There is no disadvantage for the participants if they (arbitrarily) discontinue study participation, and no further interventions are necessary for follow-up treatment.

However, if participation in the study is cancelled due to an injury sustained during participation, this will be recorded and reported to the sponsor, the head of the research laboratory, the study director, and the supervisor of the study. The report is also sent to Thim van der Laan AG's public liability insurance to ensure the appropriate follow-up treatment.

The following are recorded:

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

9.3 Procedures at each visit

9.3.1 Single measurement

The project involves multiple visits.

9.3.2 Multiple visits

The project involves multiple visits with a maximum duration of 255 minutes, which are organised as follows:

1. Subject information & informed consent

The test subjects are given comprehensive information about the procedure and possible risks before agreeing to participate in the study. They also receive the information in written form and have the opportunity to ask questions. The informed consent form explains to them what their rights and obligations are when participating and that they may withdraw from participation at any time without giving reasons and without incurring any disadvantages. More detailed information can be found in section 2.7.

2. Preparing the test subjects

If the test subject is willing to participate, she is instructed to get changed in a separate room.

First, the locations for the placement of the electrodes are marked with a skin-friendly pen (eyeliner) and, if necessary, individual locations of the EMG electrode placements are shaved (both were explained to the test subject beforehand according to a checklist). The electrodes are then attached. The electrodes are attached to the lower third of the anterior thigh muscle. Furthermore, the locations for placing the sensors for measuring skin temperature and oxygen saturation on the thigh are marked with a skin-friendly pencil (eyeliner). A heart rate monitor is applied to the test subject and to start the measurement of the core body temperature, the test subject is asked to take a capsule. It is also checked that no metal jewellery is worn on the body before the test subjects complete the jump protocol.

3. Measurements

The tests are carried out, and the data is collected in accordance with the test protocol.

4. Compensation and Conclusion

After completion of the individual measurement, the test subject is asked to change again in a separate room. Afterwards, she is asked how she feels. The subject is asked to report back to the laboratory at intervals of 24 hours, 48 hours and 72 hours to carry out the follow-up measurements. MVIC, the

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maximum jump height, skin temperature, core body temperature, heart rate, inflammation parameters, muscle swelling and oxygen saturation in the thigh are measured again. Furthermore, subjective muscle soreness and general exhaustion are recorded. After completion of the measurements, the test subject receives the compensation to which they are entitled in accordance with the information in the advertisement:

Flat rate CHF 50 after completion of the measurements (measurement time: after 72 hours).

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10. REGULATORY ASPECTS AND SAFETY

Other clinical trial with a medical device in the risk category Cat. **A** (chap. 4)

No medication is administered to the test subjects and no tissue samples are taken. Only minimally invasive methods are used.

The current regulations of the Canton of Graubünden and those of the FOPH for protection against the coronavirus will be taken into account and implemented when conducting the study.

10.1 Local regulations / Declaration of Helsinki

This study is conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP, the HRA as well as other locally relevant legal and regulatory requirements.

10.2 (Serious) Adverse Events and notification of safety and protective measures

An Adverse Event (AE) is any untoward medical occurrence in a patient or a clinical investigation subject, which does not necessarily have a causal relationship with the trial procedure. An AE can therefore be any unfavourable or unintended finding, symptom, or disease temporally associated with a trial procedure, whether or not related to it.

A Serious Adverse Event (SAE) (ClinO, Art. 63) is any untoward medical occurrence that

-Results in death or is life-threatening,

-Requires in-patient hospitalisation or prolongation of existing hospitalisation,

-Results in persistent or significant disability or incapacity, or

-Causes a congenital anomaly or birth defect

Both Investigator and Sponsor-Investigator make a causality assessment of the event to the trial intervention, (see table below based on the terms given in ICH E2A guidelines). Any event assessed as possibly, probably or definitely related is classified as related to the trial intervention.

Relationship	**Description**
Definitely	Temporal relationship Improvement after dechallenge* Recurrence after rechallenge (or other proof of drug cause)
Probably	Temporal relationship Improvement after dechallenge No other cause evident
Possibly	Temporal relationship Other cause possible
Unlikely	Any assessable reaction that does not fulfil the above conditions
Not related	Causal relationship can be ruled out
*Improvement after dechallenge only taken into consideration, if applicable to reaction	

Both Investigator and Sponsor-Investigator make a severity assessment of the event as mild, moderate or severe. Mild means the complication is tolerable, moderate means it interferes with daily activities and severe means it renders daily activities impossible.

Reporting of SAEs (see ClinO, Art. 63)

All SAEs are documented and reported immediately (within a maximum of 24 hours) to the Sponsor-Investigator of the study.

If it cannot be excluded that the SAE occurring in Switzerland is attributable to the intervention under investigation, the Investigator reports it to the Ethics Committee via BASEC within 15 days.

Notification of safety and protective measures (see ClinO, Art 62, b)

If immediate safety and protective measures have to be taken during the conduct of the study, the investigator notifies the Ethics committee of these measures, and of the circumstances necessitating them, within 7 days.

10.3 (Periodic) safety reporting

An annual safety report (ASR/DSUR) is submitted once a year to the local Ethics Committee by the Investigator (ClinO, Art. 43 para).

10.4 Amendments

Substantial changes to the study setup and study organization, the protocol and relevant study documents are submitted to the Ethics Committee for approval before implementation. Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the Ethics Committee. Such deviations shall be documented and reported to the Ethics Committee as soon as possible.

A list of all non-substantial amendments will be submitted once a year to the competent EC together with the ASR.

10.5 (Premature) termination of study

The Sponsor-Investigator may terminate the study prematurely according to certain circumstances, e.g.

- Ethical concerns,
- Insufficient participant recruitment,
- When the safety of the participants is doubtful or at risk (e.g. when the benefit-risk assessment is no longer positive),
- Alterations in accepted clinical practice that make the continuation of the study unwise, or
- Early evidence of harm or benefit of the experimental intervention

Upon regular study termination, the Ethics Committee is notified via BASEC within 90 days (ClinO, Art. 38).

Upon premature study termination or study interruption, the Ethics Committee is notified via BASEC within 15 days (ClinO, Art. 38).

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10.6 Insurance

The notification is also sent to the business liability insurance (Basler Versicherung) of Thim van der Laan AG in Landquart. Proof of this insurance policy will also be submitted.

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11. STATISTICAL METHODS

11.1 Hypothesis

Jump height (cm)

H0 The jump height is not increased by the hot water immersion compared to the cold water immersion, hypoxia group and passive control group during a 72-hour recovery phase.

H1 The jump height is increased by the hot water immersion compared to the cold water immersion, hypoxia group and passive control group during 72 hours.

MVIC (kg)

H0 MVIC performance is not increased by hot water immersion compared to cold water immersion, hypoxia group, and passive control group during a 72-hour recovery period.

H1 The MVIC efficiency is increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group during 72 hours.

Muscle swelling (mm)

H0 Muscle swelling is not increased by hot water immersion compared to cold water immersion, hypoxia group, and passive control group during a 72-hour recovery period.

H1 Muscle swelling is increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group for 72 hours.

Blood oxygen saturation (%)

H0 Blood oxygen saturation is not increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group during a 72-hour recovery period.

H1 The oxygen saturation of the blood is increased by the hot water immersion compared to the cold water immersion, hypoxia group and passive control group during 72 hours.

Oxygen Saturation Muscle (%)

H0 Oxygen saturation of the thigh muscle is not increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group during a 72-hour recovery period.

H1 The oxygen saturation of the thigh muscle is increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group during 72 hours.

Heart rate (b/min)

H0 The heart rate is not increased by the hot water immersion compared to the cold water immersion, hypoxia group and passive control group during a 72-hours of recovery.

H1 Heart rate is increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group during 72 hours.

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Skin temperature (°C)

H0 Skin temperature is not increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group during a 72-hour recovery period.

H1 Skin temperature is increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group during 72 hours.

Core Body Temperature (°C)

H0 Core body temperature is not increased by hot water immersion compared to cold water immersion, hypoxia group, and passive control group during a 72-hour recovery period.

H1 Core body temperature is increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group during 72 hours.

Inflammation parameters (CRP, IL6, CK)

H0 Inflammation levels are not increased by hot water immersion compared to cold water immersion, hypoxia group, and passive control group during a 72-hour recovery period.

H1 The inflammation rates are increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group during 72 hours.

Subjective information on shortness of breath (mod. BORG)

H0 Subjective information on shortness of breath is not increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group during a 72-hour recovery period.

H1 The subjective information on shortness of breath is increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group during 72 hours.

Subjective data on general exhaustion (BORG)

H0 The subjective data on general fatigue are not increased by the hot water immersion compared to the cold water immersion, hypoxia group and passive control group during a 72-hour recovery period.

H1 The subjective data on general fatigue are increased by the hot water immersion compared to the cold water immersion, hypoxia group and passive control group during 72 hours.

Subjective muscle soreness (VAS)

H0 Subjective muscle soreness is not increased by hot water immersion compared to cold water immersion, hypoxia group, and passive control group during a 72-hour recovery period.

H1 Subjective muscle soreness is increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group during 72 hours.

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Thermal comfort and temperature perception

H0 Thermal comfort and temperature perception are not increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group during a 72-hour recovery period.

H1 Thermal comfort and temperature perception are increased by hot water immersion compared to cold water immersion, hypoxia group and passive control group during 72 hours.

A 2-factor analysis is carried out.

Factor 1: Intervention (cold water immersion vs. hot water immersion vs. hypoxia group vs. Passive Control Intervention)

Factor 2: Time (Baseline, 0, 24 hours, 48 hours, 72 hours)

11.2 Determination of Sample Size

Based on a study with a comparable setting (Ferreira-Junior et al. 2014, Fonda et al. 2013, Hausswirth et al. 2011) and outcome, the power analysis was calculated using the G*Power App (Düsseldorf, North Rhine-Westphalia, DEU), whereby a minimum number of 33 subjects was considered necessary to ensure the statistical power (0.80) to detect a parameter difference at the 5% significance level. Taking into account a possible loss of 20% of the data sets (due to drop-out or incomplete data sets), the minimum sample size is 40 participants. The following figure shows the G-Power calculation that was used to determine the sample size.

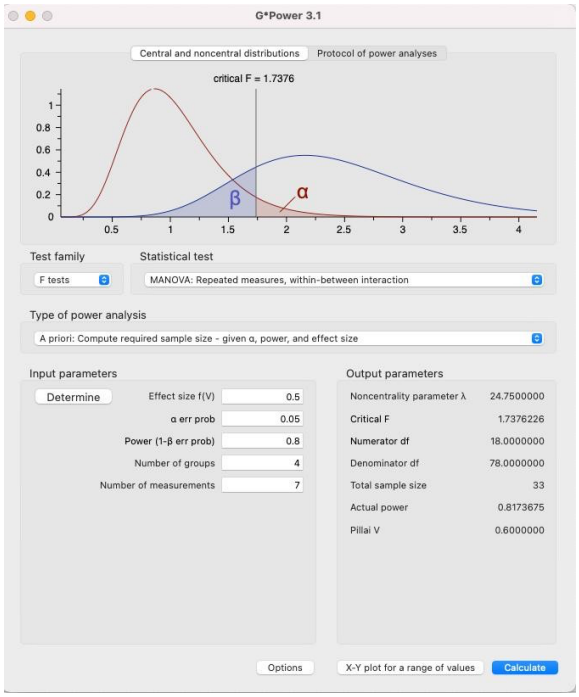

Figure: Sample Size Calculation

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11.3 Statistical criteria of termination of trial

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 Planned Analyses

11.4.1 Datasets to be analysed, analysis 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 will be carried out by Dr. Erich Hohenauer once the data collection for the entire project has been completed.

Repeated measures analysis of variance (MANOVA)

2 factors:

Factor 1: Intervention (cold water immersion vs. hot water immersion vs. hypoxia group vs. passive control intervention)

Factor 2: Time (Baseline, 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 27.

11.4.3 Secondary Analyses

Not Available.

11.4.4 Interim analyses

The data is processed once the respective sub-protocol has been completed. Thus, the data analysis for the entire study takes place in stages. No additional interim analyses are planned.

11.4.5 Safety analysis

-

11.4.6 Deviation(s) from the original statistical plan

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

11.5 Handling of missing data and drop-outs

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.

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12. QUALITY ASSURANCE AND CONTROL

12.1 Data handling and record keeping / archiving

12.1.1 Case Report Forms

Data protection and confidentiality are guaranteed, and no personal data is presented or published. The signed consent form, as well as the completed questionnaire with the other personal details and demographic and medical personal data, will be kept in the original as a study document in a locked filing cabinet. The identity of the subjects will be coded in accordance with the coding accepted by swissethics for a person participating in a research project. Only a person's year of birth (YYYY) is documented along with a coding number in the CRF.

The project leader is responsible for the secure storage of the key for the data encrypted as part of the clinical trial or research project. Direct access to personal data is only permitted to authorised persons of the CEC.

12.1.2 Specification of source documents

The data collected in writing is recorded in the form listed in the enclosures and are labelled as study data. They are therefore subject to data protection and are stored in accordance with the information described in section 12.1.1.

12.1.3 Record keeping / archiving

The data collected is stored for 10 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 using OT Bioelettronica's (MISO II) software. The oxygen measurement data is acquired with the research software "moorFLPI-2 full-field laser Perfusion Imager V1.1". The jump height is determined with a jump height measuring plate designed for this purpose (Just Jump Plate, Probotics Inc., Huntsville, USA). The data from the thermal imaging camera is processed with the "Flir Research IR max" software. The inflammation parameters are recorded with the Reflotron (Roche Diagnostic, Rotkreuz, Switzerland) and transferred to a data sheet. Dyspnea and stress assessment are recorded in writing with a questionnaire. The subjective sensation parameters regarding thermal comfort and temperature perception are asked verbally and the answers are transferred by hand to the raw data sheet. Excel is used for further data analysis. Windows® Excel will also be used for data pooling and for the graphical visualisation of the final data. The skin temperature is measured via the iButton system and stored on the PC using the software provided (iButton MMT). To assess muscle swelling, the MyLabClassC ultrasound is used (Esaote, Genoa, Italy). The images are then analysed using the OsiriX DICOM viewer software (OsiriX, Pixmeo SARL, Switzerland).Blood oxygen saturation is measured with a portable pulse oximeter with finger clip probe (Nonin 7500, Nonin medical B.V., Plymouth, USA) and transferred directly to a Windows® Excel template. The data from the core body temperature measurement is transmitted wirelessly to a monitor (BodyCAP medical, Hérouville Saint-Clair, France) and recorded.

All data is processed on the central research computer. This research computer is located in Landquart (research laboratory) at all times. Only the principal investigator and the investigators (listed in Chapter 1) know the password to unlock this computer. All digital documents are write-protected by a password. Only the study director and the examiners know the passwords of the digital documents. The digital documents are given a version number. Any changes made to the digital documents are saved as a new version number. In addition, all versions will be printed out and signed by the study director. The printed documents are stored in a separate, lockable filing cabinet. This filing cabinet also contains the password

combinations for decrypting the computer and editing the documents. Only the study director has the key to this filing cabinet.

12.2.2 Data security, access and back-up

The data is encrypted and treated confidentially; access to the personal data is not permitted 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 the "University of Applied Sciences and Arts of Southern Switzerland" is restricted to the study director and the investigators (listed in Chapter 1), as well as persons authorised by the CEC.

12.2.3 Analysis and archiving

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

12.2.4 Electronic and central data validation

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 chap. 12.2.2)
The will be monitored by the following person:

Prof. Peter Clarys, PhD
Vrije Universiteit Brussels

Faculty of Physical Therapy

Pleinlaan 2, 1050 Brussels

peter.clarys@vub.ac.be

12.4 Audits and Inspections

A report on the current status of the study is submitted orally to the study supervisor (Prof. Dr. Peter Clarys) on a monthly basis. 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. Authorised staff of the CEC have access to the data forms and the digital data on the institute's own computers at all times.

Data protection is guaranteed at all times.

12.5 Confidentiality, Data Protection

The examiners ensure that the privacy of the participant is guaranteed. 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 data is encrypted and treated confidentially; access to the personal data is not permitted to third parties. The digital data collected is stored solely on the institute's own computers and is not passed on to third parties or transferred to other computers.

Direct access to the personal data is only allowed to authorised CEC personnel.

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12.6 Storage of biological material and related health data

The blood samples are handled confidentially. After they have been analysed, they are destroyed in accordance with the applicable hygiene regulations and in compliance with anonymisation.

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13. PUBLICATION AND DISSEMINATION POLICY

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

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14. FUNDING AND SUPPORT

14.1 Funding

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14.2 Other Support

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15. INSURANCE

Thim van der Laan AG in Landquart, where the study is being conducted, is covered by public liability insurance with Basler Versicherung.

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16. REFERENCES

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17. APPENDICES

1. Subject information, informed consent & demographic subject data (Questionnaire)
2. Subject recruitment (advertisements, checklist for initial contact)
3. Data collection sheet measurements (Screening Test and Measurement Data Sheet)
4. Protocol synopsis
5. Employee list
6. CVs of all examiners