GENERAL STUDY INFORMATION AND INFORMED CONSENT

UNIVERSITY CEU CARDENAL HERRERA

Principal investigator: Dr. Francisco Javier Molina Payá

TITLE: Influence of intramuscular temperature on the textural characteristics of the B-mode ultrasound image.

Research and Ethics Committee of CEU Cardenal Herrera University Number: CEEI23/453

DATE: 06-10-2023

CONSENT DOCUMENT FOR PARTICIPATION IN A RESEARCH PROJECT

INFLUENCE OF INTRAMUSCULAR TEMPERATURE ON THE TEXTURAL CHARACTERISTICS OF THE B-MODE ULTRASOUND IMAGE

Principal investigator: Dr. Francisco Javier Molina Payá

NAME:_____

ID No._____

Freely and voluntarily

Manifest:

1. I have read and understood the fact sheet under study.
2. I have had the opportunity to ask questions.
3. My questions have been answered satisfactorily.
4. I have received sufficient information from the study and the tests to be performed.
5. I understand that participation is voluntary and I can leave the study at any time without explanation and without affecting my medical care.
6. In accordance with Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data and repealing Directive 95/46/EC, I have been informed that my personal data, obtained by completing this form as well as those resulting from my participation in the project will be processed under the responsibility of the UNIVERSITY FUNDATION SAN PABLO CEU (hereinafter, FUSP-CEU), in order to manage my participation in this research project. In addition, I have been informed of the following aspects:
    a. Profiling is planned to analyse or predict aspects of my health.
    b. That the treatments indicated are legitimized in the consent granted by me.
    c. That my personal data, obtained by completing this form, as well as those resulting from my participation in the project will be kept for the time necessary

for the development of this research, which is estimated to be1 1 months, being subsequently destroyed, without being preserved without having previously been anonymized. In any case, they may not be transferred without my express consent and I do not grant it in this act.

d. That I can contact the Data Protection Officer of FUSP-CEU, directing my written request to the postal address C/ Tutor no. 35 - 28008 Madrid or to the email address dpd@ceu.es.

e. That in accordance with the rights conferred on me by the current legislation on data protection I may contact the competent Supervisory Authority to submit the complaint that it deems appropriate, as well as I may exercise the rights of access, rectification, limitation of processing, deletion, portability and opposition to the processing of my personal data and withdraw the consent given for the processing thereof, directing my request to the responsible investigator at the contact address contained in this document.

7. I agree that my written consent and other data are available to the clinical research project in which I am participating, and the researcher responsible for it, Francisco Javier Molina Payá,, but always respecting the confidentiality and assurance that my data will not be publicly available in a way that can be identified.

8. The data collected for this study will be included, with those of other persons participating in this study, in a personal database of the CEU Cardenal Herrera University, to which only the researchers approved for this project will have access, all of them being subject to the secret inherent in their profession or derived from a confidentiality agreement.

9. I voluntarily sign this information and consent document to express my desire to participate participate in this research study on the influence of intramuscular temperature on the textural characteristics of the B-mode ultrasound image, until I decide otherwise. By signing this consent I do not waive any of my rights. I will receive a copy of this document to save for future reference.

I therefore consent and consent to the detailed study with the help of the necessary staff with due qualification and specialization.

The participant

(Signature) Name, Last Name

Elche, a .... 20....

FAMILY OR TUTOR AUTHORIZATION

In the face of the impossibility of _____

with ID_____ to provide authorization for the treatments expressed in this document freely, voluntarily, and consciously.

NAME:_____

with ID_____

As a (husband, wife, child, brother, legal guardian, family member, close friends, caregiver), I decide, within the available clinical options, to give my free, voluntary and conscious consent to the technique described for the treatments set out herein.

ELCHE, of _____of_____

Researcher

Dr. Francisco Javier Molina Payá

ID: 44751322S

Email: francisco.molina@uchceu.es

Phone: (34) 96 542 64 86 ext. 67804

Researcher at the CEU-Cardenal Herrera University of Valencia, I declare that I have provided the study participant and/or authorized person with all the information necessary for the realization of the intervention explained in this document and declare that I have confirmed, immediately prior to the application of the technique, that the participant does not incur any of the contraindication cases related above, as well as having taken all the necessary precautions for the correct intervention.

Elche,_____of _____of_____

REVOCATION OF INFORMED CONSENT

NAME:_____

with ID:_____

I revoke the consent given on the date of_____

And I do not wish to continue the treatment I give on this date.

_____, _____de _____de_____

PARTICIPANT INFORMATION

STUDY TITLE:

INFLUENCE OF INTRAMUSCULAR TEMPERATURE ON THE TEXTURAL CHARACTERISTICS OF THE B-MODE ULTRASOUND IMAGE

Principal investigator: Dr. Francisco Javier Molina Payá

INTRODUCTION

We are writing to inform you about a research study in which you are invited to participate.

Our intention is that you receive correct and sufficient information so that you can decide whether or not you agree to participate in this study. To do this, take the time necessary to read this information sheet carefully and carefully and discuss it with whoever you consider appropriate. Ask the study staff to explain any words or information you do not understand clearly, as well as any questions you may have.

If you decide you want to participate, we will ask you to sign the attached informed consent document. We will provide you with an original signed and dated copy of this document for your retention and the original document will be on file with the rest of the study documentation.

The study has been approved by the Research Ethics Committee of the CEU Cardenal Herrera University of Valencia.

Likewise, it has been designed and will be carried out in accordance with the recommendations established in the Declaration of Helsinki and the Standards of Good Clinical Practice.

You should know that your participation in this study is voluntary and that you may decide NOT to participate. If you decide to participate, you can change your decision and withdraw consent at any time, without altering your relationship with your physiotherapist.

You should also know that you may be removed from the study if the sponsor or investigators deem it appropriate, whether for safety or other reasons. In any case, you

will receive an adequate explanation of the reason that caused your withdrawal from the study.

WHY IS THE STUDY CARRIED OUT?

This study aims to observe the relationship between muscle temperature and the information extracted from ultrasound images.

HOW WILL THE STUDY BE CONDUCTED?

To carry out this study, a single group of participants will be selected. Firstly, some data related to the participant will be collected through a questionnaire. The questionnaire includes personal data (date of birth, sex, weight, height and BMI) and health to ensure that there are no known neurological, cardiovascular, metabolic and orthopedic conditions that prevent the tests from being carried out, or factors that could influence the results. or contraindications to dry needling (detailed in the following section).

Once the questionnaire is completed and it has been verified that the participation criteria are met, a prior ultrasound will be performed on the thigh. Next, using microwave equipment, a muscle located in the anterolateral part of the thigh will be heated for 20 minutes. It is a progressive and painless warm-up. Once the warm-up is complete, an ultrasound probe held by an articulated arm will be placed on the thigh in order to perform a static ultrasound examination. Once the probe is located, a fine needle will be introduced into the muscle that will detect the temperature. The temperature will passively decrease due to natural cooling after heating. For each degree of temperature that the muscle temperature drops, an ultrasound image will be saved. Once the recorded temperature reaches the basal temperature, the intervention will end.

The images obtained will be subsequently analyzed to measure variables related to the texture of the muscle and establish a correlation with temperature.

WHAT CRITERIA MUST BE MET IN ORDER TO PARTICIPATE?

The criteria that must be met to participate in this study are:

• Prior acceptance of participation in the study.

• Age exclusion: children under 18 years of age and over 60 years of age.

• Be a physiotherapist with knowledge of invasive techniques and without a subordinate relationship or employment relationship with the members of the research group.

• Not suffer from known neurological, cardiovascular, metabolic and orthopedic conditions that prevent the tests from being performed.

• Do not have metal implants in the thigh area,

• Do not wear a pacemaker.

• They will not do any physical activity for at least 48 hours before data collection*.

• Avoid drinking alcohol, coffee or energy drinks 12 hours before data collection*.

• Do not smoke in the 6 hours prior to data collection*.

• Ingestion of drugs or treatments that alter vascularization*.

• Not present any contraindication to dry needling (Belenophobia, history of abnormal reaction to the puncture or injection, anticoagulant treatment or thrombocytopenia, lymphedema over the area of intervention, severely compromised immune system, vascular diseases, diabetes mellitus, pregnancy, epilepsy, allergy to metals or that the intervention area has wounds, scars, tattoos, or stains.

*Prior information to the participant

A number of around 40 participants is expected, although the final number of participants will be determined by estimating the sample size based on a pilot study of 10 participants.

WHAT DOES MY PARTICIPATION CONSIST OF?

If you agree to participate in the study, we will ask you:

- Sign the informed consent. All information collected will be treated confidentially and its use will be purely scientific, safeguarding your identity at all times. To do this, you will need to sign your express consent through written authorization.
- Fill out a questionnaire that includes personal data (detailed in the previous point) and health to ensure that there are no known neurological, cardiovascular, metabolic and orthopedic conditions that prevent the tests from being performed, or factors that could influence the results.
- Lie down on the stretcher and place the right leg on a support to carry out the tests that consist of pre-warming the thigh using a high-frequency deep thermotherapy system (microwave), taking ultrasound images and muscle temperature at through a fine non-hollow needle during passive cooling of the thigh.

WHAT RISKS OR DISCOMFORT MAY I SUFFER BY PARTICIPATING IN THE STUDY?

Muscle heating using microwaves produces a notable increase in temperature that should be perceived as pleasant; if it is perceived as annoying, the intensity would be decreased.

Ultrasound examination is harmless and painless.

Measuring temperature using the needle probe presents less risk and discomfort than dry needling because the thickness of the needle is similar to that used in dry needling, but a single incision is made in the muscle belly before the puncture. dry that involves around 15 repetitions in the same place on the motor plate of the muscle. The data related to dry needling are: 1-10%: slight bleeding, hematoma, post-puncture pain; 0.1-1%: inflammation, swelling, severe pain, tachycardia, vomiting; <0.01%: systemic infection, disorientation. We emphasize that the technique used must present lower percentages than those shown, that is, barely existing.

WHAT BENEFITS WILL BE OBTAINED FROM THE STUDY?

This study may be of interest and provide information when considering muscle temperature as a variable that can influence the ultrasound analysis of the muscle. With this information, muscle examination protocols could be improved, which would improve the reliability of the results.

WHAT TREATMENT OPTIONS DO I HAVE IF I DO NOT PARTICIPATE IN THE STUDY?

Not applicable.

HOW ARE MY RIGHTS PROTECTED?

The study will be carried out in compliance with all current ethical and legal regulations.

Confidentiality

Researchers undertake that their personal data will be treated confidentially and will be processed in accordance with current regulations on the protection of personal data (Organic Law 3/2018, on the Protection of Personal Data and Guarantee of Digital Rights, and Regulation [EU] 2016/679 of the European Parliament and of the Council, relating to the protection of natural persons with regard to the processing of personal data and the free circulation of these data). To comply with this regulation, the data collected for the study will be identified by a code, so that it does not include information

that could identify you, and only the researchers will be able to relate said data to you and your medical history. Therefore, your identity will not be revealed to anyone except in cases of medical emergency or legal requirement. The treatment, communication and transfer of personal data of all participants will comply with the provisions of the law.

Access to your identified personal information will be restricted to researchers, health authorities, the Research Ethics Committee and personnel authorized by the promoter (study monitors, auditors), when they need it to verify the data and procedures of the study, but always maintaining its confidentiality in accordance with current legislation.

The questionnaires in which personal data appear will be kept under lock and key at all times and only researchers will have access.

The data will be collected anonymously in a research file under the responsibility of the researchers, and will be processed on computers on the network that can only be accessed with a personal password.

In accordance with the provisions of data protection legislation, you can exercise the rights of access, modification, opposition and cancellation of data, for which you must contact your study physiotherapist.

If you decide to withdraw consent to participate in this study, no new data will be added to the database, but data that has already been collected will be used.

The encrypted data may be transmitted to third parties, but in no case will it contain information that can directly identify you, such as name and surname, initials, medical record number, etc. In the event that this transfer occurs, it will be for the same purposes of the study described or for use in scientific publications, but always maintaining its confidentiality in accordance with current legislation.

The data collected will never be used for any other purpose, consequently, all data will be destroyed and/or eliminated once the research results have been published in scientific journals.

WHO CAN I CONTACT IN CASE OF DOUBT?

If you have any questions, consult with Francisco Javier Molina Payá (IP) Universidad Cardenal Herrera-CEU, telephone number 96 542 64 86 | Ext. 67804, e-mail: francisco.molina@uchceu.es, who is responsible for this research and who will answer any questions you may have related to this study.

Whatever his decision, the research team wants to thank him for his time and attention.

STUDY PROTOCOL PLAN AND STADISTICAL ANALYSIS PLAN (SAP)
UNIVERSITY CEU CARDENAL HERRERA

Principal investigator: Dr. Francisco Javier Molina Payá

TITLE: Influence of intramuscular temperature on the textural characteristics of the B-mode ultrasound image.

Research and Ethics Committee of CEU Cardenal Herrera University Number: CEEI23/453

DATE: 06-10-2023

STUDY PROTOCOL PLAN

STUDY TITLE:

INFLUENCE OF INTRAMUSCULAR TEMPERATURE ON THE TEXTURAL CHARACTERISTICS OF THE B-MODE ULTRASOUND IMAGE

Principal investigator: Dr. Francisco Javier Molina Payá

Objectives:

The objectives of our study have been the following:

-Analyze the changes in the first and second order ultrasound parameters of the vastus lateralis muscle of the quadriceps caused by muscle temperature.

Methodology:

This is a quasi-experimental analytical study on a sample of healthy, adult subjects. The selection and recruitment of the sample will be carried out by the same researcher, who will inform them about the study and give them informed consent if they accept their participation. Participants will complete a health questionnaire to ensure they meet the selection criteria.

Participants will lie on a stretcher for 10 minutes in a supine position with knees in full extension, to allow fluid redistribution before ultrasound measurements are taken. The right lower limb will be placed on an adjustable support to facilitate the subsequent positioning of the ultrasound probe and the intramuscular temperature probe. The interventions will be carried out in a controlled environment, with a room temperature of between 20ºC and 25ºC. Once the participant is acclimatized, passive heating of the vastus lateralis of the quadriceps will be performed using a local microwave diathermy device. Subsequently, an intramuscular temperature probe will be placed in the vastus lateralis of the quadriceps and ultrasound images (B-mode) of the vastus lateralis of the quadriceps will be recorded before, immediately after and each degree of temperature that decreases from the passive heating of the muscle. To maintain the position of the probe statically throughout the procedure, an articulated arm will be used that will allow images to be captured completely statically.

The percutaneous placement of the temperature probe will be carried out under strict asepsis and antisepsis measures, which include everything from the use of sterile films

on the ultrasound probe, antiseptic stockings on the skin to the use of the autoclave protocols proposed by the manufacturer on the temperature probe.

Passive warming protocol

Passive heating of the vastus lateralis of the right quadriceps will be performed using a Cosmogamma MW300-99 microwave device (Cento, Italy). The device will be set at a power of 150 W and a distance of 10-15 cm from the skin surface for 20 minutes with the purpose of increasing local muscle temperature to 40 ºC.

Ultrasound measurement

Ultrasound images will be recorded using a Telemed SmartUS ultrasound machine (Vilnius, Lithuania) with a 7-15 MHz linear probe (L15-7L40H-5). The same ultrasound image optimization parameters will be used for all participants. All ultrasound images will be taken by the same experienced investigator to minimize potential changes in probe alignment. Furthermore, to avoid changes in the position of the probe, which could alter the echogenicity of the image, an articulated arm will be used that will fix the probe in a static position throughout the ultrasound recording process. The location of the probe will be established by measuring 30% of the distance between the lateral condyle of the femur and the greater trochanter, and with an orientation transverse to the fibers of the vastus lateralis of the quadriceps.

Intramuscular temperature measurement

The intramuscular temperature of the vastus lateralis of the quadriceps will be measured directly in the right leg using a hypodermic needle probe (model MT-26/4HT, Physitemp Instruments, Clifort, USA) inserted into the muscle belly 3 cm below the skin and 2 cm from the location of the ultrasound probe. The recording will be carried out immediately after passive warming in order to continuously record the intramuscular temperature during the cooling process.

Muscle ultrasound image analysis

The analysis of the ultrasound image will be performed on the regions of interest (ROI) extracted from each stored image. To do this, an ROI located in the same place of the vastus lateralis muscle of the quadriceps will be established. The echogenicity and ecotextural variables will be calculated on each of the ROIs, using the ImageJ program (National Institute of Health, USA). An ROI will be generated that includes the largest possible muscle area, avoiding aponeurosis and bone tissue.

The measurement of subcutaneous adipose thickness will be carried out through the average of the distance from the skin to the aponeurosis coinciding with the superficial interface of the muscle. This measure will be used to develop a correction factor that allows compensating the influence of this variable on echogenicity and ecotexture, standardizing the results. The methodology that will be used is as follows: The thickness of the subcutaneous adipose tissue of 5 participants will be measured by applying 4 different levels of external pressure on the skin using a force meter connected to the probe, without deforming the muscle. Subsequently, the associations between echogenicity and echotextural variables will be compared with the different levels of thickness of the subcutaneous adipose tissue that will allow the correction factor to be calculated. Finally, the correction factor will be applied to correct the possible influence of subcutaneous fat on echogenicity.

STADISTICAL ANALYSIS PLAN (SAP)

STUDY TITLE:

INFLUENCE OF INTRAMUSCULAR TEMPERATURE ON THE TEXTURAL CHARACTERISTICS OF THE B-MODE ULTRASOUND IMAGE

Principal investigator: Dr. Francisco Javier Molina Payá

First, all the variables of interest will be detected and later they will be coded, labeled and transferred to a database. A data collection notebook (CDN) will be created where the variables, their reference values, measurement system and interpretation will be described and which will be available to all members of the working group. The programs used will be Microsoft © Access and Excel for initial data registration and IBM SPSS Statistics 19.0 for analysis.

Data debugging and control systems

The data related to the clinical information of the subjects and related to the questionnaires will be recorded in a database created for this purpose (Microsoft Access ©) with data entry protection systems. The same researcher will always record the data and a feedback review will be carried out for the detection of errors and lost cases by a third person. Missing and out-of-range screening will be performed.

The data related to the variables obtained by image analysis of ultrasounds will be exported in an automated way with a macro created for this purpose from the data analysis program in ASCII format to avoid incompatibilities between the image analysis program and the imaging programs. statistical analysis.

Exploratory Data Analysis (EDA)

Qualitative variables will be summarized in the form of counts and relative frequencies and, where appropriate, absolute frequencies and their confidence intervals (95% CI). Graphic summaries will be made using bar diagrams.

The quantitative variables will be summarized by means, standard deviations, medians, and interquartile ranges. Confidence intervals will also be provided for each of the measures. Graphical summaries will be made using box plots and bar diagrams with 95% CI.

A univariate analysis of outliers will be carried out and their influence will be verified through the Z scores. Multivariate analysis of atypical cases and their influence will be carried out using the Mahalanobis distance.

Unusual cases will be removed from the analysis only if it is certain that they are wrong.

It will be checked whether the quantitative variables of interest follow a normal distribution (Kolmogorov-Smirnof test). The normality tests will be accompanied by the Q-Q normality graphs with and without trend that will aid interpretation and decision-making. In the event that any of the variables of interest does not conform to a normal distribution, a transformation of this will be carried out until it is achieved as long as its interpretation is not compromised.

Analysis of data

Firstly, the effect of passive cooling, each 1ºC decrease that occurs after heating on echointensity and the rest of the echotextural parameters, intramuscular temperature, subcutaneous adipose tissue thickness and muscle thickness will be examined using linear models of mixed effects adjusted by restricted maximum likelihood. These procedures will be performed to test the differences between the measurements obtained at each 1ºC decrease after passive heating. Subsequently, the intra-individual relationship between temperature, ecointensity and the rest of the ecotextural parameters will be evaluated for each degree of temperature that decreases using the Bland-Altman repeated measures intra-subject correlation. Finally, a linear mixed effects model fitted by restricted maximum likelihood will be used to test the effect of temperature (centered around the mean) on ecointensity and ecotextural parameters. In addition, a model will be built that includes the effect of the thickness of the subcutaneous adipose tissue and the muscle thickness measured ultrasonographically, in order to analyze and standardize the results, avoiding the influence of these influencing variables.