**RESEARCH PROPOSAL**

**Title: Validity of Ultrasound Imaging Versus Magnetic Resonance Imaging for Measuring Anterior Thigh and Bicep Muscles and Subcutaneous Fat**

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**Protocol version:** 3, 11/05/2023

**Key Words:**

**ABSTRACT**

**Objective:** Immobilisation and inadequate nutrition after surgical trauma result in loss of lean muscle mass. Such muscle loss is traditionally quantiﬁed with techniques that require expensive equipment and/or ionizing radiation. The purpose of this study is to assess reliability and validity of ultrasound (US) to quantify muscle content for use in a randomised clinical trial assessing interventions.

**Methods:** Thirty volunteer participants will undergo assessments of muscle and subcutaneous fat thickness using US and magnetic resonance imaging (MRI). Independent raters will perform two serial US measures using US. Test–retest and inter-rater reliability will be assessed using intraclass correlation coefﬁcient (ICC). Agreement between US and MRI will be assessed with Bland–Altman analysis.

**Results**

The protocol has been developed using the SPIRIT checklist (Standard Protocol Items: Recommendations for Interventional Trials) although adapted for a validation trial, and the outcomes will be reported following the CONSORT (Consolidated Standards of Reporting Trials) guidelines. The trial will be approved by the Auckland Health Research Ethics Committee (AH26137). The results of this study will be communicated via publication.

**Discussion**

We will validate the use of US using upper and lower body landmarks against gold standard MRI measurements for muscle and fat assessment.

**Trial registration**

Registration: ANZCTRXXXXX.

**INTRODUCTION**

The assessment of body composition in a clinical and non-clinical setting continues to be a challenge. One example of the challenge is the assessment of the impact of nutritional supplementation on lean muscle mass after surgery and the identification of a reliable approach to longitudinally quantify muscle mass changes over time. There is a clear need for reliable, accurate, safe, and easily accessible tools to assess body composition as both a diagnostic and prognostic tool in clinical settings. Dual-energy x-ray is resource consuming, magnetic resonance imaging (MRI) is too expensive and may be claustrophobic for some individuals, and bioelectrical impedance analysis has fluctuating accuracy (Gomez-Perez et. al 2021, Pineau, Lalys, Pellegrini, Battistini, 2013, Sizoo, deHeide, Emous et al, 2021). Ultrasound may provide a viable option to satisfy most of these qualifying criteria.

A bedside portable ultrasound machine can offer numerous benefits in measuring prognostic and diagnostic body composition criteria, which can be especially beneficial in reducing hospital stay and assessing malnutrition outcomes (Casey, Alasmar McLaughlin, 2022; Zagzebski, 2012). One of the key advantages of using such a machine is the quick and non-invasive assessment it provides. Unlike other methods such as computed tomography (CT), ultrasound imaging does not expose patients to ionizing radiation. This can lead to faster assessments and reduced patient discomfort.

By providing quick and accurate assessments, the length of hospital stay may be reduced. For example, early detection of malnutrition using ultrasound imaging can lead to appropriate interventions being initiated sooner, which can speed up recovery and discharge (Moutzakis, Wischmeyer, 2014). This can help reduce overall healthcare costs, as well as improve patient outcomes. In addition, portable ultrasound machines are less expensive than MRI equipment thus potentially providing a cost-effective option for routine assessments of body composition.

Nijholt and colleagues (2020) investigated the reliability and validity of ultrasound to assess body composition, namely the muscle content (rectus femoris). The study found that the intra-rater reliability of ultrasound measurements was high for the different transducers when assessing both cross sectional area and muscle thickness. However, the intra-rater reliability was lower for the muscle echo intensity, a crude measure of muscle quality.

In a recent systematic review investigating the use of ultrasound to assess skeletal muscle content and how this relates to or predicts clinical outcomes was conducted by Casey et al (2022). Authors reported 37 studies which included 3100 patients, of which 22 studies were on critical care patients. 76% of the studies showed significant association between muscle content and patient functional capacity, length of stay, readmission, and survival. This highlights the potential value of ultrasound assessment of muscle content in clinical assessment.

The current investigation critically evaluates the ability of portable ultrasound (US) to reliably quantify muscle thickness compared with a standard technique. The goal is to validate a portable, relatively inexpensive, and radiation-free device to assess muscle content that in future could be used within a clinical setting to assess pre- and post-operative changes in muscle mass.

In particular, the purpose of this investigation is to determine the test–retest and inter-rater reliability of muscle thickness measured with portable ultrasound and compare with freestanding ultrasound and magnetic resonance imaging (MRI) in a cohort of volunteers.

**METHODS**

**Study Design and Participants**

The research will be conducted at the Centre of MRI Research within The University of Auckland Health Campus.

After providing written informed consent, volunteer participants from 20 to 55 years old will undergo assessment with the portable ultrasound machines by 2 reviewers (W.V and A.B.) and MRI. Participants with a range of BMI will be selected (n=10 BMI>30, n=10 BMI between 21-29, n=10 BMI < 20). MRI is a validated method of muscle thickness and cross-sectional area assessment and will be used as the criterion measure for this study. Participants will be excluded if they have: diseases and conditions affecting muscles (structure or function), musculoskeletal injuries of the lower limb and pathologies including fractures, surgical procedures, cancer, or neurological disorders, and any issue that is a contraindication for MRI will also be excluded. Examples include cardiac implant, history of welding injury, cochlear or hearing implant, prosthetic limb, magnetic dental implant. The study will be conducted at the University Centre for Advanced Magnetic Resonance Imaging (CAMRI). The US measurements will be completed before and after the MRI measurement.

Researchers will apply for ethical approval from the Auckland Health Research Ethics Committee The results will be reported using the Consolidated Standards of Reporting Trials (CONSORT) statement (XXciteXX) and communicated via publication.

**Procedures**

***Land marking***

A steel tape (Lufkin, USA) will be used to locate the mid-upper arm position of the right arm when the arm is held at 90 degrees and marked at the midpoint between the acromion and olecranon processes, a mark will be made on the skin and using the steel tape the mid-section of the bicep brachii muscle, which follows the standardised procedures described by NHANES (CDC, 2020).

The steel tape will also be used to locate the midpoint of the thigh between the anterior superior iliac spine and the top of the patella on both right and left legs. The participant will be sitting upright with knees bent at 90 degrees. The steel tape is placed in the inguinal fold at point 0 cm to the tip of the patella (end point). The mid-point is marked at the halfway mark, the two thirds point is marked two-thirds along (length from anterior superior iliac spine to top of patella x 0.66). At both points the rectus fermoris will be assessed.

***Ultrasound***

The US will be conducted using the Philips Lumify (Philips Ultrasound, Bothall, WA) handheld portable B-mode L12-4 linear array transducer that attaches to an ipad tablet and works through the Lumify App (Software Version) (Gomez-Perez, 2021). Images will be taken with the participant in a supine position and relaxed arm and leg, gel will be applied to the transducer and minimal compression applied, the image have the muscle belly centred. The image depth will be set to 5cm and adjusted if the underlying bone is not visible. Three images of each landmark will be saved for analysis.

***Magnetic Resonance Imaging***

MRI scans will be performed using a 3T Vida-Fit scanner (MAGNETOM, Siemens Healthineers; Erlangen, Germany) with a dedicated body coil. The participants will lie in a supine position with the right arm and both thighs in a neutral position. The landmarked positions and image processing will mimic the ultrasound protocol (Kim, Yoon, Choi, Jin & Cha, 2019; Borga, 2018). Landmarks will be marked by placing a vitamin E capsule at the scan site.

***Image Processing***

US and MRI images will be anonymised and analysed offline, using ImageJ software (https://imagej.nih.gov/ij/). Three images will be saved for each site, the three images will have the thickness and cross-sectional area (RF 2/3 measurements only) assessed and averaged, excluding outlier measurements (greater than 10% different to the other two images). Subcutaneous fat thickness at the thigh will be measured from the skin to the outside edge of the superficial fascial layer, while muscle thickness of the rectus femoris and vastus intermedius will be measured between the inside edges of muscle borders to exclude the perimuscular fascia (Mechelli, Arendt-Nielsen, Stokes & Agyapong-Badu, 2019).

**Accuracy and Reliability**

To determine accuracy, the ultrasound muscle and fat measures will be compared to the MRI outcomes.

To determine reliability, the two reviewers will perform ultrasound measures immediately after one another and will be blinded to the other’s results. Test–retest reliability will be assessed by calculating intraclass correlation coefﬁcient (ICC). To assess inter-rater reliability, measures from reviewer 1 and reviewer 2 will be compared using ICC.

**Statistical Analysis**

Test–retest and inter-rater reliability of ultrasound body composition measures will be assessed with intraclass correlation coefﬁcient. Agreement between the two ultrasound methods and MRI measures will be assessed using Bland–Altman analysis and limits of agreement.

**RESULTS & DISCUSSION**

This study will be reported in accordance with the CONSORT (Consolidated Standards of Reporting Trials) checklist. Our results will be communicated via publication.

**Data management, monitoring and dissemination**

The PI is responsible for project coordination and will oversee the operational aspects of the trial. The research team involved in the trial will regularly monitor study implementation, as well as data generation, documentation, and reporting. The PI will communicate protocol amendments to the ethics committee and clinical trial registration. Access to data will be granted to appropriate members of the research team and to authorised representatives from the host institution to monitor or audit the study and ensure compliance with regulations. Data will be made available to external academics on reasonable request.

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