BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-074945
Article Type:	Protocol
Date Submitted by the Author:	24-Apr-2023
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Keywords:	NUTRITION & DIETETICS, Ultrasound < RADIOLOGY & IMAGING, Nutritional support < GASTROENTEROLOGY

Protocol for a prospective cohort study on the feasibility of application of nutritional ultrasound in the diagnosis and follow-up of patients with nutritional risk at hospital discharge: Study on body composition and function (DRECO).

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ABSTRACT

Background: Nutritional ultrasound is an emerging technique in clinical nutrition for the morphological and structural study of muscle mass. Currently, all definitions of malnutrition include the measurement of muscle mass, however, there is no single way to assess it. It is necessary to develop new techniques to identify muscle involvement in malnutrition that are valid, standardized, reliable, accurate and profitable.

Objective: To value the new muscle ultrasound techniques aimed to measure muscle and functional status, to make a more accurate diagnosis and a better prediction of complications and morbidity and mortality in patients at nutritional risk. Primary outcome: to assess the feasibility of ultrasound or muscle ultrasound techniques in both nutritional diagnosis and follow-up in a nutritional intervention program.

Methods and analysis: DRECO (Disease-Related caloric-protein malnutrition EChOgraphy) is a prospective, multicenter (25 Spanish hospitals), uncontrolled clinical study in standard clinical practice to value the usefulness of nutritional ultrasound (muscle ultrasound) in the nutritional diagnosis and follow-up, over 3 to 6 months, after standard nutritional clinical practice intervention and physical activity, to control their disease-related malnutrition. 1000 patients are expected to be included in.

Discussion: This study will standardize nutritional ultrasound measures. It will validate and define specific cut-off values for nutritional ultrasound and correlate it with already well-known nutritional tools such as SGA (Subjective Global Assessment) or GLIM (Global Leadership Initiative on Malnutrition) criteria. Thus, muscle ultrasound will become not only a tool to

diagnose malnutrition, but it will be integrated in the daily practice to evaluate nutritional interventions.

Ethics and dissemination Ethical: All DRECO study materials have been approved by each of the IRB/IEC of all the sites enrolled (either approval of the own IRB/IEC or validating the approval of the IRB/IEC of another hospital). The study has been registered with ClinicalTrials.gov, on June 27th, 2022. Results from this study will be presented at scientific conferences and in peer-reviewed scientific journals.

Trial registration number: NCT05433831

Strengths and limitations of this study

DRECO strengths

- Multicentre, prospective, medium-term study in which a large sample (1000 patients) is expected to be recruited.
- Carried by a senior pool of researchers with wide experience in clinical nutrition and dietetics.
- Validation of classical tools and new morpho functional assessment techniques (ultrasound and bioelectrical impedance) are proposed.

DRECO limitations

- Non-randomized clinical practice study, so it will not be possible to adequately analyze the effect of nutritional intervention.
- It is restricted to patients upon hospital discharge, so it cannot be generalized to the entire population of people at risk of malnutrition.

Keywords: nutritional ultrasound; nutritional biomarker; ultrasound cut-off values; disease-related malnutrition; GLIM; SGA; body composition; sarcopenia; quadriceps femoris muscle; abdominal muscle area, muscle mass.

INTRODUCTION

Disease-related malnutrition (DRM) can occur when there is a deficient supply of energy, protein and/or other nutrients, depending on the nutritional needs of everyone at different times of their life cycle or health or disease circumstances. This deficiency induces effects on body composition and tissue and organ function and results in clinical consequences: increased morbidity and mortality associated with different disease processes (1).

In 2019, the GLIM criteria were published (2), providing a different vision of how to assess the malnourished patient. These criteria are divided into both phenotypic and etiological criterion:

- Phenotypic criterion
  - Weight loss (%): >5% within past 6 months, or >10% beyond 6 months
  - Low body mass index (kg/m^2): <20 if < 70 years, or <22 if >70 years. Asia: <18.5 if < 70 years, or <20 if >70 years

- Reduced muscle mass: Reduced by validated body composition measuring techniques

- Etiological criterion
  - 50% of ER (energy requirements) > 1 week, or any reduction for >2 weeks, or any chronic GI (gastrointestinal) condition
    that adversely impacts food assimilation or absorption
  - Inflammation: Acute disease/injury or chronic disease-related

There are techniques for nutritional assessment using assessment tools aimed at morpho functional diagnosis of malnutrition (3), in addition to the classical nutritional parameters, such as weight loss, BMI (body mass index), folds, circumferences, albumin, lymphocytes, cholesterol and intake. New advanced parameters are being incorporated into clinical nutrition and their incorporation into clinical practice is of increasing interest, such as measures derived from bioelectrical impedance (BIA) and phase angle (PhA), dynamometry, functional tests, CRP/prealbumin ratio and muscle ultrasound (see Figure 1).

Figure 1. Update of nutritional evolution parameters. Reproduced with permission from the authors (3).

From a scientific point of view, the following nutritional assessment techniques are being incorporated:

Muscle ultrasound

The application of ultrasound for the morphological and structural study of muscle mass is an emerging technique. Currently, there are different validation studies on the measurement technique. The ultrasound technique determines the surface area of the muscle in transverse and longitudinal position. With ultrasound analysis, it is possible to measure key parameters of muscle architecture, such as muscle volume and muscle fascicle length. Although there are different muscle structures that can be evaluated, many of the studies focus on the quadriceps rectus femoris or on combinations of various muscle groups involving large muscle bundles with functional importance to the patient in terms of gait. Measurement of the rectus femoris of the quadriceps is one of the most referenced measurements due to its correlation with strength and tests of execution or functional performance. It is necessary to develop new techniques to identify muscle involvement in malnutrition that are valid, standardised, reliable, accurate and profitable. Currently, all definitions of malnutrition include the measurement of muscle mass involvement, however, there is no single way to assess it. The classic imaging techniques such as DEXA (dual-energy x-ray absorptiometry), CT (computerised tomography) and MRI (magnetic resonance imaging) are considered the gold standard, but they have difficulties in their clinical application under normal practice conditions. Ultrasound has the advantage of being inexpensive, portable, and does not involve ionising radiation. Several studies have confirmed the reliability of this technique to

measure the size of the quadriceps muscle in a healthy population (4). Studies on the reliability of rectus femoris ultrasound have been published with an intraclass coefficient of variation (ICC) of 0.97 (95% CI: 0.92-0.99) for the test-retest reliability of ultrasound.

The American Society for Parenteral and Enteral Nutrition (ASPEN), among the criteria for the diagnosis of malnutrition in adults, recommends including an evaluation of fat and muscle deposits. Specialists must incorporate techniques that properly help to identify the loss of muscle and fat mass for a correct diagnosis of malnutrition. Implementing these evaluation techniques and instruments is challenging and remains a work in progress (5). Muscle ultrasonography correlates with body composition measurement techniques such as BIA and anthropometry in patients with cancer (6). In adults with cystic fibrosis muscle ultrasound measurements, particularly the mean muscular area rectus anterior (MARA), are related to the nutritional status and respiratory function of these patients. (7)

The Global Leadership Initiative on Malnutrition (GLIM) has recently appointed a working group to provide consensus-based guidance on assessment of skeletal muscle mass and its role in the malnutrition diagnostic and assessment process. They support the use of US (ultrasound), particularly in settings where its practical applicability provides potential for patient follow-up through repeated measurements, but it requires standardisation through experienced operators, and repeated measurements performed by the same individual. They also encourage further validation studies for the US (8).

Bioelectrical impedance (BIA)

BIA is used as a tool to obtain data that helps to better understand the patient's nutritional status, being a non-invasive, inexpensive, and easily transportable technique. Vector analysis and phase angle provide direct data, not being necessary to be later adjusted using formulas or mathematical models, as it is needed with simple or multifrequency bioelectrical impedance or multifrequency (9). This method is based on the analysis of the two bioimpedance vectors: resistance (R) and capacitive reactance (Xc). Resistance is defined as the opposition to a flow of electric current through a circuit component, medium, or substance, providing information about biological fluids, and therefore, related to tissue hydration. A decrease in the resistance/height ratio will indicate swelling or third space; conversely, an increased ratio will indicate dehydration. Reactance is the effect on an electrical current caused by a material's ability to store energy in cell membranes, so it is related to the cell mass and the integrity of its membranes. A decrease in Xc indicates loss of cell mass. This cell mass is the sum of all metabolically active cells, being the central parameter in the evaluation of nutritional status since the reduction of cell mass is typically related to malnutrition (10).

A recent study conducted by Fernandez-Jimenez, *et al* found that a low SPhA (standardised phase angle)-malnutrition value (SPhA < −0.3) was significantly associated with a higher

mortality hazards ratio (HR 7.87, 95% CI 2.56–24.24, p < 0.001). This biological marker could therefore be incorporated among the screening tools and mortality risk assessment in this population (11).

Dynamometry

Dynamometry is one of the 6 criterions to define malnutrition according to ASPEN (12). It is extremely sensitive to nutritional status changes, so it is particularly useful to track nutritional therapy or interventions results, even in the short and medium term. It has mostly been used to predict post-surgical complications including elderly patients (13). Results obtained are compared to the population averages by age and sex. Sanchez et al (14) presented reference values for hand dynamometry using a Jamar hand dynamometer for a Spanish population, providing cut-off points to define malnutrition. They concluded that hand dynamometry is associated with lean mass, which supports its usefulness in nutritional assessment.

Although the new GLIM consensus-based guidance on assessment of skeletal muscle mass do not include dynamometry as a marker of muscle mass (8), the authors hereby signing this article have previously studied dynamometry as a marker of muscle mass suggesting that GLIM criterion and dynamometry are associated to a higher mortality rate in both hospitalised and outpatient oncology patients (15, 16).

Functional tests

These tests are a series of physical activities related to mobility, walking or balance. Their results are related to those of scales that assess instrumental activities of daily living (IADL). The most common are the "Timed Up and Go test" (TUG), the "Gait Speed Test" (GST) and the "Short Physical Performance Battery (SPPB)" test that includes 3 tests (balance, gait speed and get up and walk) (17).

Besides, the decrease in physical performance, evaluated by the SPPB test or hand grip strength, has been shown to be elevated in patients with colorectal cancer prior to surgery and it was related to an increase in postoperative complications and mortality (18).

STUDY OBJECTIVES

The objective of this study is to value the new muscle ultrasound techniques aimed to measure muscle and functional status, to make a more accurate diagnosis and a better prediction of complications and morbidity and mortality in patients at nutritional risk. This main objective is developed in primary and secondary objectives as it follows:

Primary objective

- To assess the feasibility of ultrasound or muscle ultrasound techniques in both nutritional diagnosis and follow-up, over 3 to 6 months, in a nutritional intervention programme.

Secondary objectives

- To determine the association between muscle morphological parameters (nutritional ultrasound of the leg (area, circumference, axis and adipose tissue), total abdominal and

pre-peritoneal parameters measured by nutritional ultrasound and the nutritional and functional status of the patient, as well as their prognostic value in hospitalised patients.

- To establish an association between ultrasound as a diagnostic value of malnutrition as compared to the diagnostic gold standard (SGA and GLIM criteria).
- To determine the ultrasound cut-off points associated with the diagnosis of malnutrition and sarcopenia using the following tools:
    - Measurement of body composition using impedance techniques (Report: Phase angle, body cell mass (BCM), hydration, fat free mass (FFM) and lean mass index.
    - Muscle strength and capacity to perform physical activity after the intervention: dynamometry and Timed Up and Go test (TUG).
    - Criteria for sarcopenia.
    - To assess association with inflammatory activity markers: High-sensitivity C-reactive protein (CRP)/prealbumin.
- To assess ultrasound changes in patient follow-up.
- To establish an association of ultrasound results as predictors of morbidity and mortality (stay, mortality at 3 and 6 months, readmissions and in-hospital complications).

METHODS AND ANALYSIS

Study design and setting

DRECO (Disease-Related caloric-protein malnutrition EChOgraphy) is a prospective, multicentre, uncontrolled clinical study in standard clinical practice to value the usefulness of nutritional ultrasound (muscle ultrasound) in the nutritional diagnosis and follow-up of patients over a period of 3 to 6 consecutive months, after standard nutritional clinical practice intervention, and physical activity to control their disease-related malnutrition.

The study may be considered non-interventional since patients will undergo nutritional interventions and the standard treatment planned by their physician for treatment according to his/her standard clinical practice, and the only addition to the standard measurement and follow-up techniques of the patient will be the performance of a muscle ultrasound measurement using equipment provided to the centre for this purpose.

Patients and public involvement

Patients or the public will not be involved in the design, or conduct, or reporting, or dissemination plans of our research.

Recruitment

Patients over 18 years of age who, in the first week of hospital admission in medical-surgical areas, excluding critical patients, have an assessment of risk of malnutrition according to the MUST and SARC-F screening test using R-MAPP. [(MUST: Malnutrition Universal Screening Tool; SARC-F is an acronym of 5 domains included in the questionnaire: 1) Strength, 2)

Assistance with walking, 3) Rising from a chair, 4) Climbing stairs, and 5) Falls; R-MAPP (Remote consultation on MAlnutrition in the Primary Practice)].

If the results show a moderate or high risk of malnutrition, these patients will be invited to participate in the study, and will undergo the morpho functional assessment, an ultrasound study and the Subjective Global Assessment (SGA). This study is registered under ClinicalTrials.gov (NCT05433831).

Figure 2 shows the schedule of enrolment, interventions, and assessments.

Inclusion criteria

- Patients admitted to hospital who in the first week of admission have moderate or high risk of malnutrition according to the MUST and SARC-F screening test using R-MAPP.
- Patients aged 18 to 85 years.
- Patient who agrees to participate in the study and signs the informed consent.

Exclusion criteria

- Hepatic impairment - AST/ALT (aspartate aminotransferase/alanine aminotransferase) 3 x upper limit of normal.
- Chronic kidney failure - GFR (glomerular filtration rate) <45 mL/min).
- Patients with previous ICU (intensive care unit) stay during the study admission.
- Cancer patients on palliative treatment or ECOG (Eastern Cooperative Oncology Group) ≥ 3.
- Orthopaedic disease that does not allow adequate walking.
- Patients with known dementia or others not related to a significant neurological or psychiatric disorder, or any other psychological condition that may interfere with the conduct of the study.
- Patients with eating disorders.
- Life expectancy of less than 6 months.
- Patients unable to adequately complete the clinical laboratory assessments required for the study protocol.

Sample size calculation

There are no previous clinical trials focusing on this objective published in the literature. We report a study in patients with chronic kidney disease on haemodialysis (HD) (19) where measurement of the rectus femoris cross-sectional muscle area (RFCSA) was validated for the diagnosis of malnutrition related to this condition. RFCSA compared to bioimpedance spectroscopy had higher area under the curve (AUC, 0.686 vs. 0.581), sensitivity (72.8% vs. 65.8%), and specificity (55.6% vs. 53.9%). The AUC of RFCSA was higher for the risk of protein-energy wasting (PEW) in male (0.74, 95% CI: 0.66 to 0.82) and female patients (0.80, 95% CI: 0.70 to 0.90) (both p<0.001). Gender-specific RFCSA values (males <6.00 cm2;

females <4.47 cm^2) indicated that HD patients with lower RFCSA were 8 times more likely to have PEW (AOR = 8.63, 95% CI: 4.80-15.50, p<0.001).

Our study aims to establish the feasibility of nutritional ultrasound measurements at different ages in both sexes to apply to patients with nutritional risk worldwide. For this purpose, the electronic CRF will be programmed with the sample distributed by quotas to cover 50% men and 50% women, as well as 10-year age ranges. Age-stratified sampling is designed to obtain representative results of different ages and could be associated with the results of VGS, BIA, and dynamometry. Variability of measurements should be adjusted for sex, age and anthropometric parameters such as height.

It is estimated that 1,000 patients with nutritional risk will be discharged from 20-25 healthcare centres throughout Spain and that at least 60% of the population will complete the 3-to-6-month follow-up of the study. Due to the special pandemic situation, a higher-than-expected drop-out rate is expected at 6 months than under normal conditions (40% are estimated not to complete the 6-month follow-up for any reason).

Study conduct

The physicians participating in the study will be responsible for assessing the suitability of inclusion for each patient.

Patients will be consecutively recruited by the physician as they are assessed daily in their clinical practice at the hospital and found to have a risk of malnutrition according to the MUST/SARC-F (R-MAPP) screening test.

Before inclusion, the investigator must check the inclusion and exclusion criteria and obtain their informed consent.

The physician will be responsible for applying nutritional intervention and physical activity treatment according to standard clinical practice, as well as for clinical monitoring of patients. The treatment prescribed to each patient is not the objective of this study and is how the patient will experience changes that must be recorded with the different techniques described and with the muscle ultrasound involved in this study.

All physicians participating in the study must have been previously trained in the use of the ultrasound equipment and materials provided for the study, as well as in the use of the electronic CRF for data entry designed for this study.

Nutritional ultrasound techniques and measurements (ultrasound with 4-10 cm linear tube). The equipment provided for the study is UProbe L6C Ultrasound Scanner (linear transducer 7.5-10 kHz) that allows depths up to 100 mm. Manufactured by Guangzhou Sonostar Technologies Co., Ltd. PR China.

Quadriceps rectus femoris ultrasound (see Figure 3)

The measurement technique is determined for the patient lying supine with knees extended and relaxed.

Measurement technique:

- In the lower third of the imaginary line between the antero-superior iliac spine and the superior border of the patella.
- Correction of leg angle, it is important to focus the image on the rectus femoris.
- In malnourished patients, loss of muscle tone causes the muscle to move to the sides
- To minimize variability, measurements must be repeated three times.

Figure 3 – Comparison of longitudinal and transversal sections of the QRF muscle area ultrasound. Functional measures and main anatomical structures are represented.

Abdominal ultrasound (see Figure 4)

Total, superficial, and pre-peritoneal adipose tissue are measured (centimetres) for the patient lying down.

Measurement technique:

- The transducer is placed between the xiphoid process and the umbilicus in the midline (in patients with surgery without navel, this would be 10 cm from the xiphoid appendix).
- Images are taken during non-forced expiration, in a transverse plane with a variable probe depth of 4-10 cm, perpendicular to the skin.
- To minimize variability, measurements must be repeated three times.

Measurement planes:

- Measurement of subcutaneous adipose tissue: the superficial and deep layers are differentiated.
- Visceral adipose tissue measurement: it is measured in a transverse position. Measure the distance between the boundary of the parietal peritoneum to the *linea* alba on the inner side at the junction of the two-rectus straight abdominis muscles.

Figure 4 - Comparison of longitudinal and transversal sections of the abdominal area ultrasound. Functional measures and main anatomical structures are represented.

Follow-up period

The planned follow-up period for each patient will be 3 to 6 months from the inclusion visit. The investigating physician will perform at least one first inclusion visit, and a follow-up visit at 3 and 6 months for each patient.

Study duration

The study is planned to last 18 months to detect patients at risk of malnutrition, recruitment, field work, monitoring and data analysis.

An estimated 2-3 months will be needed to plan the coordination and distribution of the work in the hospitalisation and outpatient clinic areas for the selection of candidate patients. It will take 6 to 9 months to recruit patients. From the start of the study, the database will be completed, and preliminary analyses will be performed. The final analysis will be performed

when the follow-up is completed together with writing of the related work that will require 4 to 6 months to complete.

Outcome measures

A list of the outcomes of interest is provided in Table 1.

Table 1. Study outcomes.

PRIMARY OUTCOMES	
Nutritional ultrasound measurements: ultrasound with 4-10 cm linear probe *	
	• Abdominal ultrasound: total, superficial and pre-peritoneal adipose tissue (measured in centimetres)
	• Muscle ultrasound: Area, circumference, axes and adipose tissue (measured in centimetres)
SECONDARY OUTCOMES	
Sociodemographic data:	
	• Age • Sex • Educational level • Toxic habits • Medical history • Risk of sarcopenia and moderate to high malnutrition based on MUST and SARC-F screening test using R-MAPP
Subjective Global Assessment (SGA) questionnaire	
Anthropometric data:	
	• Current body weight (measured or estimated) • Usual weight • Adjusted weight (adjusted weight in obese subjects, dry weight without oedema in malnourished subjects) • Height (measured or estimated) • BMI • Arm circumference
Bioelectrical impedance data (model (50 kHz): **	
	• TBW (total body water, L) • ECW (extracellular water, L) • ICW (intracellular water, L) • FFM (lean mass, kg) • FM (fat mass, kg) • BCM (body cell mass, kg) • ASMM (appendicular skeletal muscle mass, kg) • SMI (skeletal muscle mass index, kg) • Percent hydration • Body fat (%)
Blood biochemistry data (at baseline visit, at 3 and 6 months):	
	• Albumin • Prealbumin • C-reactive protein • Total cholesterol • Lymphocytes
Bioelectrical impedance data (model (50 kHz): **	
	• Age • Sex • Educational level • Toxic habits • Medical history • Risk of sarcopenia and moderate to high malnutrition based on MUST and SARC-F screening test using R-MAPP
Functional parameters	
	• Timed Up and Go test (TUG): patient sits in a chair and is told to get up (timing starts), walks 3 metres, comes back and sits in the initial chair (timing ends). Interpretation: <20 seconds: normal,> 20 seconds: increased risk of falling. • Dynamometry. Three measurements of the dominant hand will be made recording the mean and maximum, measured in kilograms. Jamar® dynamometers are most used in international studies and have several grip positions.
Current patient status	

	• Hospital stays, mortality at 3 and 6 months, hospital readmissions and complications, if occurring, and their consequences (resolved/unresolved) must be recorded in the form.
Adherence	
	• Attendance to study follow-up visits.

*The equipment provided for the study is the UProbe L6C Ultrasound Scanner (linear transducer 7.5-10 kHz) that allows depths up to 100 mm. Manufactured by Guangzhou Sonostar Technologies Co., Ltd. PR China.
**Each healthcare center could use the BIA device they already owned. The most used device among all participants was AKERN branded.

Data analysis plan

Data analysis will be performed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA).

Quantitative variables will be expressed as mean ± standard deviation. The comparison between qualitative variables will be performed using the Chi-square test with Fisher's correction when necessary. Quantitative variables will be analysed using a Kolmogorov-Smirnov test. Differences between quantitative variables will be analysed using Student's t or ANOVA tests (for two or more samples, respectively) and non-parametric tests (Mann-Whitney or Kruskal-Wallis) will be used when the variables to be analysed do not follow a normal distribution.

Kappa coefficient will be used to assess agreement between techniques in diagnosis of malnutrition.

The association between variables will be studied using Spearman or Pearson correlations according to normality.

Several cut-off points will be estimated for prediction of diagnosis of malnutrition and sarcopenia using ultrasound by ROC curves.

The significant associations between muscle ultrasound parameters and the objective clinical variables in the univariate analysis will then be analysed in multivariate logistic regression models which also control other confounding variables. To assess which nutritional tool best predicts the risk of mortality during admission (and re-admission), we will perform multivariate logistic regression models, in which the dependent variable will be in-hospital mortality (or re-admission) based on the different tools applied (e.g. ultrasound, phase angle, SGA criteria, GLIM, LMI), also controlling for sex, the presence of previous comorbidities and other variables showing association in the univariate study.

For all calculations, a probability p less than 0.05 for two tails will be considered significant.

Recording of adverse reactions

Adverse reactions reporting is not the objective of the study. The investigator should proceed as usual and through the channels established in the healthcare system if any adverse effect occurs during follow-up. It will only be recorded in the follow-up if the patient must leave the study for this reason for statistical purposes.

Handling of missing data

No formal imputation will be made for the different analyses; therefore, all estimates will be obtained using all available data (available data only, ADO).

Since the study will be recorded using an electronic CRF (case report form), the necessary consistency filters and alerts for missing data will be programmed to validate and store the information, to minimise missing data and prevent the entry of incorrect or out of range data.

ETHICS

General aspects

This study will be conducted in accordance with current regulations, accepted international ethical standards of Good Clinical Practice (CPMP/ICH/135/95), the principles laid down in the latest version of the Declaration of Helsinki, RD 1591/2009 and Circular No. 07/2004 regulating clinical research with medical devices.

Informed consent

Before inclusion in the study and after considering the suitability of patient inclusion, all participating physicians must offer the patient information about the study using a patient information sheet, invite the patient to participate in it, answer their questions and request completion of the informed consent form that will be kept in their own file.

Evaluation by an Ethics Committee

All DRECO study materials have been approved by each of the IRB/IEC of all the sites enrolled (either approval of the own IRB/IEC or validating the approval of the IRB/IEC of another hospital).

Confidentiality

The study data will be entered into an automated file owned by the sponsor. The analysis of study results will be made from an anonymised database, that is, dissociated, with no personal data, so that no subject can be identified or identifiable. This study database will be extracted from the electronic CRF and will include data from physician records, impedance recordings, and muscle ultrasound images. Data from different sources will be linked from the patient code and will not include personal data. All data in the file owned by the sponsor will be treated confidentially. The sponsor undertakes not to transfer data to third parties.

Dissemination

Results from this study will be presented at international and national scientific conferences, and in peer-reviewed scientific journals.

DISCUSSION

There is a growing interest in the literature on the evaluation of muscle mass by ultrasound (20). Its current clinical utility focuses on measuring the involvement of muscle mass to assess the nutritional status of a patient (21). The further step that it is being investigated in this clinical study, is that muscle ultrasound becomes not only a tool to assess the diagnosis

of malnutrition but to integrate it in the routine clinical practices to evaluate nutritional interventions.

The evaluation of the nutritional ultrasound should enable clinical decisions based on its results to permit the adjustment and individualization of the nutritional therapeutic and physical exercise plan, along with functional recovery (20).

To the best of our knowledge, this is going to be the largest study (sample size=1,000) using nutritional ultrasound in patients with nutritional risk. Current scientific evidence is limited, and it is expected that such a large population will allow us to validate and define specific cut-off values for nutritional ultrasound and get its correlation with already well-known nutritional tools such as SGA or GLIM criteria (22).

The emerging field of ultrasound assessment of muscle mass only highlights the need for a standardisation of measurement technique as Perkisas, *et al* outline in their recently published 2022 SARCUS update. This update provides the approach of muscle assessment according to the most recent literature and anatomical landmarks for 39 different muscles. Besides, the discussion about 4 new muscle parameters that are added to the 5 that were previously considered is also presented (23) and some of these parameters have been correlated with PhA (24) and they will be analysed in our present protocol. Our ongoing study is intended to standardize these outstanding technique measures, to apply this technique widely soon.

Author Contributions: All authors have identified the research question and were responsible for the conception and design of the protocol and the study. JM.G.A., D.B. D.L.R, and G.O. are conducting study investigation. G.G.R has managed funding acquisition. All authors have been involved in drafting the manuscript and revising it critically for intellectual content. All authors read and approved the final manuscript.

Funding: This study will be conducted thanks to a research grant from Abbott Laboratories.

Conflicts of Interest: JM.G.A., D.B. D.L.R, and G.O declare no conflict of interest. G.G.R is an employee of Abbott Laboratories.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the following Ethical Committees: Comité de Ética de la Investigación Provincial De Málaga, Portal de Ética de la Investigación Biomédica de Andalucía (PEIBA), Comité de Ética de la Investigación con medicamentos Área De Salud Valladolid, Comité de Ética de la Investigación con medicamentos Hospital Universitario La Paz, Comité de Ética de la Investigación con medicamentos Hospital Universitario 12 de Octubre, Comité de Ética de la Investigación con medicamentos Hospital General Universitario Gregorio Marañón, Comité de Ética de la Investigación con medicamentos del Complejo Hospitalario Universitario de Canarias (Provincia de Santa Cruz de Tenerife),

Comité de Ética de la Investigación con medicamentos del Complejo Hospitalario Universitario de Las Palmas, Comité de Ética de la Investigación con medicamentos y Comisión de Proyectos de Investigación del Hospital Universitari Vall d'Hebron, Comité de Ética de la Investigación con medicamentos del Consorcio Hospital General Universitario de Valencia, Comité de Ética de la Investigación con medicamentos de la Clínica de Navarra, Comité de Ética de la Investigación con medicamentos del Hospital de Basurto and Comité de Ética de la Investigación con Medicamentos del Hospital Universitario y Politécnico la Fe de Valencia. This study is registered at clinicaltrials.gov (NCT05433831), registered on June 27th, 2022. https://clinicaltrials.gov/ct2/show/NCT05433831.

Informed Consent Statement: All participants are provided with a participant information sheet and are required to provide written consent.

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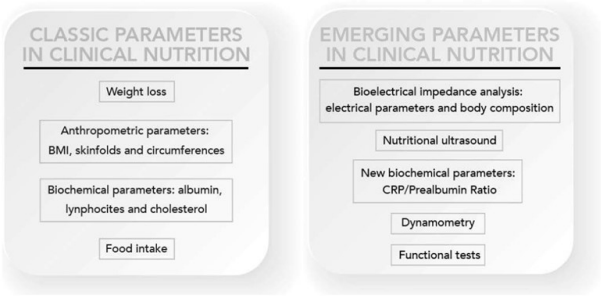

Figure 1. Update of nutritional evolution parameters. Reproduced with permission from the authors

338x190mm (96 x 96 DPI)

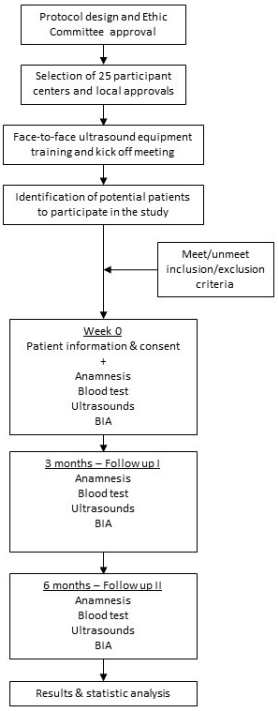

Figure 2 shows the schedule of enrolment, interventions, and assessments.

190x338mm (96 x 96 DPI)

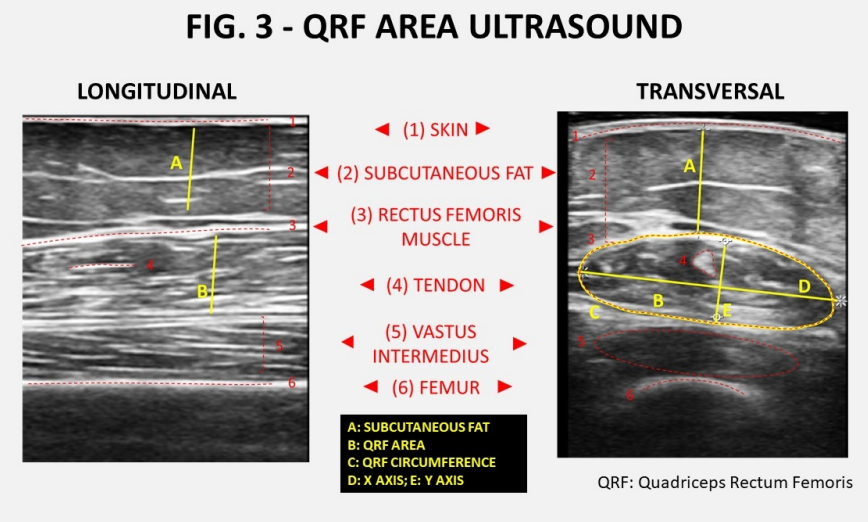

Figure 3 – Comparison of longitudinal and transversal sections of the QRF muscle area ultrasound. Functional measures and main anatomical structures are represented.

338x190mm (96 x 96 DPI)

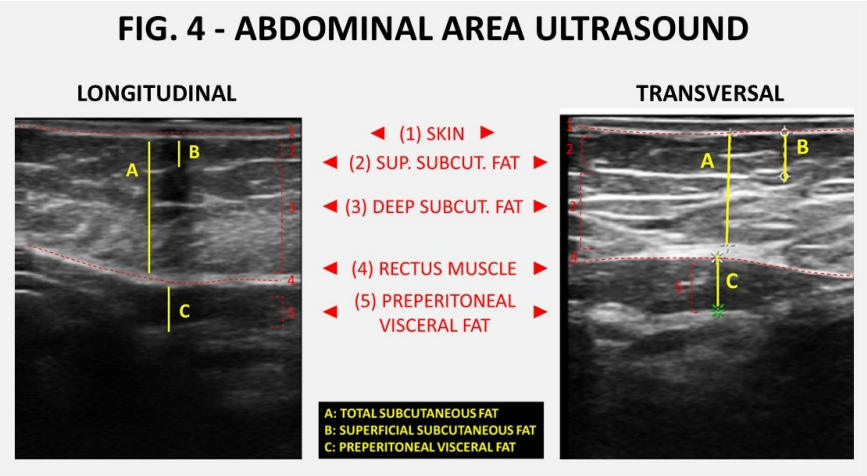

Figure 4 - Comparison of longitudinal and transversal sections of the QRF muscle area ultrasound. Functional measures and main anatomical structures are represented.

338x190mm (96 x 96 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	#3	Date and version identifier	NA
Funding	#4	Sources and types of financial, material, and other support	12
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1; 12
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1;12
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team,	1;12

		and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	1-5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	NA
Objectives	#7	Specific objectives or hypotheses	5-6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6-7
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	NO DRUG INTERVENTION
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	NO DRUG INTERVENTION
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg,	10 – TABLE 1

		systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7 – FIGURE 2
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7-8
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7-8
Methods: Assignment of interventions (for controlled trials)			NO CONTROLLED TRIAL

Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA

Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10

Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10
Methods: Monitoring			**NA**
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	

Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	11
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	11
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA

Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	11
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	12
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	11
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	11
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA

Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

BMJ Open

Protocol for a prospective cohort study on the feasibility of application of nutritional ultrasound in the diagnosis and follow-up of patients with nutritional risk at hospital discharge: Study on body composition and function (DRECO).

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-074945.R1
Article Type:	Protocol
Date Submitted by the Author:	20-Oct-2023
Complete List of Authors:	García Almeida, José Manuel; Hospital Universitario Virgen de la Victoria, Department of Endocrinology and Nutrition Bellido, Diego; Complejo Hospitalario de Ferrol, Department of Endocrinology and Nutrition De Luis, D.A; Valladolid University Hospital, Department of Endocrinology and Nutrition Guzmán Rolo, Germán ; Abbot Laboratories Olveira, Gabriel; Regional University Hospital of Málaga, Department of Endocrinology and Nutrition
Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	NUTRITION & DIETETICS, Ultrasound < RADIOLOGY & IMAGING, Nutritional support < GASTROENTEROLOGY

Protocol for a prospective cohort study on the feasibility of application of nutritional ultrasound in the diagnosis and follow-up of patients with nutritional risk at hospital discharge: Study on body composition and function (DRECO).

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ABSTRACT

Introduction: Nutritional ultrasound is an emerging technique in clinical nutrition for the morphological and structural study of muscle mass. Currently, all definitions of malnutrition include the measurement of muscle mass, however, there is no single way to assess it. It is necessary to develop new techniques to identify muscle involvement in malnutrition that are valid, standardized, reliable, accurate and profitable.

Objective: To value the new muscle ultrasound techniques aimed to measure muscle and functional status, to make a more accurate diagnosis and a better prediction of complications and morbidity and mortality in patients at nutritional risk. Primary outcome: to assess the feasibility of ultrasound or muscle ultrasound techniques in both nutritional diagnosis and follow-up in a nutritional intervention program.

Methods and analysis: DRECO (Disease-Related caloric-protein malnutrition EChOgraphy) is a prospective, multicenter (25 Spanish hospitals), uncontrolled clinical study in standard clinical practice to value the usefulness of nutritional ultrasound (muscle ultrasound) in the nutritional diagnosis and follow-up, over 3 to 6 months, after standard nutritional clinical

practice intervention and physical activity, to control their disease-related malnutrition. 1000 patients are expected to be included in.

Discussion: This study will standardize nutritional ultrasound measures. It will validate and define specific cut-off values for nutritional ultrasound and correlate it with already well-known nutritional tools such as SGA (Subjective Global Assessment) or GLIM (Global Leadership Initiative on Malnutrition) criteria. Thus, muscle ultrasound will become not only a tool to diagnose malnutrition, but it will be integrated in the daily practice to evaluate nutritional interventions.

Ethics and dissemination Ethical: All DRECO study materials have been approved by each of the IRB/IEC of all the sites enrolled (either approval of the own IRB/IEC or validating the approval of the IRB/IEC of another hospital). The study has been registered with ClinicalTrials.gov, on June 27th, 2022. Results from this study will be presented at scientific conferences and in peer-reviewed scientific journals.

Trial registration number: NCT05433831

Strengths and limitations of this study

DRECO strengths

- Multicentre, prospective, medium-term study in which a large sample (1000 patients) is expected to be recruited.
- First study designed as a real-world study to evaluate the feasibility of nutritional ultrasound, led by senior researchers wide experienced in clinical nutrition.
- Validation of classical tools and new morpho functional assessment techniques (ultrasound and bioelectrical impedance) are proposed.

DRECO limitations

- Non-randomized clinical practice study, so it will not be possible to adequately analyze the effect of nutritional intervention.
- It is restricted to patients upon hospital discharge, so it cannot be generalized to the entire population of people at risk of malnutrition.

Keywords: nutritional ultrasound; nutritional biomarker; ultrasound cut-off values; disease-related malnutrition; GLIM; SGA; body composition; sarcopenia; quadriceps femoris muscle; abdominal muscle area, muscle mass.

1. INTRODUCTION

Disease-related malnutrition (DRM) can occur when there is a deficient supply of energy, protein and/or other nutrients, depending on the nutritional needs of everyone at different times of their life cycle or health or disease circumstances. This deficiency induces effects on body composition and tissue and organ function and results in clinical consequences: increased morbidity and mortality associated with different disease processes (1).

In 2019, the GLIM criteria were published (2), providing a different vision of how to assess the malnourished patient. These criteria are divided into both phenotypic and etiological criterion:

- Phenotypic criterion
    - Weight loss (%): >5% within past 6 months, or >10% beyond 6 months
    - Low body mass index (kg/m^2): <20 if < 70 years, or <22 if >70 years. Asia: <18.5 if < 70 years, or <20 if >70 years
    - Reduced muscle mass: Reduced by validated body composition measuring techniques
- Etiological criterion
    - 50% of ER (energy requirements) > 1 week, or any reduction for >2 weeks, or any chronic GI (gastrointestinal) condition
      that adversely impacts food assimilation or absorption
    - Inflammation: Acute disease/injury or chronic disease-related

There are techniques for nutritional assessment using assessment tools aimed at morpho functional diagnosis of malnutrition (3), in addition to the classical nutritional parameters, such as weight loss, BMI (body mass index), folds, circumferences, albumin, lymphocytes, cholesterol and intake. New advanced parameters are being incorporated into clinical nutrition and their incorporation into clinical practice is of increasing interest, such as measures derived from bioelectrical impedance (BIA) and phase angle (PhA), dynamometry, functional tests, CRP/prealbumin ratio and muscle ultrasound (see Figure 1).

Figure 1. Update of nutritional evolution parameters. Reproduced with permission from the authors (3).

From a scientific point of view, the following nutritional assessment techniques are being incorporated:

Muscle ultrasound

The application of ultrasound for the morphological and structural study of muscle mass is an emerging technique. Currently, there are different validation studies on the measurement technique. The ultrasound technique determines the surface area of the muscle in transverse and longitudinal position. With ultrasound analysis, it is possible to measure key parameters of muscle architecture, such as muscle volume and muscle fascicle length. Although there are different muscle structures that can be evaluated, many of the studies focus on the quadriceps rectus femoris or on combinations of various muscle groups involving large muscle bundles with functional importance to the patient in terms of gait. Measurement of the rectus femoris of the quadriceps is one of the most referenced measurements due to its correlation with strength and tests of execution or functional performance. It is necessary to develop new techniques to identify muscle involvement in malnutrition that are valid,

standardised, reliable, accurate and profitable. Currently, all definitions of malnutrition include the measurement of muscle mass involvement, however, there is no single way to assess it. The classic imaging techniques such as DEXA (dual-energy x-ray absorptiometry), CT (computerised tomography) and MRI (magnetic resonance imaging) are considered the gold standard, but they have difficulties in their clinical application under normal practice conditions. Ultrasound has the advantage of being inexpensive, portable, and does not involve ionising radiation. Several studies have confirmed the reliability of this technique to measure the size of the quadriceps muscle in a healthy population (4). Studies on the reliability of rectus femoris ultrasound have been published with an intraclass coefficient of variation (ICC) of 0.97 (95% CI: 0.92-0.99) for the test-retest reliability of ultrasound.

The American Society for Parenteral and Enteral Nutrition (ASPEN), among the criteria for the diagnosis of malnutrition in adults, recommends including an evaluation of fat and muscle deposits. Specialists must incorporate techniques that properly help to identify the loss of muscle and fat mass for a correct diagnosis of malnutrition. Implementing these evaluation techniques and instruments is challenging and remains a work in progress (5). Muscle ultrasonography correlates with body composition measurement techniques such as BIA and anthropometry in patients with cancer (6). In adults with cystic fibrosis muscle ultrasound measurements, particularly the mean muscular area rectus anterior (MARA), are related to the nutritional status and respiratory function of these patients. (7)

The Global Leadership Initiative on Malnutrition (GLIM) has recently appointed a working group to provide consensus-based guidance on assessment of skeletal muscle mass and its role in the malnutrition diagnostic and assessment process. They support the use of US (ultrasound), particularly in settings where its practical applicability provides potential for patient follow-up through repeated measurements, but it requires standardisation through experienced operators, and repeated measurements performed by the same individual. They also encourage further validation studies for the US (8).

Bioelectrical impedance (BIA)

BIA is used as a tool to obtain data that helps to better understand the patient's nutritional status, being a non-invasive, inexpensive, and easily transportable technique. Vector analysis and phase angle provide direct data, not being necessary to be later adjusted using formulas or mathematical models, as it is needed with simple or multifrequency bioelectrical impedance or multifrequency (9). This method is based on the analysis of the two bioimpedance vectors: resistance (R) and capacitive reactance (Xc). Resistance is defined as the opposition to a flow of electric current through a circuit component, medium, or substance, providing information about biological fluids, and therefore, related to tissue hydration. A decrease in the resistance/height ratio will indicate swelling or third space; conversely, an increased ratio will indicate dehydration. Reactance is the effect on an

electrical current caused by a material's ability to store energy in cell membranes, so it is related to the cell mass and the integrity of its membranes. A decrease in Xc indicates loss of cell mass. This cell mass is the sum of all metabolically active cells, being the central parameter in the evaluation of nutritional status since the reduction of cell mass is typically related to malnutrition (10).

A recent study conducted by Fernandez-Jimenez, *et al* found that a low SPhA (standardised phase angle)-malnutrition value (SPhA < −0.3) was significantly associated with a higher mortality hazards ratio (HR 7.87, 95% CI 2.56–24.24, p < 0.001). This biological marker could therefore be incorporated among the screening tools and mortality risk assessment in this population (11).

Dynamometry

Dynamometry is one of the 6 criterions to define malnutrition according to ASPEN (12). It is extremely sensitive to nutritional status changes, so it is particularly useful to track nutritional therapy or interventions results, even in the short and medium term. It has mostly been used to predict post-surgical complications including elderly patients (13). Results obtained are compared to the population averages by age and sex. Sanchez et al (14) presented reference values for hand dynamometry using a Jamar hand dynamometer for a Spanish population, providing cut-off points to define malnutrition. They concluded that hand dynamometry is associated with lean mass, which supports its usefulness in nutritional assessment.

Although the new GLIM consensus-based guidance on assessment of skeletal muscle mass do not include dynamometry as a marker of muscle mass (8), the authors hereby signing this article have previously studied dynamometry as a marker of muscle mass suggesting that GLIM criterion and dynamometry are associated to a higher mortality rate in both hospitalised and outpatient oncology patients (15, 16).

Functional tests

These tests are a series of physical activities related to mobility, walking or balance. Their results are related to those of scales that assess instrumental activities of daily living (IADL). The most common are the "Timed Up and Go test" (TUG), the "Gait Speed Test" (GST) and the "Short Physical Performance Battery (SPPB)" test that includes 3 tests (balance, gait speed and get up and walk) (17).

Besides, the decrease in physical performance, evaluated by the SPPB test or hand grip strength, has been shown to be elevated in patients with colorectal cancer prior to surgery and it was related to an increase in postoperative complications and mortality (18).

1.1. STUDY OBJECTIVES

The objective of this study is to value the new muscle ultrasound techniques aimed to measure muscle and functional status, to make a more accurate diagnosis and a better

prediction of complications and morbidity and mortality in patients at nutritional risk. This main objective is developed in primary and secondary objectives as it follows:

1.1.1. Primary objective

- To assess the feasibility of ultrasound or muscle ultrasound techniques in both nutritional diagnosis and follow-up, over 3 to 6 months, in a nutritional intervention programme.

1.1.2. Secondary objectives

- To determine the association between muscle morphological parameters (nutritional ultrasound of the leg (area, circumference, axis and adipose tissue), total abdominal and pre-peritoneal parameters measured by nutritional ultrasound and the nutritional and functional status of the patient, as well as their prognostic value in hospitalised patients.
- To establish an association between ultrasound as a diagnostic value of malnutrition as compared to the diagnostic gold standard (SGA and GLIM criteria).
- To determine the ultrasound cut-off points associated with the diagnosis of malnutrition and sarcopenia using the following tools:
    - Measurement of body composition using impedance techniques (Report: Phase angle, body cell mass (BCM), hydration, fat free mass (FFM) and lean mass index.
    - Muscle strength and capacity to perform physical activity after the intervention: dynamometry and Timed Up and Go test (TUG).
    - Criteria for sarcopenia.
    - To assess association with inflammatory activity markers: High-sensitivity C-reactive protein (CRP)/prealbumin.
- To assess ultrasound changes in patient follow-up.
- To establish an association of ultrasound results as predictors of morbidity and mortality (stay, mortality at 3 and 6 months, readmissions and in-hospital complications).

2. PATIENT PARTICIPANT INVOLVEMENT AND FEASIBILITY OF STUDY DESIGN

DRECO (Disease-Related caloric-protein malnutrition EChOgraphy) is a prospective, multicentre, uncontrolled clinical study in standard clinical practice to value the usefulness of nutritional ultrasound (muscle ultrasound) in the nutritional diagnosis and follow-up of patients over a period of 3 to 6 consecutive months, after standard nutritional clinical practice intervention, and physical activity to control their disease-related malnutrition.

The study may be considered non-interventional since patients will undergo nutritional interventions and the standard treatment planned by their physician for treatment according to his/her standard clinical practice, and the only addition to the standard measurement and follow-up techniques of the patient will be the performance of a muscle ultrasound measurement using equipment provided to the centre for this purpose.

Patients over 18 years of age who, in the first week of hospital admission in medical-surgical areas, excluding critical patients, have an assessment of risk of malnutrition according to the

MUST and SARC-F screening test using R-MAPP. [(MUST: Malnutrition Universal Screening Tool; SARC-F is an acronym of 5 domains included in the questionnaire: 1) Strength, 2) Assistance with walking, 3) Rising from a chair, 4) Climbing stairs, and 5) Falls; R-MAPP (Remote consultation on MAlnutrition in the Primary Practice)].

If the results show a moderate or high risk of malnutrition, these patients will be invited to participate in the study, and will undergo the morpho functional assessment, an ultrasound study and the Subjective Global Assessment (SGA). This study is registered under ClinicalTrials.gov (NCT05433831).

Figure 2 shows the schedule of enrolment, interventions, and assessments.

2.1. Inclusion criteria

- Patients admitted to hospital who in the first week of admission have moderate or high risk of malnutrition according to the MUST and SARC-F screening test using R-MAPP.
- Patients aged 18 to 85 years.
- Patient who agrees to participate in the study and signs the informed consent.

2.2. Exclusion criteria

- Hepatic impairment - AST/ALT (aspartate aminotransferase/alanine aminotransferase) 3 x upper limit of normal.
- Chronic kidney failure - GFR (glomerular filtration rate) <45 mL/min).
- Patients with previous ICU (intensive care unit) stay during the study admission.
- Cancer patients on palliative treatment or ECOG (Eastern Cooperative Oncology Group) ≥ 3.
- Orthopaedic disease that does not allow adequate walking.
- Patients with known dementia or others not related to a significant neurological or psychiatric disorder, or any other psychological condition that may interfere with the conduct of the study.
- Patients with eating disorders.
- Life expectancy of less than 6 months.
- Patients unable to adequately complete the clinical laboratory assessments required for the study protocol.

2.3. Sample size calculation

There are no previous clinical trials focusing on this objective published in the literature. We report a study in patients with chronic kidney disease on haemodialysis (HD) (19) where measurement of the rectus femoris cross-sectional muscle area (RFCSA) was validated for the diagnosis of malnutrition related to this condition. RFCSA compared to bioimpedance spectroscopy had higher area under the curve (AUC, 0.686 vs. 0.581), sensitivity (72.8% vs. 65.8%), and specificity (55.6% vs. 53.9%). The AUC of RFCSA was higher for the risk of

protein-energy wasting (PEW) in male (0.74, 95% CI: 0.66 to 0.82) and female patients (0.80, 95% CI: 0.70 to 0.90) (both p<0.001). Gender-specific RFCSA values (males <6.00 cm2; females <4.47 cm^2) indicated that HD patients with lower RFCSA were 8 times more likely to have PEW (AOR = 8.63, 95% CI: 4.80-15.50, p<0.001).

Our study aims to establish the feasibility of nutritional ultrasound measurements at different ages in both sexes to apply to patients with nutritional risk worldwide. For this purpose, the electronic CRF will be programmed with the sample distributed by quotas to cover 50% men and 50% women, as well as 10-year age ranges. Age-stratified sampling is designed to obtain representative results of different ages and could be associated with the results of VGS, BIA, and dynamometry. Variability of measurements should be adjusted for sex, age and anthropometric parameters such as height.

It is estimated that 1,000 patients with nutritional risk will be discharged from 20-25 healthcare centres throughout Spain and that at least 60% of the population will complete the 3-to-6-month follow-up of the study. Due to the special pandemic situation, a higher-than-expected drop-out rate is expected at 6 months than under normal conditions (40% are estimated not to complete the 6-month follow-up for any reason).

2.4. Study conduct

The physicians participating in the study will be responsible for assessing the suitability of inclusion for each patient.

Patients will be consecutively recruited by the physician as they are assessed daily in their clinical practice at the hospital and found to have a risk of malnutrition according to the MUST/SARC-F (R-MAPP) screening test.

Before inclusion, the investigator must check the inclusion and exclusion criteria and obtain their informed consent.

The physician will be responsible for applying nutritional intervention and physical activity treatment according to standard clinical practice, as well as for clinical monitoring of patients. The treatment prescribed to each patient is not the objective of this study and is how the patient will experience changes that must be recorded with the different techniques described and with the muscle ultrasound involved in this study.

All physicians participating in the study must have been previously trained in the use of the ultrasound equipment and materials provided for the study, as well as in the use of the electronic CRF for data entry designed for this study.

Throughout the entire study, monthly meetings are hold with all participants on Thursdays at 8:30 a.m., and on Fridays at 8:30 a.m. with the study's central committee. The objective of these meetings is to monitor the status of the study at each participating center, to resolve doubts, and to make sure that all techniques and measurements are properly made according to previous training.

2.4.1. Nutritional ultrasound techniques and measurements

US accuracy highly depends on the skills of the technician. Point training using rectus femoris phantom have shown to improve the accuracy of measurements. (20) Before starting the study, a training session was held. All study participants were required to attend, and they had the opportunity to practice with the same ultrasound machine that was going to be used in the study in phantom patients. Besides, several videos explaining detailed measurements technique were recorded. These videos were proactively shared with all researchers and available anytime at the study on-line electronic data capture (EDC) platform.

Beyond, once the study finishes, all DICOM images gathered will be analysed to develop a semi-automated algorithm that helps diagnose the patient's nutritional status. Subsequently, once the algorithm is available, the individual and manual US measurements will be contrasted with the data showed by the automatic algorithm, thus minimizing the inter and intra observer correlation. This work will have its own analysis and publication plan.

Abdominal and anterior thigh muscle measurements are performed using a commercially available portable ultrasound system with a 4-10 cm linear tube (UProbe L6C Ultrasound Scanner, Guangzhou Sonostar Technologies Co., Ltd., Guangzhou, Guangdong, P.R. China). The funder of the study provided an ultrasound machine to each of the participants hospitals.

2.4.1a. Quadriceps rectus femoris (QRF) ultrasound (see Figure 3)

With the patient lying supine with knees extended and relaxed, ultrasound measurements of unilateral (right side) quadriceps rectus femoris is performed at each participating center by an experienced medical sonographer blinded to the clinical data and other results of nutritional assessment. The acquisition site is located two-thirds of the way along the femur length, measured between the anterior superior iliac spine and the upper edge of the patella. The transducer is placed perpendicular to the long axis of the thigh with excessive use of contact gel and minimal pressure to avoid compression of the muscle. All parameters are taken as an average of three consecutive measurements in the dominant leg. We measure the transversal axis of the cross-sectional area (CSA) in cm^2, the X-axis and Y-axis in mm, which corresponded to the linear measurement of the distance between the muscular limits of the rectus femoris (lateral and anteroposterior), the X-axis/Y-axis ratio, and the total fat tissue in mm. All US parameters were also standardized divided by height squared (in cm^2 for rectus femoris). The DICOM images of the QRF ultrasounds will be kept for later analysis.

Figure 3 – Comparison of longitudinal and transversal sections of the QRF muscle area ultrasound. Functional measures and main anatomical structures are represented.

2.4.1b. Abdominal ultrasound (see Figure 4)

The second component of nutritional ultrasound is the evaluation of fat at the level of the abdominal wall. (21) The location of the measurement point is set at the midpoint between the xiphoid appendix and the navel on the midline. The patient remains in a supine position in a situation of relaxation and the image is taken during the unforced expiration, in a transverse plane using the same linear probe perpendicular to the skin. In the cross-section, the anatomical structures that are visualized are ordered from the most superficial layer corresponding to the epidermis, followed by the layer of subcutaneous, superficial, and deep adipose tissue. Then the two muscles of the anterior rectum of the abdomen that join in the central part in the linea alba are identified. (21) We measure both total and superficial subcutaneous adipose tissue and the pre-peritoneal visceral adipose tissue. The DICOM images of the abdominal ultrasounds will be kept for later analysis.

Figure 4 - Comparison of longitudinal and transversal sections of the abdominal area ultrasound. Functional measures and main anatomical structures are represented.

- **2.4.2 Bioelectrical Impedance (BIA)**

Total body BIA (50-kHz frequency) (Tanita BC-420MA BIA analyzer, Tanita Corporation, Arlington Heights, IL, USA) was used to determine phase angle (degrees), total body water (%), fat mass (kg), lean mass (kg), body cell mass (kg), and appendicular skeletal muscle mass (ASMM) (kg).

Since interval fluid balance is more sensible to the change of edema, bioelectrical impedance analysis can be affected in edematous patients. (22) Therefore, extreme phase angle values and/or non-coherent reactance/resistance ratios will be discarded, as a control measure, to detect patients with edema and fluid balance change.

- **2.4.3 Timed Up and Go test (TUG)**

The TUG test was used to assess functionality. A coloured tape was marked 3 m away from an armless chair in which participants were sitting. Participants were asked to walk 3 m, turn around the marked tape, and return to the chair as fast as they could. A timer was set as soon as the patient stood up from the chair and was stopped when the patient was seated again. At least one practice trial was performed before the test. Being that a TUG-score of ≥ 20 s is identified as a cut-off point for severe sarcopenia, TUG was considered in this study. (23)

- **2.4.4 Handgrip strength test**

Handgrip strength was determined using the Jamar dynamometer (J A Preston Corporation, New York, NY, USA). The dominant hand was tested. Three measurements of both media and maximum value were taken. The American Society for Parenteral and Enteral Nutrition has included the assessment of grip strength by dynamometer as one of

the six criteria to define malnutrition. (24) In this study, the cut-off values defined for the Spanish population will be considered. (14)

Although some quality-of-life test, such as SF-36 or ADL test (activities of daily living), were initially considered in the study protocol, they were finally rejected because, in real clinical practice, these tests are not used with the patient profile included in this study.

- **2.4.5 Follow-up period**

The planned follow-up period for each patient will be 3 to 6 months from the inclusion visit. The investigating physician will perform at least one first inclusion visit, and a follow-up visit at 3 and 6 months for each patient. A follow-up period of 6 months was established since it is common clinical practice in these patients, and with the aim of making the results more generalizable.

It is planned that the same physician attends the three visits to the patient (baseline, 3 and 6 months), to minimise the interpersonal variability in the measurements.

- **2.4.6. Study duration**

The study is planned to last 18 months to detect patients at risk of malnutrition, recruitment, field work, monitoring and data analysis.

An estimated 2-3 months will be needed to plan the coordination and distribution of the work in the hospitalisation and outpatient clinic areas for the selection of candidate patients. It will take 6 to 9 months to recruit patients. From the start of the study, the database will be completed, and preliminary analyses will be performed. The final analysis will be performed when the follow-up is completed together with writing of the related work that will require 4 to 6 months to complete.

2.5. Outcome measures

A list of the outcomes of interest is provided in Table 1.

Table 1. Study outcomes.

PRIMARY OUTCOMES	
Nutritional ultrasound measurements: ultrasound with 4-10 cm linear probe *	
	• Abdominal ultrasound: total, superficial and pre-peritoneal adipose tissue (measured in centimetres)
	• Muscle ultrasound: Area, circumference, axes and adipose tissue (measured in centimetres)
SECONDARY OUTCOMES	
Sociodemographic data:	
	• Age • Sex • Educational level • Toxic habits • Medical history • Risk of sarcopenia and moderate to high malnutrition based on MUST and SARC-F screening test using R-MAPP

Subjective Global Assessment (SGA) questionnaire	
Anthropometric data:	
	• Current body weight (measured or estimated) • Usual weight • Adjusted weight (adjusted weight in obese subjects, dry weight without oedema in malnourished subjects) • Height (measured or estimated) • BMI • Arm circumference
Bioelectrical impedance data (model (50 kHz): **	
	• TBW (total body water, L) • ECW (extracellular water, L) • ICW (intracellular water, L) • FFM (lean mass, kg) • FM (fat mass, kg) • BCM (body cell mass, kg) • ASMM (appendicular skeletal muscle mass, kg) • SMI (skeletal muscle mass index, kg) • Percent hydration • Body fat (%)
Blood biochemistry data (at baseline visit, at 3 and 6 months):	
	• Albumin • Prealbumin • C-reactive protein • Total cholesterol • Lymphocytes
Bioelectrical impedance data (model (50 kHz):	
	• Age • Sex • Educational level • Toxic habits • Medical history • Risk of sarcopenia and moderate to high malnutrition based on MUST and SARC-F screening test using R-MAPP
Functional parameters	
	• Timed Up and Go test (TUG): patient sits in a chair and is told to get up (timing starts), walks 3 metres, comes back and sits in the initial chair (timing ends). Interpretation: <20 seconds: normal,> 20 seconds: increased risk of falling. • Dynamometry. Three measurements of the dominant hand will be made recording the mean and maximum, measured in kilograms. Jamar® dynamometers are most used in international studies and have several grip positions.
Current patient status	
	• Hospital stays, mortality at 3 and 6 months, hospital readmissions and complications, if occurring, and their consequences (resolved/unresolved) must be recorded in the form.
Adherence	
	• Attendance to study follow-up visits.

*The equipment provided for the study is the UProbe L6C Ultrasound Scanner (linear transducer 7.5-10 kHz) that allows depths up to 100 mm. Manufactured by Guangzhou Sonostar Technologies Co., Ltd. PR China.

2.6. Data analysis plan

Data analysis will be performed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Quantitative variables will be expressed as mean ± standard deviation. The comparison between qualitative variables will be performed using the Chi-square test with Fisher's correction when necessary. Quantitative variables will be analysed using a Kolmogorov-Smirnov test. Differences between quantitative variables will be analysed using Student's t or ANOVA tests (for two or more samples, respectively) and non-parametric tests (Mann-Whitney or Kruskal-Wallis) will be used when the variables to be analysed do not follow a normal distribution.

Kappa coefficient will be used to assess agreement between techniques in diagnosis of malnutrition.

The association between variables will be studied using Spearman or Pearson correlations according to normality.

The thresholds for translation into clinical practice will be presented as cut-off points that will be estimated by AUC ROC curves. Centiles will be also considered.

The significant associations between muscle ultrasound parameters and the objective clinical variables in the univariate analysis will then be analysed in multivariate logistic regression models which also control other confounding variables. To assess which nutritional tool best predicts the risk of mortality during admission (and re-admission), we will perform multivariate logistic regression models, in which the dependent variable will be in-hospital mortality (or re-admission) based on the different tools applied (e.g. ultrasound, phase angle, SGA criteria, GLIM, LMI), also controlling for sex, the presence of previous comorbidities and other variables showing association in the univariate study.

For all calculations, a probability p less than 0.05 for two tails will be considered significant.

- **2.6.1. Recording of adverse reactions**

Adverse reactions reporting is not the objective of the study. The investigator should proceed as usual and through the channels established in the healthcare system if any adverse effect occurs during follow-up. It will only be recorded in the follow-up if the patient must leave the study for this reason for statistical purposes.

- **2.6.2. Handling of missing data**

No formal imputation will be made for the different analyses; therefore, all estimates will be obtained using all available data (available data only, ADO).

Since the study will be recorded using an electronic CRF (case report form), the necessary consistency filters and alerts for missing data will be programmed to validate and store the information, to minimise missing data and prevent the entry of incorrect or out of range data.

3. ETHICS

3.1. General aspects

This study will be conducted in accordance with current regulations, accepted international ethical standards of Good Clinical Practice (CPMP/ICH/135/95), the principles laid down in the latest version of the Declaration of Helsinki, RD 1591/2009 and Circular No. 07/2004 regulating clinical research with medical devices.

3.2. Informed consent

Before inclusion in the study and after considering the suitability of patient inclusion, all participating physicians must offer the patient information about the study using a patient information sheet, invite the patient to participate in it, answer their questions and request completion of the informed consent form that will be kept in their own file.

3.3. Evaluation by an Ethics Committee

All DRECO study materials have been approved by each of the IRB/IEC of all the sites enrolled (either approval of the own IRB/IEC or validating the approval of the IRB/IEC of another hospital).

3.4. Confidentiality

The study data will be entered into an automated file owned by the sponsor. The analysis of study results will be made from an anonymised database, that is, dissociated, with no personal data, so that no subject can be identified or identifiable. This study database will be extracted from the electronic CRF and will include data from physician records, impedance recordings, and muscle ultrasound images. Data from different sources will be linked from the patient code and will not include personal data. All data in the file owned by the sponsor will be treated confidentially. The sponsor undertakes not to transfer data to third parties.

3.5. Dissemination

Results from this study will be presented at international and national scientific conferences, and in peer-reviewed scientific journals.

3.6. Patient and Public Involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

4. DISCUSSION

There is a growing interest in the literature on the evaluation of muscle mass by ultrasound (21). Its current clinical utility focuses on measuring the involvement of muscle mass to assess the nutritional status of a patient (25). The further step that it is being investigated in this clinical study, is that muscle ultrasound becomes not only a tool to assess the diagnosis of malnutrition but to integrate it in the routine clinical practices to evaluate nutritional interventions.

The evaluation of the nutritional ultrasound should enable clinical decisions based on its results to permit the adjustment and individualization of the nutritional therapeutic and physical exercise plan, along with functional recovery (21).

To the best of our knowledge, this is going to be the largest study (sample size=1,000) using nutritional ultrasound in patients with nutritional risk. Current scientific evidence is limited, and it is expected that such a large population will allow us to validate and define specific cut-off values for nutritional ultrasound and get its correlation with already well-known nutritional tools such as SGA or GLIM criteria (26).

This study stands out for the use of several morphofunctional assessment techniques in patients with disease-related malnutrition in real clinical practice. Beyond its large sample, it is the first study with this design, as a real-world study, to evaluate the feasibility of nutritional ultrasound.

The emerging field of ultrasound assessment of muscle mass only highlights the need for a standardisation of measurement technique as Perkisas, *et al* outline in their recently published 2022 SARCUS update. This update provides the approach of muscle assessment according to the most recent literature and anatomical landmarks for 39 different muscles. Besides, the discussion about 4 new muscle parameters that are added to the 5 that were previously considered is also presented (27) and some of these parameters have been correlated with PhA (28) and they will be analysed in our present protocol. Our ongoing study is intended to standardize these outstanding technique measures, to apply this technique widely soon. Recruited patients were at risk of malnutrition so the results will be very interesting for routine clinical practice and nutritional care, in this patient profile, easily generalizable and free to use with publication.

Author Contributions: All authors have identified the research question and were responsible for the conception and design of the protocol and the study. JM.G.A., D.B. D.L.R, and G.O. are conducting study investigation. G.G.R has managed funding acquisition. All authors have been involved in drafting the manuscript and revising it critically for intellectual content. All authors read and approved the final manuscript.

Funding: This study will be conducted thanks to Abbott Laboratories funding.

Conflicts of Interest: JM.G.A., D.B. D.L.R, and G.O declare no conflict of interest. G.G.R is an employee of Abbott Laboratories.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the following Ethical Committees: Comité de Ética de la Investigación Provincial De Málaga, Portal de Ética de la Investigación Biomédica de Andalucía (PEIBA), Comité de Ética de la Investigación con medicamentos Área De Salud Valladolid, Comité de Ética de la Investigación con medicamentos Hospital Universitario La Paz, Comité de Ética de la Investigación con medicamentos Hospital Universitario 12 de Octubre, Comité de Ética de la Investigación con medicamentos Hospital General Universitario Gregorio Marañón, Comité de Ética de la Investigación con medicamentos del Complejo Hospitalario Universitario de Canarias (Provincia de Santa Cruz de Tenerife), Comité de Ética de la Investigación con medicamentos del Complejo Hospitalario Universitario de Las Palmas, Comité de Ética de la Investigación con medicamentos y Comisión de Proyectos de Investigación del Hospital Universitari Vall d'Hebron, Comité de Ética de la Investigación con medicamentos del Consorcio Hospital General Universitario de Valencia, Comité de Ética de la Investigación con medicamentos de la Clínica de Navarra, Comité de Ética de la Investigación con medicamentos del Hospital de Basurto and Comité de Ética de la Investigación con Medicamentos del Hospital Universitario y Politécnico la Fe

de Valencia. This study is registered at clinicaltrials.gov (NCT05433831), registered on June 27th, 2022. https://clinicaltrials.gov/ct2/show/NCT05433831.

Informed Consent Statement: All participants are provided with a participant information sheet and are required to provide written consent.

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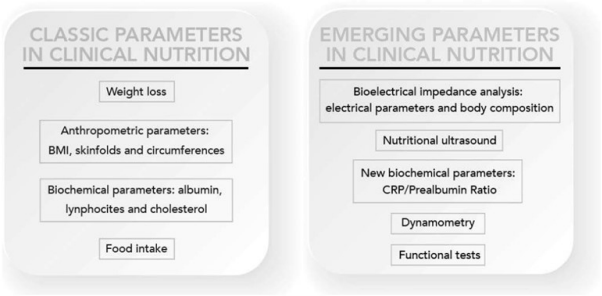

Figure 1. Update of nutritional evolution parameters. Reproduced with permission from the authors

338x190mm (96 x 96 DPI)

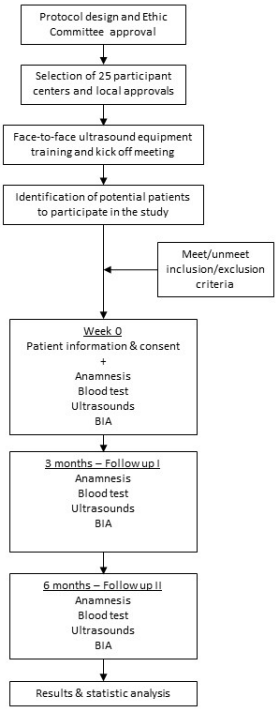

Figure 2 shows the schedule of enrolment, interventions, and assessments.

190x338mm (96 x 96 DPI)

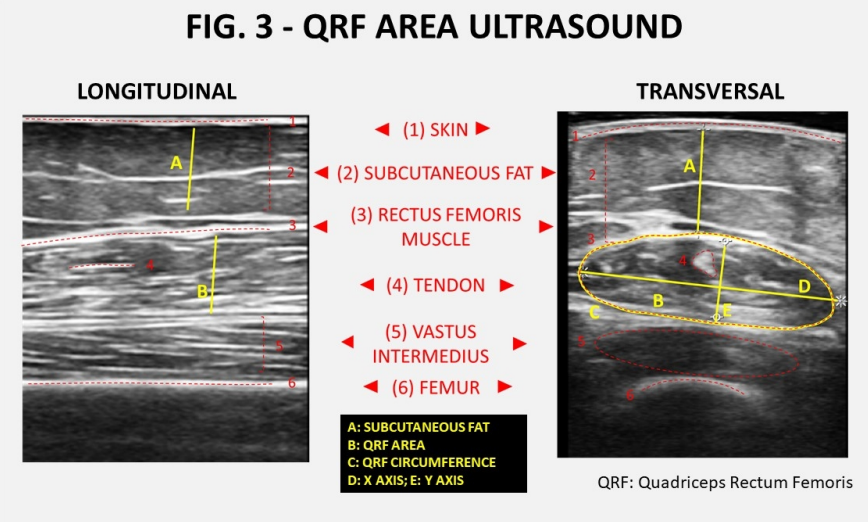

Figure 3 – Comparison of longitudinal and transversal sections of the QRF muscle area ultrasound. Functional measures and main anatomical structures are represented.

338x190mm (96 x 96 DPI)

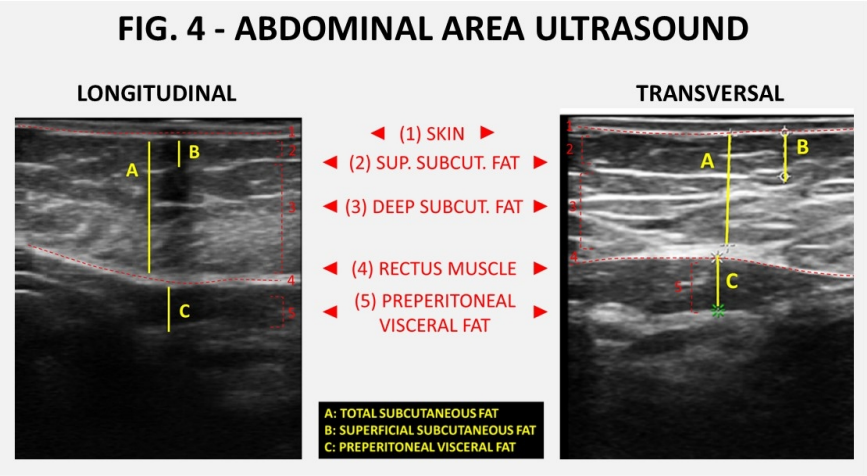

Figure 4 - Comparison of longitudinal and transversal sections of the QRF muscle area ultrasound. Functional measures and main anatomical structures are represented.

338x190mm (96 x 96 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	#3	Date and version identifier	NA
Funding	#4	Sources and types of financial, material, and other support	12
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1; 12
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1;12
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team,	1;12

		and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	1-5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	NA
Objectives	#7	Specific objectives or hypotheses	5-6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6-7
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	NO DRUG INTERVENTION
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	NO DRUG INTERVENTION
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg,	10 – TABLE 1

		systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7 – FIGURE 2
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7-8
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7-8
Methods: Assignment of interventions (for controlled trials)			NO CONTROLLED TRIAL

Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA

Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10

Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10
Methods: Monitoring			**NA**
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	

Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	11
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	11
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA

Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	11
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	12
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	11
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	11
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA

Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA