Novel Methods to Monitor Nutrition Status and Determine Protein Needs in Clinical Populations

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ABSTRACT

Malnutrition and muscle loss in hospitalized patients is a significant problem. The ability to accurately identify the development of malnutrition and monitor changes in muscle at the bedside are essential to optimizing nutrition interventions throughout treatment course and hospitalization; however, there is currently a lack of valid bedside tools. Furthermore, the provision of adequate protein and amino acids is crucial for maintaining muscle and optimizing outcomes in clinical settings. Yet, the methodologies used to determine protein needs have significant limitations, and current recommendations for dietary protein intake in clinical populations are not supported by strong evidence. This dissertation project consists of a series of studies that explore novel approaches for evaluating lean tissue and muscle (as core components of nutritional status) at the bedside, and determining protein requirements in the clinical setting.

In the first study, using a large and ethnically-diverse healthy population sample (NHANES 1999-2004), it was determined that ethnicity significantly influences the values of phase angle (PA) and impedance ratio (IR), two bioimpedance parameters currently being investigated as clinical markers. Based on the findings from this study, cut-points for PA and IR corresponding to low muscle mass defined by dual-energy X-ray absorptiometry (DXA) were established that can potentially serve as reference data for future clinical studies investigating the applications of PA and IR as markers of lean tissue and/or nutritional status.

In the second study, it was determined that PA and IR could be used to assess low muscularity and predict clinical outcomes in a large sample of critically ill patients. PA

and IR were moderately associated with muscle cross-sectional area (CSA) as determined by computed tomography (CT). Furthermore, PA and IR appeared to predict low CT-derived muscle CSA. In summary, PA and IR show promise in being able to aid in the identification of low muscularity and poor nutritional status in the ICU setting.

In the third and ongoing study, a novel stable amino acid isotope multi-step feeding protocol is being employed to determine the protein intake required to prevent net protein loss (anabolic threshold) and to evaluate the relationship between protein intake and net protein synthesis (anabolic capacity) of individuals with head and neck cancer (HNC), following chemoradiation therapy. Moreover, a force-measuring ultrasound (US) device is being used to assess changes in muscle quantity and quality due to chemoradiation.

There is a vital need to develop objective bedside methods capable of assessing muscle mass in hospitalized patients. Results from the described studies show the potential utility of bioimpedance and US to characterize changes in muscle mass in various clinical populations. Furthermore, as clinicians become better equipped at detecting changes in muscle, appropriate nutritional intervention is needed to stave off the loss of muscle. Amino acid tracer methods that can estimate whole body protein synthesis and breakdown are fundamental to the determination of more accurate protein and amino acid recommendations in clinical populations in order to improve patient outcomes.

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LIST OF ABBREVIATIONS

BIA – Bioelectrical impedance analysis

SF-BIA – Single-frequency bioelectrical impedance analysis

MF-BIA – Multiple-frequency bioelectrical impedance analysis

BIS – Bioelectrical impedance spectroscopy

PA – Phase angle at 50 kHz

IR – Impedance ratio (impedance at 200 kHz/impedance at 5 kHz)

ICW – Intracellular water

ECW – Extracellular water

ASPEN – American Society for Parenteral and Enteral Nutrition

APACHE II – Acute Physiology and Chronic Health Evaluation II

SOFA – Sequential Organ Failure Assessment

BMI – Body mass index

PG-SGA – Patient-Generated Subjective Global Assessment

CSA – Cross-sectional area

CT – Computed tomography

HU – Hounsfield Unit

US – Ultrasound

QMLT – Quadriceps muscle layer thickness

LST – Lean soft tissue

NHLT – Non-hydrated lean tissue

FFMI – Fat-free mass index

DXA – Dual-energy x-ray absorptiometry

ICU – Intensive care unit

COPD – Chronic obstructive pulmonary disease

DPA – Dual-photon absorptiometry

L3 – 3rd lumbar

MRI – Magnetic resonance imaging

ROC – Receiver operating characteristic

NHANES – National Health and Nutrition Examination Survey

N – Nitrogen

NB – Net Balance

UUN – Urinary urea nitrogen

EAA – Essential amino acid

WHO – World Health Organization

EAR – Estimated average requirement

RDA – Recommended daily allowance

IAAO – Indispensable amino acid oxidation

PHE – Phenylalanine

TYR – Tyrosine

TTR – Tracer/Tracee Ratio

NPO – Nil per os (nothing by mouth)

HNC – Head and neck cancer

CVC – Central venous catheter

IV – Intravenous

CRT – Chemoradiation therapy

CHAPTER 1: INTRODUCTION

Malnutrition and muscle loss in various clinical populations is a major problem that adversely impacts quality of life, functional status, survival, and health-care costs.[1–4] Hospitalized populations are uniquely susceptible to these issues due to prolonged and intermittent NPO status, location of various diseases, nutrition-specific effects of treatment, and other important factors.[5,6] It has been well observed that malnutrition is associated with suboptimal outcomes such as reduced quality of life, decreased functional status, increased treatment related adverse events, reduced survival, and increased health-related costs.[1,7]

Although there is not a universal consensus on the definition of malnutrition, it is accepted that loss of lean tissue, and skeletal muscle mass in particular, is the most important defining characteristic.[8] Muscle is an essential reservoir of amino acids used by the body to support tissue repair and respond to immunologic challenges.[9–12] Provision of adequate protein to support muscle maintenance and anabolism is thus critical for healing during times of stress; however, current protein recommendations are based on limited data generated using inaccurate methods.[13–17] Furthermore, the ability to monitor changes in muscle in response to nutritional interventions has been limited by the lack of valid bedside assessment methods.

This dissertation will address the above concerns through a series of related studies. Chapter 2 presents a literature review that will discuss relevant background for these concerns. Chapter 3, Chapter 4, and Chapter 5 are publications based on recently completed studies that explore the potential utility of various bedside methods to describe changes in muscle mass compared to reference methods and how they may be associated with patient outcomes. Chapter 6 presents conclusions gathered from the completed,

published work of this dissertation project. In addition, future directions are addressed by describing the methods from a current ongoing study that applies a novel method that can be used to more accurately define protein requirements and a new device that may remedy bedside assessment of muscle mass in a stage III and IV HNC.

CHAPTER 2: LITERATURE REVIEW, PART I*

*Publication Citation (portions of this Literature Review have been published): Teigen LM, Kuchnia AJ, Mourtzakis M, et al. The Use of Technology for Estimating Body Composition: Strengths and Weaknesses of Common Modalities in a Clinical Setting [Epub ahead of print]. *Nutr Clin Pract*. 2016. doi:10.1177/0884533616676264. http://journals.sagepub.com/doi/pdf/10.1177/0884533616676264

CLINICAL MALNUTRITION

Malnutrition is an under-appreciated problem that is typically described as some sort of ambiguous state of health resulting from inadequate or excessive intake leading to a change in body composition and reduced function.[18] It has crippled clinical populations for centuries and has been estimated to be prevalent among 13 – 78% of all hospitalized patients.[19,20] The consequences of malnutrition are not trivial and have been associated with poor patient outcomes including increased infections, longer length of hospital stay, higher frequency of readmission, and increased mortality.[1,21,22] Such poor outcomes directly correlate to an estimated 60% increase in hospital costs in affected patients[20,23] and likely contributes to the inability of patients to return to work after a stay in the intensive care unit.[24] Malnutrition has been suggested to be caused by diffusion of responsibility, prolonged NPO status, poor documentation of dietary intake, failure to recognize or diagnose those at risk, and failure to provide nutrition support in a timely manner.[20,25]

Prevalence of malnutrition in vulnerable populations, specifically HNC, has been estimated to occur in up to 40 – 67% individuals even before starting treatment.[26–29] Individuals with HNC are uniquely susceptible to nutritional related issues due to the location of malignancy, the nutrition-specific effects of treatment in that area, and the high likelihood of concurrent chemoradiation therapy.[5,6,30–34] There is also a well-recognized and staggering cycle of anorexia due to an apathetic attitude toward food, along with absorption abnormalities, which likely intensify nutritional insufficiencies.[6] A prospective study of 114 patients with HNC showed that patients with nutritional deficits had a 2-year survival of 7.5%. That was compared to patients without deficits, who had a

2-year survival of 57%.[35] These results highlight the critical role nutrition plays as a prognostic indicator in HNC.

The definition and clinical diagnosis of malnutrition, however, is a challenging endeavor. Consensus definitions have been proposed by prominent organizations, but diagnostic criteria still varies between institutions.[18,36,37] A seemingly universal criterion, regardless of institution, is absolute weight change over a specified time-period. The use of absolute weight change, however, lacks the ability to meaningfully characterize an individual's nutrition status, primarily due to its inability to identify specific body composition changes – lean tissue being central to nutrition status.[38] Body composition technologies, such as CT, DXA, bioimpedance, and US, may provide a more sensitive way to characterize and monitor nutrition status in a clinical setting.[39] Body composition data can be particularly useful when rounding out other assessment information, including handgrip strength and other functional measures.

The importance of body composition, specifically skeletal muscle, is reinforced in the *Academy of Nutrition and Dietetics/American Society for Parenteral and Enteral Nutrition Consensus Statement*.[8] The most recent *European Society of Enteral and Parenteral Nutrition Consensus Statement* on diagnostic criteria for malnutrition includes a criterion for low fat-free mass index (FFMI) – based on gender-specific cut-offs.[37,40] In research settings, the most ideal in vivo body composition research uses a number of different methods to measure body composition based on a "four-compartment model".[41–43] Clinically, however, this is not feasible. Instead, clinicians are forced to rely on simpler, easier-to-obtain tissue-level measurements. CT and DXA offer robust measures of body composition, and are often used as reference standards against which

field techniques (e.g. bioimpedance, US) are validated.[11] Bioimpedance and ultrasound techniques, although not as well-established as DXA or CT, are more cost-effective and accessible, and offer the ability to obtain real-time and repeatable measures at the bedside without any radiation exposure. The first portions of this literature review will give a general understanding of lean tissue, muscle and the use of CT, DXA, bioimpedance techniques, and US as they apply to body composition assessment in clinical settings.

LEAN TISSUE ASSESSMENT

Lean tissue, with skeletal muscle as its principal constituent, is critical to the body's healthy response to both acute and chronic illnesses.[10,11,44] Lean tissue depletion has been associated with increased morbidity and mortality,[45–48] infections,[49] hospital length of stay,[50,51] and decreased functional capabilities.[52] Despite the significance of lean tissue (and muscle) loss as a marker of malnutrition and prognosis in chronic and acute disease, its estimation is not commonly performed in hospital settings in part due to the limited availability of valid bedside methods.[11,51] Instead, clinicians often rely on subjective physical examination techniques, [36,53,54] alone or as part of the Patient-Generated Subjective Global Assessment (PG-SGA).[55,56] Subjective evaluation of muscle mass, however, is potentially error-prone, particularly in obese and edematous states.[11] It was recently demonstrated that 60% of individuals who were deemed normally nourished at admission by SGA were actually sarcopenic (i.e. had low muscle mass) as defined by CT.[57] Approximately 33% of individuals who were misclassified were overweight or obese.[57] Clearly, there is a significant and growing need for easily accessible and accurate methods for assessing lean tissue in the clinical setting.

REFERENCE METHODS FOR ESTIMATING BODY COMPOSITION

Dual-energy X-ray Absorptiometry

DXA began to replace dual-photon absorptiometry (DPA) for estimation of bone mineral density in the late 1980s.[58] Although DXA is still primarily used to measure bone mineral density, it has also been widely employed for body composition estimates. DXA relies on principles of x-ray absorptiometry similar to CT, but is associated with a much lower radiation dose; as a result, DXA is able to be utilized in a much wider range of clinical populations.[59–63] In fact, due to the safety and availability of DXA scans, they are often used to validate various other body composition-estimating devices and equations – including CT.[64–68]

DXA, similar to CT, relies on measured changes in x-ray attenuation to estimate body composition.[69] With DXA scans, however, the energy beam is only passed along one-side of the body producing a planar image. DXA imaging relies on attenuation measured at two different energy spectra – a "high" and a "low" energy level. The form of x-ray beam administration varies, but technological improvements have resulted in a progression from a pencil beam to a fan beam, and most recently, to a narrow fan beam.[70] The measured difference in x-ray beam attenuation between the two energy levels is used to delineate specific tissues – with increased attenuation occurring with increased tissue density. Pixel-based images are generated and, based on algorithms incorporating mass attenuation coefficients, allow for the calculation of both mass and volume.[43] These algorithms can differ among DXA instruments and software. The two energy levels relied upon for DXA primarily allow for differentiation of bone mineral and soft tissue. Unique

attenuation characteristics of fat and lean soft tissue allow for additional delineation, but this is limited to the pixels that do not contain bone. Overall, DXA is capable of separating the body into three compartments: bone mineral content, fat and lean soft tissue mass. Measurements are able to be obtained at either a whole-body or regional level can occur.[70–72]

The safety of DXA scans has allowed for the collection of large quantities of healthy "normal" body composition data that can be used to generate healthy reference values.[73,74] In general, DXA tends to be used in stable clinical populations or employed to measure long-term changes in body composition associated with aging. One of the most notable uses for DXA measures of body composition has been its use in defining sarcopenia.[75,76] One of the primary features of sarcopenia is the loss of skeletal muscle mass, which is difficult to reliably define using subjective methods.[77] The ability of DXA to safely obtain whole-body imaging on a large, population-based scale has provided the data necessary to objectively define sarcopenia.[76]

Due to its reliance on x-ray absorptiometry, DXA, similar to CT, has a number of technical and physiological limitations that influence attenuation measurements. Technological limitations include patient positioning and device and software differences.[78–81] Physiological limitations include depth and length of the patient, hydration status, and timing of the scan in relation to activities of daily living (meal timing, bathroom habits, and exercise).[71,82,83] Although mild changes in hydration have not been demonstrated to influence body composition measurements, disease-associated fluid accumulation, such as ascites or edema, have been shown to influence body composition measures.[82,84] In addition, weight and size limitations of scanners present

challenges when scanning individuals with obesity or individuals who are especially tall. For example, it may not be possible to adequately separate the limbs within the scanning area, thus limiting the ability to define sarcopenia based on DXA-derived appendicular lean mass cutpoints.[85] It should be noted, however, that some investigators have identified methods to accommodate the weight and size limitations of scanners[86], and larger capacity instruments (e.g. iDXA, GE) that can accommodate heavier and wider individuals are now available.

In summary, DXA imaging technology has proven to be uniquely suited for body composition measurement in a wide range of populations. A number of assumptions associated with DXA imaging, however, can make interpretation of results challenging. Similar to CT scanning it is important to document scanner type and version of software, and make every attempt to maintain consistency in device and measuring technician for longitudinal measures. In addition, establishment of a standardized protocol, which controls for both technical and physiological factors, can minimize error in DXA measures. Establishing a collaborative relationship with radiologists and medical physicists before utilizing DXA scanning for body composition assessment in your patient population is suggested.

Computed Tomography Scans

CT is another reference technique used since the late 1970s to determine body composition in the research setting. CT continues to provide important clinical insights on body composition and outcomes.[87–89] The primary limitation with the use of prospective CT scans for body composition assessment is the high-dose of radiation to

which the subject is exposed. Therefore, the application is limited to individuals who have had CT scans completed as part of their medical treatment. To date, the majority of published research utilizing CT scans for body composition assessment has been conducted in cancer populations,[90–94] but recent work has extended this to other clinical populations.[47,95–100]

CT scanners rely on a rotating x-ray beam to produce cross-sectional images of the individual being scanned.[101] Each of these cross-sectional images has a depth associated with it, which is referred to as "slice-thickness". In order to generate a cross-sectional image, individual x-ray images—obtained as the x-ray beam rotates—are reconstructed, based on algorithms programmed in the device. Each image is composed of volumetric units called "voxels", which are associated with "Hounsfield Units" (HUs). The HUs reflect the attenuation the tissue exerts on the x-ray beam during the scan and generally reflects the density of that particular tissue. Each voxel is logarithmically assigned to a single HU. If there are multiple tissue types in a voxel, the calculated HU will reflect an average of the tissues based on the resolution limit of the scanner. In order to create the two-dimensional images viewed on the screen following the procedure, the HUs of each voxel are used to produce "pixels". Pixels are square units with pre-specified dimensions and colors along the gray-scale based on their associated HUs. The HUs allow for differentiation of tissue, based on tissue attenuation; the area associated with each pixel allows for the calculation of cross-sectional area.

The two primary software packages utilized for CT analysis in nutrition research are *ImageJ* and *Sliceomatic*.[102] A recent comparison study found no difference in soft-tissue measurements between the two programs.[103] Although both these programs are

user friendly, and could be used by clinicians, there are differences in how the scan is manipulated to obtain body composition data. It is also important to note that they are not the only way to obtain body composition data from CT images. In fact, most radiology departments rely on much more advanced software for CT analysis. In general, body composition research utilizing CT images relies on single axial image analysis, which is purported to reflect whole body tissue distribution.[104,105] Although a number of different anatomical sites have been used in body composition research, investigators have identified the L3 region on CT scans as being particularly reflective of whole-body tissue distribution and this has become the most commonly assessed site in the literature.[68, 98,106–108] Current sarcopenia cut-points are based on tissue distribution measurements at the L3 vertebra.[95]

Most studies that measure body composition with CT analysis attempt to identify the prognostic ability of skeletal muscle cross-sectional area, or changes in skeletal muscle attenuation values (i.e. HUs) at a single time-point.[88, 90–99,108–111] In general, skeletal muscle cross-sectional area and muscle attenuation are often demonstrated to have strong prognostic values – with lower cross-sectional area or attenuation values being associated with worse outcomes. Investigators have standardized skeletal muscle cross-sectional area for height by dividing skeletal muscle cross-sectional area (cm^2) by height in meters squared (m^2) to produce what is commonly referred to as a skeletal muscle index (SMI) value - similar to BMI. The ability to obtain average HU values for tissues of interest on CT images has led researchers to pursue studies assessing tissue attenuation values as a surrogate for muscle quality (e.g. a lower average HU value may reflect increased fatty infiltration within skeletal muscle).[112] A recent study found tissue

attenuation to be more prognostic than SMI, which reinforces the potential utility of this measure.[89]

CT scans are considered by many to provide reference measures of body composition[72], but, like any indirect assessment method, CT has limitations that are important to consider when interpreting results. As stated earlier, the primary limitation to the use of CT scans for body composition assessment is the high-dose radiation associated with the procedure, which inherently limits its use to individuals needing it for diagnostic purposes. In addition, several variables specific to the CT image can introduce error and should be taken into consideration. For example, slice thickness and patient positioning are two important variables that need to be controlled in order to minimize errors in determination of cross-sectional area. Slice thickness (often 3-8 mm) will determine the number of slices that exist at the L3 region. The research team of Mourtzakis et al. demonstrated that the average height of L3 is nearly 4 cm.[68] Therefore, multiple slices will exist at the L3 region for each patient and it is important to consider that although each slice represents a portion of the L3 region, it does not encompass the entire L3. Some studies have attempted to account for the multiple slices obtained at the L3 by averaging two adjacent images, but this still fails to ensure that the exact same location is assessed in each patient. It is unclear, however, if small changes in the exact location of the analyzed slice at the L3 will result in clinically meaningful measurement differences.[107] Patient positioning can also influence cross-sectional area. Although the patient is in a recumbent position during the scan, changes in positioning will influence the relaxation state of the muscles in the L3 region, and, subsequently, the cross-sectional area.

Some investigators have attempted to utilize average CT HUs as a way to evaluate muscle *quality*, but the validity of this practice is not clear.[88,89, 109,110,113] This will need to be validated through studies that include muscle biopsies. The major concern is that, although HUs predominately reflect tissue attenuation, they are known to be influenced by a wide variety of factors (e.g. scanner, tube voltage, edema, patient size and shape).[114–120] Furthermore, there is no standard definition for HUs.[121] Only air and water have specific HUs associated with them: -1000 and 0, respectively (in fact, water's HU of 0 is often used to calibrate CT scanners).[119] Rather than relying on specific attenuation values, tissues are differentiated based on ranges of HUs. The range most frequently used for skeletal muscle and adipose are -29 to +150 and -190 to -30, respectively; visceral adipose is often defined by a narrower range, -50 to -150. It should be noted, however, that a variety of ranges have been used throughout the literature, which should be taken into account when interpreting results.[105] Although these wide ranges accommodate the inherent variation in HUs, tissue measures based on these units are likely to include more than just the tissue of interest.

In summary, CT images offer unique insights on the tissue distribution of the body, but rely on a number of assumptions for body composition estimation. The biggest assumption is that a single slice represents whole body muscle distribution. To minimize error in longitudinal CT measures, the ideal would be to have scans performed using the same scanner by the same technician; this is rarely possible in research or clinical settings. From a practical standpoint, it is important that technical information such as tube voltage, scanner type, and slice thickness are documented given that inconsistencies in these variables could impact interpretation. Prospective research using CT, in addition

to the aforementioned technical variables, should attempt to control for patient positioning by establishing a standard protocol with the imaging department. Although it may not be feasible, establishing a collaborative relationship with radiologists and medical physicists before attempting to use CT scans to assess body composition is also suggested.

BEDSIDE METHODS FOR ASSESSING LEAN TISSUE

Bioimpedance Analysis

In a basic sense, bioimpedance involves the application of a small electrical current to the body to estimate body water and composition. Because of its relative simplicity and accessibility, bioimpedance techniques remain the primary method for body composition assessment in clinical settings.[122] Bioimpedance techniques have been comprehensively reviewed by others[123–125]; this section will provide an overview of basic concepts.

There are three types of bioimpedance devices available commercially: single- and multiple-frequency bioelectrical impedance analysis (SF- and MF-BIA), and bioimpedance spectroscopy (BIS).[126] Regardless of the device, bioimpedance treats the body as an electrical circuit and devices measure changes in voltage across the body.[127] The change in voltage is referred to as impedance, which consists of resistance and reactance. Resistance generally refers to the ability of the current to flow through fluid and space and reactance reflects the interaction of the current with membrane interfaces (i.e. cell membranes).[123] Equations that employ these impedance measures are used to generate estimates of fluid and other body composition compartments.[124]

Although SF-BIA, MF-BIA, and BIS all rely on the same principle that impedance relates to body composition, each approach has different underlying assumptions. Different devices allow for whole body or segmental estimates of various body compartments. SF-BIA relies on the use of a single frequency, generally 50 kHz, to generate impedance data that can be applied to regression-derived prediction equations to estimate total body water, fat-free mass, and/or fat mass.[128] Due to the use of a single frequency, however, SF-BIA is theoretically unable to differentiate between intracellular (ICW) and extracellular water (ECW).[11] In contrast, MF-BIA involves the application of the electric current at a number of defined frequencies (typically two to six) from 5 to 500 kHz, which theoretically allows for differentiation of ICW and ECW. Impedance data at 5 kHz can be applied to a regression-derived equation to predict ECW while that from higher frequencies can be used to predict total body water; and ICW can be derived by subtraction.[128] An understanding of both ICW and ECW can be useful when evaluating fluid status. The ability to estimate ICW, in particular, is clinically relevant because it provides an estimate of body cell mass, which reflects the amount of metabolically active tissue.[128] Finally, BIS differs fundamentally from SF- and MF-BIA techniques in that it involves the utilization of impedance data measured over an entire spectrum of frequencies from 5 kHz to 1200 kHz, and utilizes the data from 50 to up to 250 of those frequencies.[128] Rather than being applied to linear equations, BIS-measured spectral data undergo complex modeling based on the classic Cole model[129]; Cole model terms can then by applied to complex algorithms to generate ICW and ECW.[123] Similarly, lean tissue and fat mass estimates can also be derived in various ways.[11,130] Notably, BIS techniques have recently shown promise in assessing body composition and mortality

risk in people on dialysis,[130] and in managing fluid status in dialysis[131–136] as well as surgery[135] and critically illness.[134]

Whole body estimates of lean tissue have traditionally utilized single- and multi-frequency BIA techniques, require the use of population-specific prediction equations and are often erroneous in clinical populations due to violation of underlying assumptions (e.g. normal body geometry, hydration of FFM, and fluid distribution).[11,122,123] MF-BIA offers the potential for rapid assessment of lean tissue and fluid status with minimal technical expertise required and can also be done at the bedside. MF-BIA has been evaluated for its validity in healthy populations,[137–139] and some clinical populations including HIV,[140] hemodialysis,[141] and surgery[142] and is purported to be a method that may be used at the bed-side to monitor changes in fluid distribution as well as body composition.[122,124] Little has been done in cancer populations and valid, cancer-specific prediction equations to predict whole body lean tissue are lacking.[143] Inaccuracies in whole-body lean tissue estimates have led to the investigation of raw BIA parameters (e.g. 50 kHz PA and 200/5 kHz IR) as potential markers of nutritional status, prognosis, and clinical outcomes in cancer[144–147] and other populations.[148–158] A number of investigators have reported that PA and IR may be useful for assessing nutritional status and/or predicting clinical outcomes.[159,160] For example, IR has been shown to correlate with quadriceps muscle weakness in COPD and disease severity in heart failure,[158] to predict post-operative edema after major abdominal surgery,[156] and to predict dry-weight after hemodialysis.[11] PA has been reported to predict nutritional status and/or post-operative complications[161–164] and prognosis in advanced cancer.[146,147]

At this time, bioimpedance offers one of the most convenient clinical tools for objective estimates of body composition at the bedside. Regardless of the approach, when taking bioimpedance measurements, it is important to implement a standardized measurement protocol[11,122] and to utilize the same device and equation/algorithm by the same technician for longitudinal measures.

Ultrasound

US imaging, also called sonography, is the measurement of high-frequency sound waves as they encounter various underlying tissues, to visualize the body.[165,166] The production of the image is based on the amplitude of reflected sound waves and the speed at which they travel through the body.[166] It has been used in medical applications for decades and has seen rapid growth in almost all fields of medicine, including bedside quantification of various tissues.[167] US shares many of the same advantages as bioimpedance compared to CT and DXA, including the noninvasive, nondestructive, portable nature of the measurements and the ability to be used in real-time at the bedside.[11,165]

The assessment of body composition by ultrasound is an emerging modality with great clinical potential. There has been increasing interest in using US to assess skeletal muscle, such as to quantify thickness or cross-sectional area, with intent to further its utility in determining the presence and severity of malnutrition.[168–170] It has been shown to provide reasonable estimates of muscle mass in various clinical populations when compared to other reference methods.[169,171–175] However, lack of consistency within protocol has limited its prognostic value in diagnosing longitudinal muscle loss; primarily

due to variability in muscle compression, selection site, and fluid status of the patient.[72, 168,176]

There is currently no clear consensus on an appropriate protocol for assessing muscle mass, but some groups have suggested the use of minimal compression with minimal deviation of the muscle group being measured.[169, 172,173,177] Questioning whether edema and increased extracellular fluid affects muscle measurements, recent work has evaluated whether maximal compression may more accurately reflect quantity;[168] this is a significant limitation to the advancement of body composition analysis by US, as researchers have clearly demonstrated substantial differences in thickness with the application of various forces.[178,179] And although mechanical systems have been introduced to remedy this concern,[172,178,179] a more pragmatic and practical approach may be needed to improve the bedside applicability of US.

Anatomical site selection is also not consistent between researchers. Abe and colleagues[173] developed a 9-site measurement protocol which was shown to have excellent correlation with whole body muscle mass as determined by DXA,[174] but again, practicality becomes a concern. Protocols measuring fewer sites have been described, in comparison to reference methods, with mixed results.[168,180] Regardless of which muscle groups are assessed, replication of image location on repeat US is essential to accurate longitudinal monitoring;[169] these limitations continue to persist.

The rapid expansion of US use within the clinical setting reflects the evolution of its diagnostic capabilities[181,182] and allows for the assessment of muscle *quality*. This is important, because skeletal muscle function is not solely determined by quantity.[170] Echogenicity, the characteristic of tissue to reflect ultrasound waves, has been proposed

as a sonographic estimate of quality.[181] Through grayscale histogram analysis, US has been used for years to help identify tissue pathologies typical in children affected by Duchenne Muscular Dystrophy[167,181] and other various inflammatory myopathies.[183,184] Recent evidence also suggests ultrasound may be a particularly attractive technology to objectively assess muscle architecture breakdown and necrosis when compared to muscle biopsy,[170,181] which has been shown to be reflective of functional status.[169] US-based determination of muscle quality, however, is limited by transducer anisotropy, or transducer orientation, among other factors.[178] It is not typical for devices routinely used in clinical sonography to control for anisotropy; and this may limit the use of US in diagnosing muscle quality.[185]

Moreover, US compression-based elastography is a developing area of clinical research and allows for the determination of mechanical tissue properties.[165] This non-invasive technique may help elucidate the physiological changes that occur in muscle tissue during disease, disuse and treatment course. However, these types of technologies are atypical and not available to most clinicians. Such advances in US-based imaging are expected to appear in the near future[165] and may prove to be useful in determining the nutritional status of clinical populations.

In summary, US imaging is a developing technology that is gaining ground for the bedside analysis of body composition in various clinical populations. At this point, however, uncertainties about image repeatability, caused by variation in transducer surface force, measurement site, anisotropy and protocol practicality, limit its diagnostic and monitoring capabilities for nutritional status. Innovative US technologies are being created to remedy these concerns, but until they are available to a plethora of researchers,

a concerted effort must be made to reach consensus on protocol that is able to provide some level of utility in monitoring muscle loss. Caution should be taken with the application of this modality, but the future seems promising.

SUMMARY

A growing appreciation for the clinical relevance of lean tissue has generated a need for objective, clinically feasible measurement methods.[38] Technologies such as CT, DXA, bioimpedance, and US offer clinically viable methods of measuring body composition, but a good understanding of each modality's strengths and weaknesses is important for clinicians to have as they interpret measurements in a clinical setting. The reader is referred to the full publication by Teigen et al. (2016)[186] for full table of strengths and weaknesses. Ideally, technologies to estimate body composition will ultimately be capable of two types of measures: a single time-point measurement capable of contributing to the diagnosis of malnutrition, and repeated measures over time in order to monitor changes in body composition – which will function as a surrogate for the adequacy of nutrition intervention. The next section will focus on adequate nutrition intervention of protein and amino acids to promote lean tissue and muscle maintenance.

CHAPTER 2: LITERATURE REVIEW, PART II*

*This portion of the Literature Review is submitted and under review.

PROTEIN IN THE HOSPITAL

Synopsis

Provision of adequate protein is crucial for optimizing outcomes in hospitalized patients. However, the methodologies upon which current recommendations are based have limitations and little is known about true requirements in any clinical population. In this tutorial, we aim to give clinicians an understanding of how current protein recommendations were developed, an appreciation for the limitations of these recommendations, and an overview of more sophisticated approaches that can be applied to better define protein requirements. A broader perspective of the challenges and opportunities in determining clinical protein requirements can help clinicians think critically about the individualized nutrition care they provide to their patients with the goal of administering adequate protein to optimize outcomes.

Introduction

Skeletal muscle plays a principal role in the body's normal response to illness and injury.[187,188] During times of stress, muscle protein degrades to release amino acids into circulation in order to maintain protein synthesis at vital tissues, organs and at other locations to regulate inflammatory and immune responses. [187,189,190] The increased rate of proteolysis, resulting from enhanced metabolic demand, leads to a net negative protein balance and subsequent muscle wasting; this is amplified by severity of illness, organ dysfunction, insulin resistance, sepsis, immobility, and lack of adequate nutrition.[10, 188,191] The metabolic cost of this muscle protein breakdown is of great clinical consequence and has been shown to be associated with negative patient outcomes in various clinical populations.[45, 49, 97,188] Despite the significance of muscle wasting, little is known about

the amount of amino acid and protein required in clinical settings to preserve muscle mass and function. Here, we address the evidence upon which current protein recommendations are based, review new methods capable of improving protein recommendations, and present potential strategies to help patients achieve existing requirements.

Background

Nitrogen Balance

Dating back to the early 1900s, the nitrogen balance technique has been used as the classical method for determining protein requirements in healthy and clinical populations.[192–194] Protein requirement in adults has been defined as the minimum amount of protein needed to produce nitrogen (N) equilibrium: the balance between N (protein) intake and N output.[194] Because a N containing amino group is the defining structural characteristic of animal protein and N makes up roughly 16% of dietary protein (6.25 gm dietary protein for every gm N), N metabolism is often associated with protein metabolism.[193] Urea is the primary excreted product of N metabolism, the majority being excreted in the urine as urinary urea nitrogen (UUN).[195,196] N balance is then the net difference between the amount of N consumed in the diet and the amount of N excreted from all potential sources.[197] However, because capturing all sources of N loss is difficult and often not feasible,[198] the most commonly accepted approach is to add a constant of 4 gm N to a measured UUN.[199] This constant makes the assumption that 2 gm N loss is from non-urea urinary N losses (ammonia, creatinine, and uric acid), 1.5 gm N loss is

estimated from fecal N losses, and 0.5 gm N loss is estimated from other miscellaneous losses.[194,200] The N balance equation is as follows:

$$N\ Balance = \left(\frac{protein\ consumed\ gms}{6.25}\right) - (UUN + 4)$$

At a glance, N balance methodology seems simplistic and uncomplicated. However, the complexity in determining protein requirements lies within the interpretation and application of such data and continues to spark controversy to this day.[194,201]

Practical Concerns

Nitrogen balance methods have provided the foundation for understanding protein metabolism and continue to provide important physiological insight used for clinical guidance.[16,202] However, there are important limitations that make this method susceptible to error.[187,193,194] An underappreciation of these limitations narrows clinical perspective and creates complacency with current nutritional guidelines, often at the expense of patient care.

One major fault of nitrogen balance methodology is that it favors an overestimation of N utilization, [16,198] leading to an exaggerated positive balance and thus, potentially an underestimation of protein requirements.[203,204] Although conceptually perspicuous, accounting for the totality of N consumed, metabolized, and excreted is difficult. N can inevitably be lost at any step in the process between preparation to consumption, from cooking losses to wastage and spillage of food contents;[194,205] this

concern is partially ameliorated when patients are on full enteral tube feedings. Moreover, amino acids that pass through the small bowel may be metabolized by colonic bacteria, rendering them largely non-contributive to protein synthesis.[206] Further, although the majority of N excretion is collected via urine, a failure to capture all other routes of excretion and the difficulty quantifying miscellaneous losses may lead to further inaccuracies in determining protein utilization; recycling of an unknown amount of ammonia N from the colon for reuse in liver has also been shown to convolute an already difficult task.[207] Taken together, these complexities contribute to a potentially unreliable N balance, even under the most meticulous protocols, and results must be interpreted with caution.[198]

Nutritional Adaptation

Nutritional adaptation has been defined as a process by which a new steady state is reached in response to varying levels of intake.[208] The ability to adapt to a range of protein intakes is advantageous but complicates the N balance method for quantifying protein requirements.[194] In the face of reduced protein intake, the body first prevents the loss of lean mass and maintains N balance by reducing total N excretion, provided intake does not fall below a critical level.[209] This metabolic adaptation is driven by the severity of dietary restriction and reduced amounts of amino acids available for protein synthesis. Therefore, in a protein deficient diet the oxidation rate of essential amino acids is decreased, which limits urea excretion.[210] A reduction in urea cycle enzymes and enzymes responsible for transporting N into the urea cycle also contribute to the adaptive process.[211,212]

The most shocking example of this adaptive process was shown by physicians studying starvation in the Jewish ghetto in Warsaw, Poland in the early 1940s. Under extreme conditions of protein deficiency, physicians noticed a surprising reduction in urea excretion in order to maintain N balance.[213] Clinically speaking, patients often experience prolonged periods of protein energy malnutrition prior to hospitalization but may also be unintentionally restricted during admission due to intermittent NPO status. To account for such changes in protein intake, N balance experiments must include an adaptation period that allows for the urea body pool to completely turnover and achieve a new steady state of urea N excretion.[205]

Although no consensus has been agreed upon, 1-2 weeks is commonly used as an adaptation period to a test diet;[198,214,215] other studies of varying protein intakes show non steady state urea N excretion up to 28 – 40 days.[216] In practice, N balance studies typically do not extend beyond a few hours within the clinical setting. Further, it is questionable whether there is enough stability to perform a balance study in the hospital. Taken together, these factors question whether clinical N balance studies are merely a representation of incomplete nutritional adaptation and if they are capable of determining protein requirements.[194,217]

Insulin and Protein Metabolism

The amount of energy provided in a mixed meal is an important determinant of N balance and deserves careful consideration when evaluating protein utilization.[218] It has long been accepted that energy balance influences N balance and protein requirements, in part because protein synthesis and protein breakdown are both energy dependent

processes.[194,219] Thus, it is nearly impossible to separate the interrelationship between dietary energy and protein; when protein (and essential amino acids; EAAs) is adequately dosed, the adequacy of dietary energy determines N balance, and when energy is adequately dosed, the protein level will determine N balance.[220] Consequently, the amount of protein needed to achieve N equilibrium increases with inadequate energy intake, which calls into question the validity of historic N balance studies that did not appropriately control for variations in energy provision.

Insulin secretion in response to energy intake has been shown to promote anabolism by inhibiting protein breakdown.[17,205] Moreover, exogenous insulin infusions, beyond postabsorptive levels, have also been shown to be sufficient at inhibiting protein breakdown in young adults but do not stimulate protein synthesis;[191,221] further studies have shown that insulin can reduce protein breakdown in a dose-dependent manner.[222] The anti-catabolic role of insulin seems to be partially attributed to its ability to inhibit the ubiquitin-proteasome pathway and the lysosomal proteolysis pathway, both of which play primary roles in breaking down intracellular proteins in stressed states.[219] It is postulated that the deficiency of insulin, potentially induced by prolonged periods of inadequate energy intake, could result in an enhanced rate of muscle breakdown;[219] insulin resistance caused by severe illness or trauma has also been shown to create a catabolic state of muscle depletion.[219] This is particularly important when considering the individual response to protein supplementation in the context of a mixed meal or tube feeding, where underfeeding calories can significantly reduce the insulin response.[17]

Protein Turnover

Black box is a term often used to describe a complex or unknown transition step between input and output.[223] Relevant to N balance, the *black box* is the metabolic fate of N between intake and excretion. The lack of clarity within this transition step makes the N balance method inherently problematic for measuring anything other than the specific net balance of body N.[193,194] Thus, it is important to appreciate that the N balance method is not a direct measure of whole body protein turnover; it is a direct measure of body N from which protein requirements are often estimated.

Whole body protein turnover describes a dynamic and continuous process of simultaneous synthesis and breakdown of all body lean tissues, neither of which can be quantified by N balance.[17,202] Calculating whole body protein turnover is an informative way to determine protein requirements but requires advanced methods that will be discussed later. Therefore, if employed to estimate protein needs, N balance study findings should be interpreted with an appreciation for what they are capable of directly measuring (balance of body N) and what they can only estimate (whole body protein turnover).[202,224]

Protein Requirements

Current Guidelines

Regardless of the shortcomings of N balance methodology, it continues to provide the basis for protein requirements in healthy and clinical populations and will continue to do so until an alternative method is validated and accepted;[205,225] such methods are being employed in clinical trials.[203,226,227] A meta-analysis published in 2003 by Rand, Pellet and Young[228] forms the foundation of current protein recommendations for healthy adults

set forth in a report of a joint WHO/FAO/UNU expert consultation.[205] Data from 235 individuals in 19 N balance studies was gathered to conclude the median (estimated average requirement, EAR) and 97.5th percentile safe (recommended dietary allowance, RDA) recommendation for good-quality protein was 0.65 and 0.83 gm protein/kg/day, respectively;[228] these values have been disputed as significantly too low for various healthy adult populations.[204,229] When investigators reanalyzed the same N balance data as Rand et al. using a different, and potentially more appropriate, statistical approach, they found an elevated mean and safe protein requirement of 0.91 and 0.99 gm protein/kg/day, respectively.[204]

Regardless of much dispute, and similar to current guidelines, the protein recommendation for metabolically normal hospitalized patients is also 0.8 gm protein/kg/day.[16] The most common recommendations in various stressed states, including critical illness, are 1.2 to 1.5 gm protein/kg/day, but may increase to over 2.0 gm protein/kg/day in special catabolic cases.[230,231] These recommendations, however, are grounded in N balance with no consideration given to clinically relevant endpoints, which renders them susceptible to both skepticism and criticism.[16,232] Higher protein intake beyond current guidelines has been shown to safely improve N balance, however, this practice does not consider pertinent clinical outcomes.[233] In a pediatric study evaluating whole body protein turnover in septic adolescents receiving either 1.5 gm protein/kg/day or 3.0 gm protein/kg/day, investigators report an improved protein balance attributable to increased whole body protein synthesis at the higher protein level;[234] unfortunately, clinical outcome measures were again overlooked. Although it is unknown if such high levels are appropriate in adult patient populations, increasing protein beyond

1.2 gm protein/kg may improve clinical outcomes based on observational data.[235,236] Intervention studies comparing similar protein levels and measure clinically relevant endpoints are desperately needed to support the development of evidence-based recommendations.[237]

Difficulties in Determining a Requirement

Amino Acid Composition

Although it is easy to point out flaws in the current protein dosing guidelines, it is much more difficult to propose new recommendations capable of maintaining muscle mass because of the complexity of an individual's response to protein intake. There are many factors capable of altering the body's ability to convert dietary protein into cellular protein,[238] including: protein quality (i.e. dietary protein amino acid composition and digestibility), digestion rate, non-protein calorie intake, anabolic resistance, disuse, timing of ingestion, and inflammatory status.[189,239–242] The sum of these factors make defining protein requirements for a homogeneous population very difficult.

Protein quality refers to a combination of factors, including the balance between essential and nonessential amino acids, the digestibility of the protein to provide amino acids for absorption, and the availability of the amino acids for protein synthesis.[226] The predominant belief is that high quality proteins—containing high amounts of EAAs that are readily absorbed in the small bowel— consumed in smaller quantities, compared with lower quality proteins (i.e. plant proteins), will continue to meet EAA requirements.[226]

Recent studies have shown that EAA supplementation, beyond that provided with a complete protein source of whey, casein, or soy, is the primary driver of whole body

protein turnover;[243,244] leucine-enriched EAA supplementation appears to be particularly promising in stimulating muscle synthesis.[239,245] There is controversy, however, as to the degree of importance protein quality has on protein synthesis.[239] Some investigators have proposed that protein digestion rate and speed of absorption are important determinants of whole body protein turnover, regardless of amino acid composition.[246] In order to evaluate the importance of digestion rate, researchers characterized amino acid kinetics following ingestion of slow and fast digesting proteins with similar amino acid profiles.[240] They found that fast digesting protein was an independent factor promoting protein deposition;[240] this is because an increase in the concentration of intracellular amino acids facilitates protein synthesis.[247] Thus, patients with the same metabolic demand may require differing protein amounts depending on the amino acid composition of the supplied protein source, the digestibility of the protein, and rate of amino acid absorption.

Protein and Calorie Provision in the Hospital

Hypocaloric feeding, not to be confused with "permissive underfeeding", is a feeding strategy used within the clinical setting to selectively restrict energy with a compensatory increase in protein provision.[16] The intent is to promote an adequate anabolic response while preventing the metabolic complications of overfeeding (e.g. hyperglycemia, weight gain, hypercapnia, etc.).[197] Defining the appropriate protein prescription, however, requires an understanding of the relationship between protein and energy intake.

Based on N balance studies, increasing energy intake decreases protein needs due to the protein-sparing effect of non-protein calories. Similarly, if caloric provision is inadequate, protein requirements increase to meet the energy and N demand of the body.[189,194] Investigating the dynamic relationship between protein and energy with N balance, Elwyn and Shaw[248–250] showed a rapid rate of N retention with increasing caloric intake at fixed protein levels, until calorie requirements reached 50 – 60% of total energy expenditure. The provision of additional calories beyond 60% is met with little improvements in N retention,[251] which supports current caloric guidelines.[231] Notably, although improvements in N balance ceased with additional energy provisions, any potential improvements in clinical outcome were not addressed.

In contrast to current guidelines, sophisticated methods determining whole body protein turnover in critically ill patients have shown that hypocaloric feeding is associated with a more negative protein balance than normocaloric feeding.[252] Further evaluations show the interconnected importance of adequate intake of goal calories and protein on mortality.[235] Clinically, it is prudent to appreciate the importance of energy needs with respect to protein dosing. If caloric restriction (< 80% of measured energy expenditure) persists beyond the first 4 days of admission, it should be acknowledged that the current protein recommendations may be below that needed to prevent muscle wasting.[253]

Although not discussed in detail, anabolic resistance, immobility, timing of ingestion, and inflammatory status are crucial elements that shape protein requirements.[242,254,255] Individual variations in any of these variables may cause substantial differences in amino acid and protein needs, even among individuals of similar disease states. Accounting for these variables throughout a hospital stay and over

the time-period needed to conduct optimal N balance studies is not feasible. A variety of advanced protein kinetic methods, however, have been used to estimate protein requirements in shorter time-periods and represent significant advantages over the classic N balance technique.[227]

New Methods

A number of methods, beyond N balance, have been created to estimate protein requirements in both healthy and clinical populations. Focus will be placed on popular methods that may be most appropriate in a clinical setting; the reader is referred to publications by Wolfe et al. for additional methods.[227,256]

Whole-Body Protein Synthesis and Breakdown

The inherent limitations of the N balance technique have prompted the creation of alternative methods capable of estimating protein requirements.[194] The measurement of whole body protein synthesis and breakdown was the first approach to quantify various elements of protein metabolism in humans and represents a significant advancement over N balance.[227] This approach utilizes stable amino acid isotopes to quantify whole body net protein balance, using the equation:

$$Net\ Protein\ Balance = Protein\ Synthesis - Protein\ Breakdown$$

Traditionally, anabolic response is equated with muscle protein synthesis, so measuring protein synthesis and breakdown in muscle can be very informative.[17] However, a more

appropriate measure of anabolic response associated with protein intake is quantifying the balance between protein breakdown and synthesis at the whole body level.[257] This is because various high turnover soft tissues, such as the gut, are capable of retaining amino acids after a meal.[258] In the postabsorptive phase following a meal, these tissues redistribute amino acids that fuel protein synthesis in other organs and tissues, such as muscle.[259] Thus, protein turnover in various soft tissues functions in a complementary capacity to amplify a sustained anabolic response in muscle and may be important when determining protein needs.[17,259]

The most relevant whole body method for the estimation of protein requirements utilizes an infused essential amino acid tracer to calculate protein breakdown and protein synthesis. Proposed by Clarke and Bier in 1982,[260] labeled phenylalanine (PHE) became an ideal tracer due to its limited metabolic fates in the body: incorporation into muscle (protein synthesis) and hydroxylation to tyrosine (TYR) – the first committed step in PHE oxidation.[261] Once infused intravenously (peripherally), the dilution of the labeled PHE with unlabeled PHE from muscle (tracee), gives a measure of muscle protein breakdown. Knowing the proportional contribution of PHE to the total body amino acid pool allows for the estimation of whole body protein breakdown.[256,262] Additionally, because whole body protein synthesis correlates with muscle protein synthesis, whole body protein synthesis can be obtained by deducting total PHE oxidation (from the hydroxylation PHE label into TYR, using a simultaneous infusion of labeled TYR) from the appearance of the labelled PHE in plasma.[227 256] This method can be adapted to assess the effect of various levels of protein on whole body protein turnover.[263] A recent study by Kim et al. (2016)[257] estimated net protein balance in healthy young adults given either

a moderate (40 gm) or high protein (70 gm) meal. Whole body protein turnover was increased in the high protein group due to a greater reduction of protein breakdown.[257] A similar study in elderly adults corroborated these results showing increased protein turnover in a group consuming 1.5 gm protein/kg (2 x RDA) compared with a group consuming only 0.8 gm protein/kg (1 x RDA).[263] These findings are consistent with other studies showing a linear association between protein consumption and whole body protein turnover in various clinical populations.[264–266]

Indispensable Amino Acid Oxidation

The indicator amino acid oxidation (IAAO) technique is an adapted model of the whole body protein turnover approach and has been used to estimate both protein and individual amino acid requirements.[267] It is based on the concept that when an essential (or indispensable) amino acid is deficient in the diet, a limited amount of protein can be synthesized by the body. In this circumstance of deficiency, the body has no choice other than to oxidize all other amino acids, including an indicator amino acid isotope tracer (typically PHE).[268] Consequently, the more deficient the diet in a particular essential amino acid, the greater the oxidation of all other amino acids.[262] However, with intake nearing the requirement of the limiting amino acid, oxidation of the indicator amino acid will decrease to a minimum, reflecting incorporation of PHE into protein (i.e. protein synthesis).[203] Thus, when oxidation of the indicator amino acid is absent or plateaus, protein requirements are postulated to be met. Elango and colleagues verified this in healthy children by showing that oxidation plateaus or protein requirements are met at 1.3 to 1.5 gm protein/kg, which is much higher than the N balance determined RDA.[269] This

method, however, seems well-suited only for the determination of individual amino acid requirements[270–272] or minimum protein requirements in healthy individuals.[227,269] Clinical applicability of this method to determine protein requirements may be limited because the IAAO technique ignores the contribution of protein breakdown to whole body protein turnover in response to varying levels of protein intake.[227] As described above, net protein balance is the sum of synthesis minus breakdown, and solely basing protein requirements on protein synthesis ignores half the equation for determining anabolic response.[17]

The importance of measuring simultaneous protein synthesis and protein breakdown is illustrated by common clinical situations of increased muscle breakdown (e.g. cancer and cardiac cachexia, burn injury, wounds, etc.), where protein synthesis, for the most part, remains unaffected.[273] As stated above, this is due to the ability of the body to recycle amino acids from protein breakdown into protein synthesis. As such, the IAAO method, although very useful in certain circumstances, may not allow for the quantification of the optimal amount of protein needed for healing during disease and illness.[17,254]

Final Thoughts and Considerations for Clinicians

Regardless of the validity of dosing guidelines, providing adequate protein and amino acids is difficult. Hoffer and Bistrian showed that despite the diligent efforts of clinicians, the median protein and amino acid intake in critically ill adults ranged from 0.1 to 0.7 gm protein/kg, which is less than half the most common recommendation of 1.5 gm protein/kg.[16] Consequently, even if new methods to estimate protein requirements

reveal the need for an increased level of protein intake during illness, clinicians must develop the strategies required to achieve those levels. Often, clinicians are reluctant to provide high levels of protein for fear of worsening clinical status. With respect to renal function, Dickerson[197] makes an important observation that compromising renal function with elevated protein intake is inconsequential if a patient is not given sufficient protein to survive the insult that led to hospital admission.[197]

Acknowledging the inherent limitations in the current protein guidelines and understanding new methods capable of measuring physiological relevant aspects of protein metabolism will allow clinicians to better advocate for the well-being of the patient. In the future, conducting clinical trials that examine whole body protein turnover and are capable of quantifying both protein synthesis and breakdown will help inform new protein and amino acid recommendations in clinical populations.[227] Such trials must consider muscle mass, muscle quality, and clinical endpoints.[16,187]

In the meantime, adoption of various practices may help clinicians attempt to meet existing protein recommendations in hospitalized patients; these practices must be adopted while considering a patient's clinical status with agreement from all members of the healthcare team. The authors suggest clinicians consider the following:

- Understand the distinction between protein requirement and optimal protein intake: requirement has been determined as the minimum amount of protein needed to maintain N balance.[273] This may be much less than the optimal amount of protein needed to maximize protein turnover, maintain muscle, and stave off acute or chronic illness.

- Adopt a proactive approach to oral and enteral feeding when appropriate: examine current protocols as they relate to NPO status.
- Recognize the importance of nutritional intervention with protein modulars or high protein oral supplements early in admission.
- Consider adopting practices that encourage early utilization of intravenous amino acids.
- Examine the use of piggyback intravenous amino acids with a high ratio of EAAs to help promote muscle accretion, without overfeeding calories.[16,189]
- Consider an increase in the provision of free amino acids when parenteral nutrition is indicated: it has been shown that free amino acids provide 17% less substrate needed to make formed protein.[16,274] For example, if clinicians are aiming for 1.2 – 1.5 gm protein/kg, 1.5 – 1.8 gm protein/kg would be administered.[274]

While additional research that incorporates whole body and muscle protein turnover is needed to elucidate more accurate protein requirements for clinical populations, understanding the limitations of current recommendations and the methodology behind their inception is essential for clinicians. Although better evidence-based guidelines are needed, clinicians in the meantime must remain diligent in implementing strategies to ensure that patients are at a minimum meeting current protein recommendations.

CHAPTER 3: PHASE ANGLE AND IMPEDANCE RATIO: REFERENCE CUT-POINTS FROM THE UNITED STATES NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY 1999–2004 FROM BIOIMPEDANCE SPECTROSCOPY DATA*

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CHAPTER SYNOPSIS

Background: Raw bioimpedance parameters, e.g. 50 kHz phase angle (PA) and 200 kHz/5 kHz impedance ratio (IR) have been investigated as predictors of nutritional status and/or clinical outcomes. However, their validity as prognostic measures depends on the availability of appropriate reference data. Using a large and ethnically diverse data set, we aimed to determine if ethnicity influences these measures, and provide expanded bioimpedance reference data for the US population.

Methods: The National Health and Nutrition Examination Survey (NHANES) is an ongoing compilation of studies conducted by the *United States Centers for Disease Control and Prevention* designed to monitor nutritional status of the US population. The NHANES datasets analyzed were from the years1999-2000, 2001-2002, and 2003-2004.

Results: Multivariate analysis showed that PA and IR differed by BMI, age, gender, and ethnicity ($n = 6237$; $R^2 = 41.2\%$, $P < 0.0001$). Suggested reference cut-points for PA stratified by age decade, ethnicity, and gender are provided.

Conclusion: Ethnicity is an important variable that should be accounted for when determining population reference values for PA and IR. We have provided gender, ethnicity, and age decade specific reference values from PA for use by future studies in US populations. Inter-device differences are likely to be important contributors to variability across published population-specific reference data and where possible, should be evaluated in future research. Ultimately, further validation with physiologically-relevant reference measures (e.g. Dual-energy X-ray Absorptiometry) are necessary to determine if PA/IR are appropriate bedside tools for the assessment of nutrition status in a clinical population.

INTRODUCTION

Malnutrition and lean tissue depletion are associated with a number of adverse outcomes in clinical populations, which may include decreased functional capacity, increased morbidity and hospital length of stay, substantial healthcare costs, and increased mortality.[45, 49–51,97] Despite the importance of muscle loss as a key marker of malnutrition,[8] the ability to accurately assess and monitor lean tissue in clinical settings has been limited by the lack of valid bedside methods.[11] Single- and multiple-frequency bioimpedance techniques have been the most widely studied option, but have been shown to be erroneous for whole body estimates in clinical populations, particularly when fluid abnormalities and extreme adiposity are present.[11] Bioimpedance spectroscopy (BIS) modeling approaches have also not been fully realized for whole body lean tissue measurement in clinical settings due to similar concerns and limited availability, despite promising developments in the management of fluid balance in individuals on dialysis.[275,276]

There has been growing interest in the use of raw bioimpedance parameters, such as phase angle (PA) at 50 kHz and impedance ratio (IR) at 200/5 kHz), as surrogate markers of nutritional status and/or clinical outcomes in clinical populations.[146, 148,149, 156–158, 161,162,277,278] The practical application of PA and/or IR measurements to define nutritional status requires reference cut-points from a relevant healthy population. Before these cut-points can by implemented, however, they will need to be evaluated for their sensitivity to identify poor nutritional status and to monitor response to nutritional interventions.

Several researchers have published reference cut-points for PA derived from large healthy population data sets in mostly Caucasian German[279–281] and Swiss[161] individuals. Reference cut-points for PA have also been published from one racially diverse, but relatively small, American data set.[282] Very little has been done with regard to reference cut-points for IR, but some have suggested it may be a more robust measure of lean tissue and/or nutritional status.[154,156–158]

Most investigators have established PA cut-points, using methods similar to this study, based on age, gender and when sample size allows, BMI. Published cut-points, however, vary across studies; additional variables such as ethnicity and device-specificity may need to be considered. Using BIS data from a large and ethnically-diverse healthy population sample (NHANES 1999-2004), we aimed to determine if ethnicity significantly influences PA and IR values. Based on these findings, we established cut-points for PA and IR that could potentially serve as reference data for future clinical studies investigating the applications of PA and IR as markers of lean tissue and/or nutritional status in diverse US samples.

METHODS

Dataset

The National Health and Nutrition Examination Survey (NHANES) is an ongoing compilation of studies conducted by the *United States Centers for Disease Control and Prevention* designed to monitor the nutritional status of the United States population. NHANES collects a wide variety of measurements, including body composition. The NHANES datasets used for this analysis were the 1999-2000, 2001-2002, and 2003-2004

datasets, when BIS data were collected using a HYDRA ECW/ICW Bio-Impedance Spectrum Analyzer (Hydra Model 4200, Xitron Technologies, Inc., San Diego, California). In these years, whole body DXA measurements were conducted using a Hologic QDR 4500 A Fan-beam densitometer (Hologic, Inc., Bedford, MA). BIS measurements were only conducted on individuals from 8-49 years of age who were not pregnant, did not have any amputations (other than fingers and toes), did not have any types of metal objects in the body, did not have pacemakers or automatic defibrillators, did not have coronary stents or metal suture material in the heart, and weighed less than 300 pounds. Impedance was measured at 50 frequencies logarithmically spaced from 5 kHz to 1 mHz.

This data set was further limited to ages 18-49 years for this analysis. Using impedance (Z) data at the 5 KHz and 200 kHz frequencies, the 200/5 kHz impedance ratio (IR) was calculated:

$$IR = (Z\ at\ 200\ kHz)\ /\ (Z\ at\ 5\ kHz)$$

Using bioimpedance data at 50 kHz, PA was calculated from the arctangent of the ratio of reactance to resistance using the following equation:[278]

$$PA = arctangent\left({}^{X}/_{R}\right) \times {}^{180°}/_{\pi}$$

Statistical Analysis

Data were assessed for normality and found to be normally distributed. To determine if ethnicity influenced PA and IR measures, a multivariate analysis was conducted using a general linear model that included BMI, age, ethnicity, and gender. Data were stratified into the following categories: age by decade (18-19, 20-29, 30-39, 40-49), BMI class (<18.5, 18.5-24.9, 25-29.9, 30-39.9, ≥40), gender (male/female), and ethnicity (Hispanic, White, Black, Other). With age, gender, and BMI included in the regression model, ethnicity was determined to be a significant predictor of the model. Post hoc analysis was conducted to determine which ethnicities differed from one another; multiple comparisons were accounted for using Tukey's procedure correction.

Mean and standard deviation were calculated for age, weight, height, and BMI, stratified by gender and ethnicity. Mean and standard deviation for PA and IR measurements, stratified by gender and ethnicity, were also calculated. In addition, percentiles of PA were determined for grouped ethnicities (Hispanic/Black, White/Other), stratified by age decade and gender.

Fat-free mass index (FFMI) was generated by dividing DXA derived fat-free mass (FFM) by height squared (FFM (kg)/height (meters)^2). Stratifying by ethnicity and gender, percentiles of DXA FFMI were generated. Within the individuals falling at or below the 5th percentile of FFMI, stratified by ethnicity and gender, we identified mean PA and IR measures. In the cohort of individuals falling at or above the 25th percentile and at or below the 50th percentile of FFMI, stratified by ethnicity and gender, we identified mean PA and IR measures. Simple t-tests were conducted to test for differences between the 25th – 50th percentile and the ≤ 5th percentile groups.

The cut-points for PA and IR derived from the DXA FFMI data were further evaluated by converting to standardized scores (Z-scores). Using population mean PA and IR, stratified by ethnicity and gender, Z-scores were generated by the following equation:

$$Z = (Observed\ Value - Mean\ Value)/SD$$

The observed values were the mean PA (and IR) values from the DXA ≤ 5th % FFMI mean values; and the mean and SD for PA (and IR) were calculated from the overall sample, stratified by ethnicity and gender.

The data analysis was generated using SAS software, version 9.4 of the SAS system (Copyright, SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA).

RESULTS

The mean and standard deviation (SD) for age, weight, height, and BMI of the 6237 NHANES subjects are shown in **Table 3.1**. Ethnicities were distributed as follows: 40% white, 23% black, 33% Hispanic, and 4% of another race. There were significant differences in age, weight, height, and BMI ($p < .0001$) between ethnic groups. Gender differences were observed in weight, height, and BMI ($p < .0001$). Overall, mean characteristic frequency (Fc) was 43.9 ± 8.2. Fc, measured by the BIS device, represents the frequency at which the reactance is maximal.

Table 3. 1: Subject Characteristics

	Race-Ethnicity				
Variables	**White**	**Black**	**Hispanic**	**Other**	**P-value***
Men					$p < 0.0001$
N	1292	729	1058	156	
Age (years)	32.4 ± 9.7	30.4 ± 10.6	30.3 ± 10.1	28.4 ± 9.2	
Weight (kg)	84.6 ± 16.1	83.0 ±18.6	78.3 ± 15.8	78.2 ± 19.1	
Height (cm)	178.2 ± 6.9	177.5 ± 7.2	170.3 ± 7.1	173.5 ± 7.3	
BMI (kg/m^2)	26.6 ± 4.7	26.3 ± 5.6	26.9 ± 4.9	25.8 ± 5.3	
Women					$p < 0.0001$
N	1227	685	986	104	
Age (years)	33.1 ± 9.7	31.5 ± 10.6	30.5 ± 10.4	30.5 ± 10.5	
Weight (kg)	72.3 ± 18.5	80.0 ± 20.7	70.2 ± 16.5	63.9 ± 18.4	
Height (cm)	164.3 ± 6.2	163.4 ± 6.2	158.4 ± 6.5	159.8 ± 6.9	
BMI (kg/m^2)	27.8 ± 6.7	29.9 ± 7.6	28.0 ± 6.3	24.9 ± 6.3	

All numbers are Mean ± S.D**.**
*****P-value indicates significant differences in variables between race ethnicity
Abbreviations: BMI, body mass index

PA and IR values (mean ± SD), stratified by ethnicity and gender, are presented in

Table 3.2. Crude analysis of PA and IR from all 6237 subjects found significant

differences between ethnicity ($p < .0001$). Further multivariate analysis, adding BMI, age, and gender to the model, showed that PA and IR continued to significantly differ between ethnicities (R^2 = 41% and 45 %, respectively; $p < .0001$). Albeit to a small degree (1 – 2% change in R^2), the model lost power when ethnicity was discarded. A similar change in the model was seen when BMI and age were removed. Gender was the most predictive variable in the model, describing 32 – 36% of the variance in PA and IR.

Table 3. 2: Mean PA at 50 kHz and IR at 200 kHz/5 kHz by Race and Gender*

	Men		**Women**	
Race-Ethnicity	PA (50 kHz)	IR (200/5)	PA (50 kHz)	IR (200/5)
White	7.36 ± 0.71	0.76 ± 0.02	6.30 ± 0.67	0.79 ± 0.02
Black	7.55 ± 0.78	0.75 ± 0.02	6.61 ± 0.73	0.78 ± 0.02
Hispanic	7.58 ± 0.68	0.75 ± 0.02	6.54 ± 0.70	0.78 ± 0.02
Other	7.37 ± 0.76	0.76 ± 0.02	6.30 ± 0.59	0.79 ±0.02

All numbers are Mean ± S.D**.**
* Although Age and BMI are not included in the above table, when added to the model, race-ethnicity IR values remained significantly different
PA and IR values generated by the Hydra Model 4200 bioimpedance spectroscopy device, Xitron Technologies (San Diego, CA)
Abbreviations: PA, phase angle; IR, impedance ratio

Suggested reference cut-points for PA, stratified by age decade, ethnicity, and gender, are provided in **Table 3.3**. Mean BMI ± SD are also provided for each cohort. BMI was not included in the stratification model due to sample size limitations. Additionally, ethnicities were grouped based on post hoc analysis to increase sample size of each cohort. The PA cut-points presented are the 5^{th} percentile values.

Table 3. 3: Cut-points Defining Low* PA at 50 kHz from NHANES Reference Data.

Ethnicity	Age Group			
	18-19 y	**20-29 y**	**30-39 y**	**40-49 y**
Males (n=3,253)				
Hispanic/Black	6.42	6.47	6.68	6.13
N	441	477	404	465
Mean BMI	24.4 ± 5.1	26.5 ± 5.2	27.8 ± 4.7	28.1 ± 4.8
White/Other	6.35	6.30	6.32	6.08
N	213	423	405	407
Mean BMI	24.6 ± 4.6	25.8 ± 4.7	27.0 ± 4.6	27.9 ± 4.6
Females (n=3,002)				
Hispanic/Black	5.43	5.52	5.60	5.37
N	412	404	370	485
Mean BMI	26.0 ± 6.6	28.1 ± 6.9	29.7 ± 6.8	30.9 ± 6.4
White/Other	5.29	5.47	5.38	5.11
N	172	350	403	406
Mean BMI	24.9 ± 6.2	26.0 ± 6.5	27.0 ± 6.9	27.4 ± 6.5

All numbers are Mean ± S.D**.**
*Defined as <5th percentile
PA values generated by the Hydra Model 4200 bioimpedance spectroscopy device, Xitron Technologies (San Diego, CA)
Abbreviations: BMI, body mass index; PA, phase angle

The values for FFMI were generated from available DXA and anthropometric data. FFMI was found to be significantly correlated with PA and IR measures (r = 0.55 and - 0.58, respectively; p < 0.0001). Using the <5th percentile and 25th-50th percentile for FFMI stratified by ethnicity and gender, the mean PA and IR values were calculated for the respective percentiles and are presented in **Table 3.4**. A comparison of the mean values within each gender and ethnicity category were conducted and the mean PA and IR values from the <5th percentile compared to the 25th-50th percentile numbers were found to be significantly different (p < 0.0013).

Table 3. 4: Comparison of mean PA at 50 kHz and IR at 200 kHz/5 kHz obtained from the ≤5th percentile of FFMI and 25th-50th percentile of FFMI.

	Men			**Women**		
Race-Ethnicity and IR/PA	**≤5th % of FFMI**	**25th-50th % of FFMI**	**P-value***	**≤5th % of FFMI**	**25th-50th % of FFMI**	**P-value***
White						
IR	0.78 ± 0.02	0.76 ± 0.02	<0.0001	0.81 ± 0.02	0.79 ± 0.02	<0.0001
PA	6.77 ± 0.76	7.35 ± 0.73	<0.0001	5.79 ± 0.64	6.26 ± 0.63	<0.0001
	(n=59)	(n=296)		(n=56)	(n=283)	
Black						

IR	0.78 ± 0.03	0.75 ± 0.02	<0.0001	0.80 ± 0.02	0.78 ± 0.02	<0.0001
PA	6.62 ± 1.02	7.53 ± 0.68	<0.0001	5.92 ± 0.52	6.58 ± 0.66	<0.0001
	(n=33)	(n=165)		(n=29)	(n=147)	
Hispanic						
IR	0.77 ± 0.02	0.75 ± 0.02	<0.0001	0.80 ± 0.02	0.79 ± 0.02	<0.0001
PA	6.97 ± 0.64	7.58 ± 0.70	<0.0001	5.79 ± 0.61	6.48 ± 0.70	<0.0001
	(n=50)	(n=250)		(n=47)	(n=231)	
Other						
IR	0.79 ± 0.02	0.76 ± 0.02	<0.0001	0.82 ± 0.01	0.79 ± 0.01	0.0013
PA	6.33 ± 0.56	7.28 ± 0.59	0.0001	5.59 ± 0.37	6.48 ± 0.46	0.0008
	(n=8)	(n=39)		(n=4)	(n=24)	

All numbers are Mean ± S.D**.**
*P-values calculated with paired t-tests
PA and IR values generated by the Hydra Model 4200 bioimpedance spectroscopy device, Xitron Technologies (San Diego, CA); FFMI values generated from FFM measured by dual-energy X-ray absorptiometry, using a Hologic QDR 4500 A Fan-beam densitometer (Hologic, Inc., Bedford, MA)
Abbreviations: PA, phase angle; IR, impedance ratio; FFMI, fat-free mass index

Z-scores are provided in **Table 3.5**. As expected, PA decreases with decreasing FFMI, thus there is a negative Z-score; the opposite is true of IR. PA Z-scores range from – 0.8 to – 1.4. IR measures ranged from 1.0 – 1.5.

Table 3. 5: Standardized scores of population mean PA and IR using the DXA ≤5th percentile cut-points from Table 4 as the observed values and the population mean and SD, stratified by gender and ethnicity, reported in Table 3.2.

	Men	Women
Race-Ethnicity and IR/PA	**Z-score**	**Z-score**
White		
IR	**1.0**	**1.0**
PA	**-0.8**	**-0.8**
Black		
IR	**1.5**	**1.0**
PA	**-1.2**	**-1.0**
Hispanic		
IR	**1.0**	**1.0**
PA	**-0.9**	**-1.1**
Other		
IR	**1.5**	**1.5**
PA	**-1.4**	**-1.2**

$Z = (Observed\ PA - Mean\ PA)/SD$

Abbreviations: PA, phase angle; IR, impedance ratio

DISCUSSION

PA cut-points (defined as < 5th percentile), established using a large ethnically diverse dataset, fell between previously published cut-points from other large population data.[281,282] In addition, PA and IR cut-points, based on physiologically relevant endpoints (DXA FFMI), were created. Fc (43.9 ± 8.2) was found to be within the range that has

been observed in other healthy populations, which suggests this NHANES data set is a representative sample of healthy individuals.[11,123]

The application of bioimpedance analysis for the estimation of whole body composition has been accepted for the assessment of healthy, non-obese individuals and large epidemiologic studies, but has been questioned for its applicability to the clinical setting due to its variability at the individual level. Limitations to bioimpedance analyses for the assessment of whole body composition in clinical populations have been reviewed elsewhere,[124, 277,278,282,283] and have led investigators to explore the use of raw bioimpedance data in nutrition assessment.[277,278]

PA and IR are simple bioimpedance parameters that have been reported to be prognostic indicators of disease and nutritional status in a variety of clinical populations.[146, 149, 157,161,162] Low PA and high IR have been associated with poor nutritional status and poor disease outcome.[148,149, 157,161] A dearth of available reference values, however, has limited research aimed at validating the use of PA and IR in clinical settings.

Normative reference values for PA have been published based on healthy American,[282] German,[277] and Swiss[284] populations. Although these investigators used similar regression modeling, differences between these published values suggest that there are variables other than BMI, age, and gender that affect these raw impedance parameters.[285] Barbosa-Silva and colleagues[282] identified ethnicity as a significant predictor with univariate analysis, but not in a multivariate model (with BMI and age). We believe, however, that the lack of significance in their multivariate model was due to an inadequate sample size. Using a large and ethnically diverse NHANES data set, we

were able to demonstrate that PA and IR are significantly different between ethnicity when added to a multivariate regression model, which included BMI, age, gender and ethnicity. These results suggest that ethnicity influences PA/IR values and should be included as a variable in determination of population reference values.

Various reference techniques for assessing body composition, such as computed tomography, magnetic resonance imaging, and DXA, are frequently used in research settings.[72] DXA-derived fat-free mass from the NHANES datasets analyzed in this study were used to objectively ground our PA and IR cut-points. As expected, PA and IR were positively and negatively associated with FFMI, respectively. This seems to suggest that raw bioimpedance parameters may be useful tools in assessing lean tissue[286]; and more specifically, may function as surrogate markers for FFMI in the diagnosis of malnutrition - as proposed by the European Society for Parenteral and Enteral Nutrition.[37] However, this has yet to be determined.

The use of the 5th percentile for PA values has been determined as a possible cut-point for malnutrition by other investigators[277,282] and was therefore reported in Table 2. It should be noted, however, that a specific percentile has not been defined to identify malnutrition in clinical populations when using healthy reference values. Therefore, the use of z-scores, specifically derived from PA (standardized phase angle), have been proposed as a more meaningful way to define malnutrition.[163, 279,287,288] More research is needed to determine clinically meaningful values for PA and IR z-scores at single time points, but the transformation of PA and IR measurements into z-scores seems to offer a more meaningful method for quantification of longitudinal changes in nutrition status - regardless of the initial value.

There are a number of limitations in our study design and presentation of results that need to be acknowledged. The results of our study were based on an age and weight limited (17<age<50 and less than 300 pounds, respectively) cohort that limits the generalizability of the results. In addition, measures were only conducted using one bioimpedance device which limits the ability to account for inter-device variability. Finally, the reference ranges included in Table 3 are only stratified by gender, age decade, and ethnicity. The sample sizes became too small to provide meaningful values with additional stratification. Because BMI is an established predictor of PA and IR values we provided the means (±SD) for each group in Table 3, but the lack of inclusion in our stratification model makes our results incomplete.

This study is unique for several reasons; it is the first to develop physiologically relevant PA and IR cut-points from a large ethnically diverse population data set, using reference measures of lean tissue (DXA-derived FFMI) to ground the data. Furthermore, these are the first published reference data generated from a BIS device.

CONCLUSION

The development of appropriate reference values for PA and IR are essential for future research aimed at determining if raw bioimpedance parameters can be used in a meaningful way at a clinical level – particularly with regard to the diagnosis and treatment of malnutrition. We have demonstrated, using a large and ethnically diverse data set, that ethnicity is an important variable that should be accounted for when determining population reference values for PA and IR. We have provided reference data, which includes ethnicity as a variable, from a large ethnically diverse data set (Table 3 &

4). These need to be further evaluated against clinical outcomes data to ascertain their sensitivity in identifying malnourished individuals and monitoring response to nutritional interventions compared to existing tools.

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CHAPTER 4: EVALUATION OF BIOELECTRICAL IMPEDANCE ANALYSIS IN CRITICALLY ILL PATIENTS: RESULTS OF A MULTICENTER PROSPECTIVE STUDY*

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CHAPTER SYNOPSIS

Background: In critically ill patients, muscle loss is associated with adverse outcomes. Raw bioelectrical impedance analysis (BIA) parameters (e.g. phase angle (PA) and impedance ratio (IR)) have received attention as potential markers of muscularity, nutritional status and clinical outcomes. Our objective was to test whether PA and IR could be used to assess low muscularity and predict clinical outcomes.

Methods: Patients (≥ 18 years) having an abdominal CT scan and admitted to intensive care underwent multi-frequency BIA within 72 hours of scan. CT scans were landmarked at the 3rd lumbar vertebra and analyzed for skeletal muscle cross-sectional area (CSA). CSA ≤ 170cm^2 for males and ≤ 110cm^2 for females defined low muscularity. The relationship between PA (and IR) and CT muscle CSA were evaluated using multivariate regression and included adjustments for age, sex, BMI, Charlson comorbidity index, and admission type. PA and IR were also evaluated for predicting discharge status using DXA-derived cut-points for low fat free mass index.

Results: Of 171 potentially eligible patients, 71 had BIA and CT Scans within 72 hours. Area under the receiver operator characteristic (c-index) curve to predict CT-defined low muscularity was 0.67 ($p \leq 0.05$) for both PA and IR. With covariates added to logistic regression models, PA and IR c-indexes were 0.78 and 0.76 ($p < 0.05$), respectively. Low PA and high IR predicted time to live ICU discharge.

Conclusion: Our study highlights the potential utility of PA and IR as markers to identify patients with low muscularity who may benefit from early and rigorous intervention.

INTRODUCTION

Lean tissue, primarily consisting of skeletal muscle, is central to the body's healthy response to injury and illness.[10,11] Muscle wasting often occurs in the setting of illness and inflammation due to increased metabolic demands on the body. Critically ill patients often experience major losses of lean tissue due to severity of illness and organ dysfunction, prolonged immobility, and malnutrition.[3] Loss of this highly metabolic tissue is associated with increased morbidity and mortality,[45,97] infections,[49] and length of stay.[50] For ICU survivors, muscle atrophy may lead to decreased functional capabilities.[52]

Despite the prognostic significance of lean tissue loss in acute and chronic disease, its estimation is not commonly performed in hospital settings in part due to the limited availability of valid objective bedside methods.[11,51] Instead, clinicians often rely on subjective physical examination techniques,[36,54] alone or as part of the Subjective Global Assessment (SGA).[56] Subjective evaluation of muscle mass, however, is potentially error-prone, particularly in obese and edematous states.[11,289]

Clearly, there is a significant and growing need for easily accessible and accurate methods for assessing lean tissue in the clinical setting. Very few available techniques are appropriate for use at the bedside or for repeated measures. Bioimpedance spectroscopy, and the more commonly available multifrequency bioelectrical impedance analysis (MF-BIA) technique offer the potential for easy, rapid, and portable assessment of lean tissue and fluid status in the clinical setting. The MF-BIA approach has been evaluated for its validity in healthy populations,[137,139] and multiple clinical populations.[141,290] The MF-BIA approach for whole body estimates of lean tissue requires the use of population-specific

prediction equations and is predicated upon meeting various underlying assumptions (e.g. normal body geometry, hydration of lean tissue, and fluid distribution), which are often violated in critical illness; typically due to fluid shifts and edema present during illness and trauma.[123]

Inaccuracies in whole-body lean tissue estimates have led to recent interest in the use of raw BIA parameters (e.g. 50 kHz phase angle (PA) and 200/5 kHz impedance ratio (IR)) as potential markers of nutritional status, disease severity, and outcomes in various clinical populations.[146, 154–156, 158,290] It has been hypothesized that PA may be related to cell membrane integrity;[160] and IR may be related to hydration status.[11] In order to obtain PA, reactance (Xc) and resistance (R) at 50 kHz must be obtained from the BIA device. Xc is the resistive force that cell membranes and tissue interfaces have on an electric current; R is the inability of an electric current to flow through the body, inversely related to the volume of fluid within tissues.[160] Subsequently, PA is calculated from the arctangent of the ratio of Xc to R at 50 kHz.

$$PA = arctangent\ (Xc/R) \times 180°/\pi$$

There is growing interest in the use of IR at 200 kHz/5 kHz as a potential marker nutritional and clinical status.[11]

$$IR = \frac{\sqrt{Xc^2 + R^2\ at\ 200\ kHz}}{\sqrt{Xc^2 + R^2\ at\ 5\ kHz}}$$

Grounding of these raw bioimpedance parameters by comparison to a reference technique such as dual energy X-ray absorptiometry (DXA) or CT, particularly for the ability to identify low muscle mass and monitor response to targeted nutrition interventions are necessary steps before they can be accepted as useful tools in any clinical population. CT imaging has been shown to be reliable and precise in quantifying skeletal muscle mass.[291] Moreover, the cross-sectional area (CSA) of skeletal muscle in a single transverse CT image at the 3rd lumbar (L3) region has excellent correlation with whole body skeletal muscle mass.[68,292] However, CT is limited in its bedside applicability. MF-BIA generated PA and IR offer the advantages of non-invasiveness, portability, and repeatability.

We are interested to know if PA and IR can be used as surrogate markers of muscle mass as measured by abdominal CT scans. Ultimately, as low muscularity is one of the key defining criteria to determine malnutrition, we are interested in whether these parameters can give insight into the nutritional status and adequacy of nutritional intervention throughout hospitalization. Furthermore, we investigated whether PA and IR had any prognostic capabilities to predict low CT-derived muscle CSA or clinical outcomes, such as ICU and hospital length of stay. Finally, we determined whether the creation of reference values (defined by mean PA and IR for healthy individuals falling at or below the 5th percentile for fat-free mass index (FFMI; FFM/height) measured by DXA in the most recent NHANES) could improve their prediction capabilities of clinical outcomes. We hypothesized that PA and IR would have a strong linear association with abdominal CT muscle CSA and subsequent strong capabilities in predicting low muscularity and live hospital and ICU discharge.

METHODS

Study design

This was a prospective, multicenter observational study that was conducted across 3 ICU's, including the University of Minnesota Health, Minneapolis-St. Paul, Minnesota; VU University Medical Center Amsterdam, Amsterdam, The Netherlands; and Rush University Medical Center, Chicago, Illinois. This study was approved by the local and institutional Research Ethics Committees. Patients ≥ 18 years of age were included if they were admitted to the ICU and had a CT scan of the L3 vertebra performed for clinical reasons <24 hours prior to or < 72 hours after admission to the ICU. Moribund patients not expected to survive and other vulnerable populations including pregnant women, prisoners, and mentally-disabled individuals were excluded.

Once enrolled, patients underwent BIA within 72 hours of the initial CT scan. Physical data, including age, sex, height and weight was obtained for all patients. All patients being bedridden, height was taken by a registered nurse with the patient lying flat and weight was calculated using a bed scale; bed items zeroed prior to measurement. Clinical data obtained or calculated upon ICU admittance included admission type (surgical vs medical), Acute Physiology and Chronic Evaluation (APACHE II) Score (a score to assess the severity of disease in the ICU),[293] Sequential Organ Failure Assessment (SOFA) Score (a score to assess the degree of organ dysfunction in the ICU),[293] Charlson Comorbidity Index (designed to predict mortality by categorizing severity of comorbidities),[294] Functional Comorbidity Index (designed to predict physical function, not mortality, by categorizing severity of comorbidities),[295] number of mechanical ventilation days, ICU length of stay, hospital length of stay, ICU mortality

and hospital mortality. Height and weight were used to calculate BMI (kg/m^2) for each patient, which was further classified into the following categories: underweight (BMI <18.5 kg/m^2), normal weight (BMI 18.5 to 24.9 kg/m^2), overweight (BMI 25 to 29.9 kg/m^2) and obese (BMI >30 kg/m^2).

Bioimpedance analysis

Bioimpedance analysis, in order to acquire PA and IR, was done using a MF-BIA QuadScan 4000 (Bodystat LTD, Isle of Man, UK) and was conducted as close as possible to a CT scan done for clinical reasons. PA and IR were directly measured by the Quadscan 4000. Electrodes were placed in a standard tetrapolar position (on the hands and feet), and patients were placed in a recumbent position with arms abducted from the body, and legs separated, using rolled blankets as needed to ensure physical separation, as described previously.[11] MF-BIA was not performed on individuals with pacemakers or electronic implantable devices. For improved interpretation of the PA and IR data, we utilized bioimpedance and DXA data from NHANES (1999-2000, 2001-2002, and 2003-2004) to generate reference cut-points. Data generated from a spectroscopy device (Hydra Model 4200, Xitron Technologies, Inc., San Diego, California) and a whole body DXA scanner (Hologic QDR 4500 A, Hologic, Inc., Bedford, MA) were available in a healthy ethnically-diverse sample of men (n = 149) and women (n = 137) between the ages of 18 and 49 years. Cut-points were defined by mean PA and IR for individuals falling at or below the 5th percentile for DXA-derived FFMI.

CT scan analysis

PA and IR were compared, separately, to CT-derived muscle CSA of the L3 region. CT images were sent to the University of Waterloo, Waterloo, ON, Canada for analysis of muscle volume. Scans were analyzed using SliceOmatic image analysis software (TomoVision, Montreal, QB, Canada – version 4.3). All scans were analyzed by trained analysts and reliability measures were performed. The mean coefficient of variation for intra- and inter-rater reliability for muscle CSA was 1.0% and 1.9%, respectively. Intra- and inter-rater reliability measures completed were similar to the coefficients of variation described in the literature.[68] Patients were categorized as having low muscularity if their skeletal muscle CSA was <110 cm^2 for females and <170 cm^2 for males. These cut-points have been previously established and associated with mortality in an ICU population.[97]

Statistics

Descriptive statistics were reported as mean±standard deviation (SD) or median and interquartile range (Q1 – Q3). PA and IR were further stratified by age (< and > 65 years) and gender to compare differences between groups using Student's t-test. Linear regression, with and without consideration of covariates (BMI, age, gender, Charlson comorbidity index, and admission type) was performed to assess the ability of PA and IR to predict the variability in abdomen CT-derived muscle area. BMI, age, and gender are known to affect BIA parameters.[282] These and the other two covariates were retained in the regression model regardless of their statistical significance in predicting the dependent variable. Once patients were classified as having low muscularity (<110 cm^2

for females and <170 cm^2 for males), logistic regression, with and without consideration of the same covariates, was performed to assess the capabilities of PA and IR to predict CT derived muscle area. Similarly, area under the receiver operating characteristic (ROC) curve was performed to assess prognostic capabilities, again taking into account covariates as described previously. Finally, Cox regression was performed to determine the predictive capabilities of PA and IR, with respect to live ICU and live hospital discharge. All analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA) and all p-values are two-sided without correction for multiplicity.

RESULTS

Descriptive characteristics

Seventy one out of a total of 171 enrolled had both BIA and readable CT scans for comparison and were included for analysis, of whom 62% were male and all fell at or below Class I obesity. Overall, patients had a mean age of 57±16 years old and had a mean BMI of 29±8 kg/m^2. Mean APACHE II score was 16±7 (Table 4.1). According to BMI, 39% were classified as normal weight, 31% were overweight, and 30% were class I obese. Median ICU and hospital length of stay were 3 (2 – 7) and 10 (6 – 20) days, respectively, while ICU and hospital mortality were 4% and 6%, respectively (Table 4.1). There was no difference in PA or IR between males and females (p=0.09 and p=0.07, respectively). There was a trend toward differences between PA in young versus elderly (p=0.05), but no significant differences were seen between IR when stratified by age (p=0.09) (Table 4.2). CT scans revealed that 57% of patients had lower than normal

muscularity, defined as CSA <110 cm^2 for females and <170 cm^2 for males. BIA occurred within 1.3 days to the CT scan.

Table 4. 1: Patient clinical and physical characteristics.

Characteristics	All patients (n=71)
Age (years)	57±16 (21-87)
Sex	
Male	44 (62.0%)
Female	27 (38.0%)
Height (cm)	172±10 (152-196)
Usual Weight (kg)	85±24 (51-186)
BMI (kg/m^2)	29± 8 (19-57)
Normal	28 (39.4%)
Overweight	22 (31.0%)
Obesity class I	21 (29.6%)
APACHE II score	16± 7 (3-31)
SOFA score	5± 4 (0-15)
Charlson comorbidity index	1± 1 (0- 5)
Functional comorbidity index	1± 1 (0- 4)
Admission type	
Medical	54 (76.1%)
Surgical	17 (23.9%)
Primary ICU admission	
Cardiovascular/Vascular	17 (23.9%)
Respiratory	12 (16.9%)
Gastrointestinal	16 (22.5%)
Neurologic	2 (2.8%)
Sepsis	9 (12.7%)
Trauma	10 (14.1%)
Metabolic	1 (1.4%)
Other	4 (5.6%)
Mechanical ventilation duration (days)	3[1 to 6] (0-39)
Intensive care unit length of stay (days)	3[2 to 7] (1-22)
Hospital length of stay (days)	10[6 to 20] (2-72)
ICU mortality	4 (5.6%)
Hospital mortality	6 (8.5%)

Numbers are reported as (n) mean±SD (range) or median [Q1 to Q3] (range) or n (%).

BMI = body mass index; APACHE II = acute physiology and chronic health evaluation II; SOFA= sequential organ failure assessment; ICU = intensive care unit.

Table 4. 2: Descriptive data of phase angle and impedance ratio stratified by gender and age.

Measurement Mean ± SD	All patients (n=71)	Males (n=44)	Females (n=27)	p-value	Young (<65 years) (n=45)	Elderly (≥65 years) (n=26)	p-value
Impedance Ratio	0.85±0.04 (69)	0.85±0.04 (42)	0.86±0.05 (27)	0.07	0.85±0.04 (43)	0.87±0.04 (26)	0.09
Phase Angle	4.34±1.40 (71)	4.54±1.36 (44)	4.01±1.42 (27)	0.09	4.63±1.42 (45)	3.85±1.24 (26)	0.05

Numbers are reported as mean±SD (n)

Phase angle and Impedance ratio

Mean PA and IR were 4.34±1.40 and 0.85±0.04, respectively, for all patients. Using linear regression, PA alone was able to predict 20% of the variance in CT muscle CSA, and 61% of the variance when covariates (age, sex, BMI, Charlson comorbidity index, and admission type) were added to the model (Table 4.3 and 4.4). Similarly, IR alone was also able to predict 20% of the variance in CT muscle CSA, and 60% of the variance when covariates were added. The area under the ROC (c-index) curve to predict CT-defined low muscle area was 0.67 for both PA and IR. With covariates added to logistic regression models including PA and IR, the c-indexes were 0.78 and 0.76, respectively (Figures 1A and 1B). PA and IR were both able to predict live ICU discharge (c-index=0.611 and c-index=0.608, p=0.008 and p=0.009, respectively), but not live hospital discharge (Table 4.5). When low FFMI cut-points for PA and IR derived from NHANES data were used, they were able to predict live ICU discharge with greater

certainty (c-index=0.77 and c-index=0.79, p=0.04 and p=0.01, respectively) than that found without using low FFMI cut-points (Table 4.5).

Table 4. 3: Linear regression of PA to predict CT-derived muscle area

Outcome	Predictors	R^2	Adjusted R^2	RMSE	p-value model	P-value PA
CT muscle area (cm^2)	PA	0.21	0.20	37.7	<0.0001	<0.0001
CT muscle area (cm^2)	covariates*	0.60	0.57	27.5	<0.0001	NA
CT muscle area (cm^2)	covariates+PA	0.65	0.61	26.2	<0.0001	0.008

Covariates are: age (linear), sex (binary), BMI (linear), Charlson comorbidity index (linear) and admission type (binary). NA = not applicable, R^2 = % of variance in outcome explained by model. RMSE=root mean square error. CT= computed tomography

Table 4. 4: Linear regression of IR to predict CT-derived muscle area

Outcome	Predictors	R^2	Adjusted R^2	RMSE	p-value model	P-value IR
CT muscle area (cm^2)	IR	0.21	0.20	36.9	<0.0001	<0.0001
CT muscle area (cm^2)	covariates*	0.60	0.57	27.1	<0.0001	NA
CT muscle area (cm^2)	covariates+IR	0.63	0.60	26.1	<0.0001	0.02

Covariates are: age (linear), sex (binary), BMI (linear), Charlson comorbidity index (linear) and admission type (binary).
NA = not applicable, R^2 = % of variance in outcome explained by model. RMSE=root mean square error. CT= computed tomography

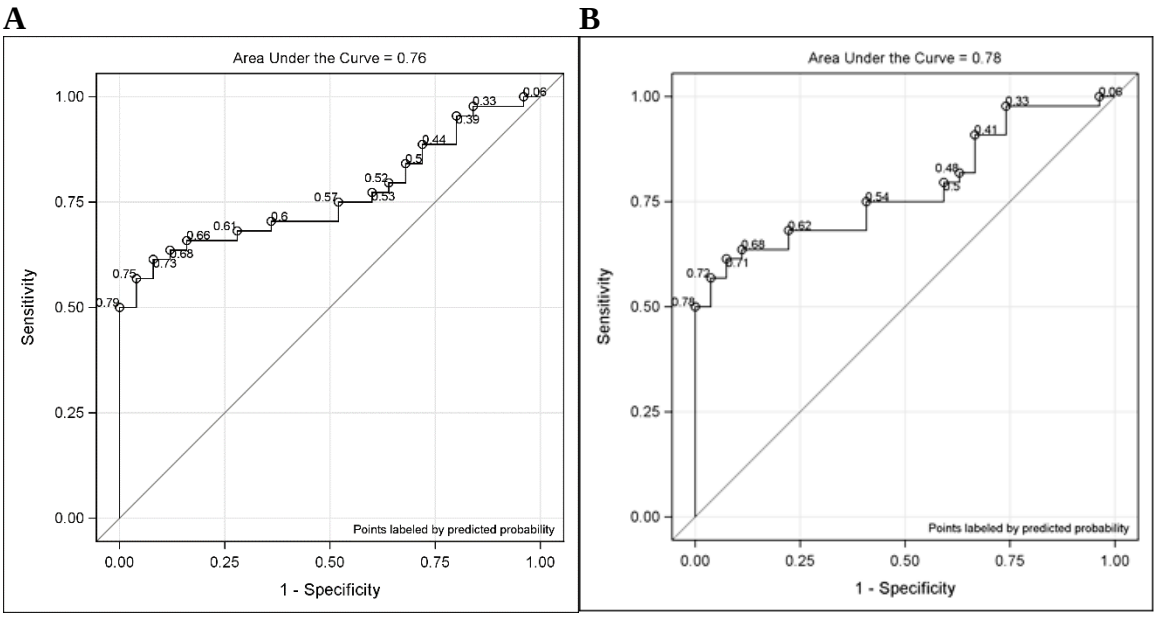

Figure 4. 1: (A) Receiver operating characteristic curve for impedance ratio with covariates to predict low muscle area. (B) Receiver operating characteristic curve for phase angle with covariates to predict low muscle area. Receiver operating characteristic curve analysis is used to quantify how accurately medical diagnostic tests can discriminate between 2 patient states—in this case, low muscle mass or normal muscle mass. Area under the curve or c-index of 0.5 indicates no ability of the model to discriminate between low and normal muscle mass, while a c-index of 1 implies the ability to perfectly discriminate lower and normal muscle mass.

Table 4. 5: Cox regression for the ability of PA and IR to predict relative time to live discharge.

Predictors	Outcome	c-index of model	p-value of PA or IR
PA (continuous)	Time to live ICU discharge	0.599	**0.028**
PA (continuous)	Time to live hospital discharge	0.528	0.49
PA (continuous)+covars*	Time to live ICU discharge	0.611	**0.008**
PA (continuous)+covars*	Time to live hospital discharge	0.599	0.43
PA<5% cut-point	Time to live ICU discharge	0.774	**0.04**

PA<5% cut-point	Time to live hospital discharge	0.613	0.70
IR (continuous)	Time to live ICU discharge	0.584	**0.032**
IR (continuous)	Time to live hospital discharge	0.513	0.46
IR (continuous)+covars*	Time to live ICU discharge	0.608	**0.0095**
IR (continuous)+covars*	Time to live hospital discharge	0.587	0.39
IR<5% cut-point	Time to live ICU discharge	0.786	**0.01**
IR<5% cut-point	Time to live hospital discharge	0.563	0.75

* covariates in model include sex, age (as linear) and BMI (as linear).
PA cut-point is 6.75 for men and 5.85 for female
IR cut-point is 0.78 for men and 0.81 for women
Abbreviations: PA = phase angle, IR = impedance ratio

DISCUSSION

We conducted a critical comparison between BIA parameters and CT CSA analysis for muscle quantification in ICU patients. In a heterogeneous sample of 71 critically ill patients, we found that PA and IR accounted for 61% and 60% of the variance in CT muscle CSA (p=0.008 and p=0.02), respectively. Prediction of abdominal CT muscle CSA was improved when age, sex, BMI, Charlson Comorbidity Index and admission type were added to the multivariate regression model. Based on ICU-derived gender-specific cut-points from CT analysis,[97] 57% of patients had low muscularity at admission to the ICU, which is similar to that seen in the literature for other ICU-specific studies.[45,97] ROC curve analysis showed PA and IR had moderate capabilities in predicting low CT muscle CSA when controlling for covariates, appropriately diagnosing low muscularity 78% and 76% of the time, respectively. Furthermore, when DXA-derived low FFMI cut-points for PA and IR from an NHANES dataset was applied in a logistic model, both were able to significantly predict live ICU discharge 77% and 79%

of the time. Prediction of live ICU discharge was improved when applying reference cut-points from a large dataset.

Loss of muscle and functional status are two of the defining criteria to determine the presence and severity of malnutrition in the Academy of Nutrition and Dietetics/American Society for Parenteral and Enteral Nutrition Consensus definition.[54] PA and IR pose intriguing potential as markers to determine nutritional status and monitor nutrition intervention adequacy throughout treatment course. In the context of this study, PA performed well and is in agreement with previously published literature.[158, 160,290] When patients with heart failure were classified by severity of disease, a lower PA was significantly associated with the severity of disease. Moreover, handgrip strength was lower and correlated with PA and severity of disease; similar results were seen with IR.[158] This points to the potential usefulness of PA and IR in not only predicting disease status but also functional status in this population. In individuals undergoing cardiac surgery, lower pre-operative PA was associated with undernutrition (as specified by low BMI) and prolonged ICU and hospital length of stay.[290] This is consistent with the findings of our study. Furthermore, a lower PA has been shown to predict post-operative complications, nutritional risk, length of stay, and prognosis in various other clinical populations, including surgery,[163] HIV[149] and various cancers.[145,146] Although fewer studies have been conducted looking at IR, some researchers suggest it may be a more robust marker of nutritional status and disease severity.[156] It has also been reported to identify malnutrition as defined by low total body nitrogen with greater sensitivity (79%) than PA (23%).[154] Our findings in light of published literature underscore the potential of PA and IR as clinical tools and additional clinical evaluation is warranted.

Implementation of phase angle and impedance ratio reference cut-points derived from NHANES data

The practical application of PA and IR measurements as biomarkers to define nutritional status requires that reference cut-points from healthy population data be established. Several researchers have published reference cut-points for PA in select populations including German,[277,280] Swiss[284] and American;[282] very little has been done with IR. In order to further establish the use of healthy population cut-points, the Continuous NHANES dataset was utilized to produce reference cut-points for PA and IR to be applied in this study;[296] Grounding BIA parameters in a physiologically relevant endpoint, all individuals falling at or below the 5th percentile for FFMI as determined by DXA were grouped together. Mean PA and IR values were then found for this group of individuals with low muscularity, which subsequently became the reference cut-points applied to our critically ill population. Upon inspecting the ability of PA and IR to predict live ICU and hospital discharge, we found that when we applied our healthy reference cut-points, PA and IR predictive capabilities improved. However, more work is needed to establish appropriate cut-points based on age, BMI, gender, and ethnicity from larger, more robust datasets comprised of DXA or other reference methods, if raw bioimpedance parameters are going to be used in a meaningful way at the clinical level – particularly with regard to the identification of malnutrition.

Strengths and limitations

The strengths of this study include the use of expert analysis of abdominal muscle CSA using CT images in order to evaluate our BIA parameters. Additionally, the multi-site nature of this collaboration that recruited a heterogeneous ICU population with

multiple operators enhances the generalizability of the findings. Admitting diagnoses included patients with a plethora of medical and surgical conditions. We believe the heterogeneous nature of our population adds strength to the results, as we would expect to see even stronger associations and predictive capabilities of PA and IR with a more homogeneous sample. Another strength to our study was that we attempted to generate physiologically relevant cut-points based on a large U.S. population sample in order to better interpret our data. However, it must be noted that the reference data were generated by a different bioimpedance device than the device used in our study; inter-device differences are potential sources of error but are not well documented. The study was limited by its relatively small sample size and the small number of confounding variables that were collected at the start of the study for inclusion into statistical modeling. Given that a pragmatic approach was taken with the implementation of the BIA protocol, logistical challenges in patient positioning and measurement procedures in light of the ICU setting likely contributed to the variability in measurements. Furthermore, our final sample did not include individuals with Class II and III obesity due to the inability to obtain both BIA and CT scans for comparison in these individuals.

CONCLUSIONS

PA and IR were moderately associated with CT-derived muscle CSA at the L3 level with covariates added to the model. That is, lower PA and higher IR values were associated with lower muscle CSA. Furthermore, PA and IR appeared to predict low CT-derived muscle CSA. Using a Healthy population dataset, DXA-derived cut-points of low FFMI for PA and IR seemed to predict ICU discharge with greater certainty than did PA

and IR (with covariates) alone. From a clinical standpoint, the simplicity, non-invasiveness, and easy repeatability of BIA has substantial appeal as a bedside assessment tool, and the idea that a PA below a certain value or an IR above a certain value could indicate low muscle mass, poor nutritional status, and/or predict clinical outcomes is compelling. However, it remains to be established if these parameters can be effectively used to assess and monitor muscle mass and nutrition status with greater sensitivity than other clinically accessible techniques. Successful implementation of PA and IR as assessment tools in the ICU will require adherence to standardized measurement protocol and utilization of appropriate reference cut-points for these parameters. In summary, PA and IR show promise in being able to aide in the identification of low muscularity and poor nutritional status in the ICU setting; additional research is warranted.

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CHAPTER 5: SHORT COMMUNICATION OF ADDITIONAL MULTICENTER RESULTS: VALIDATION OF BEDSIDE ULTRASOUND OF MUSCLE LAYER THICKNESS OF THE QUADRICEPS IN THE CRITICALLY ILL PATIENT (VALIDUM STUDY)

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INTRODUCTION

Body composition measures are critical for comprehensive evaluations of nutritional status. Body weight, body mass index and change in weight are often used for these assessments, however, they cannot distinguish specific tissue compartments from muscle. Identifying an objective modality that is readily available for assessing muscularity is of critical importance. Given the impact of muscle wasting on clinical outcomes, recent research has focused on objective, non-invasive methods to help quantitate muscle loss within clinical populations.[2] Ultrasound measures of muscle thickness at various sites has been shown to correlate well with reference measures of total body lean tissue and muscle mass.[172, 174,180] As part of the previous study, ultrasound measurements of quadriceps muscle layer thickness (QMLT) were measured to validate its ability to predict muscle as determined by CT-derived CSA. Results of this study have been published. For more detail on study design and methods, the reader is referred to the publication by Paris et al. 2017.[2]

RESULTS

Physical and clinical characteristics

Two hundred four patients who had a cross-sectional abdominal CT scan of the L3 vertebra for clinical reasons within 24 hours prior or less than 72 hours after ICU admission were recruited for further ultrasound analysis. Of the 204 patients recruited, 41 had a non-analyzable CT scan and 14 had incomplete ultrasound data or it was performed greater than 72 hours after initial CT scan, resulting in a total of 149 patients for further analysis. A sensitivity analysis was performed and identified no differences between the data of the 2 studies; as such, all data were combined. Overall, 42% of patients were

female. Patients were 59 ±19 years old, had a BMI of 29 ±8 kg/m^2, and had an average APACHE II and SOFA Scores of 17 ±8 and 5 ±4. Median ICU and hospital length of stay were 3 [2-7] and 8 [5-17] days respectively, while ICU and hospital mortality were 9% and 11%, respectively. Based on BMI classifications, 68.5% of the population was overweight or obese, whereas only 2.7% of the population was underweight.

Mean CT abdominal skeletal muscle CSA was 108.5±24.5 cm^2 for females, which was significantly different from males 168.4±36.6 cm^2 ($p<0.001$). Approximately 58% (59% of females and 57% of males) of patients had low muscularity, despite that only 2.7% of the population was classified as underweight by BMI. Significant differences were found between young (157.4 ±45.6 cm^2) and elderly (126.0 ±34.1 cm^2) groups ($p<0.001$). However, for QMLT, significant differences were found between males (1.5 ±0.6 cm) and females (1.1 ±0.6 cm) ($p<0.001$), but not between young (1.4 ±0.7 cm) and elderly (1.2 ±0.5 cm) age groups ($p=0.57$).

QMLT is moderately correlated with abdominal muscle CSA

On average, QMLT measures were performed 1.1 ±1.0 days after the CT scan. Overall, QMLT was moderately ($r=0.45$, $p<0.001$) correlated with abdominal skeletal muscle CSA. Further sub-analysis examining correlations for young females, older females, young males and older males revealed that this correlation was likely driven by the largest group - young males ($n=55$; $r=0.51$, $p<0.001$); the remaining groups, young females, elderly males and females had non-significant correlations of $r=0.13$, $r=0.24$ and $r=0.26$, respectively.

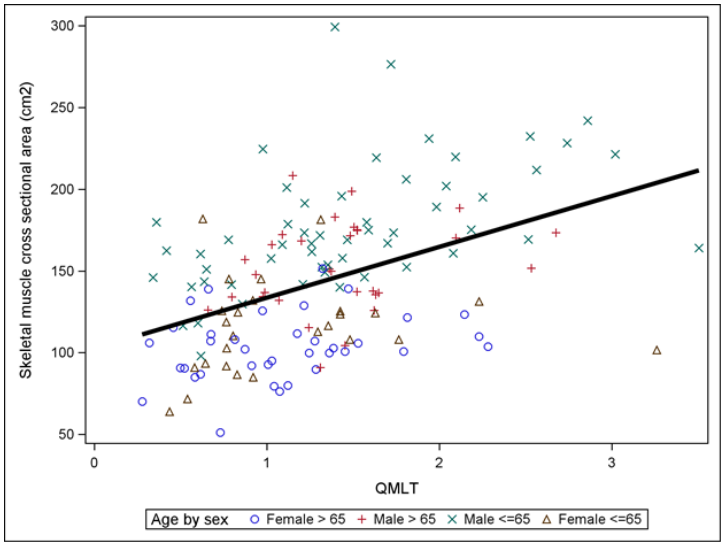

Figure 5. 1: Association between CT skeletal muscle cross sectional area and ultrasound QMLT with linear regression lines superimposed. Overall regression fit: CT skeletal muscle cross sectional area=102.5+31.1 x QMLT. Overall Pearson correlation coefficient between CT skeletal muscle cross sectional area and Ultrasound QMLT index is 0.45, p<0.0001; L3, 3rd lumbar.

With the addition of age, sex, BMI, Charlson Comorbidity Index and admission type (surgical or medical) to the regression model, prediction of abdominal skeletal muscle CSA improved to R^2=0.61 (r=0.78, p<0.001) (Table 5.1). A multivariate logistic regression to predict low muscularity from CT skeletal muscle area demonstrated that QMLT alone resulted in a concordance index of c=0.67 (p<0.002), and this improved to c=0.77 with the addition of the pre-selected covariates (p<0.001) (Table 5.2).

Table 5. 1: Ability of QMLT to predict CT skeletal muscle CSA by linear regression

Outcome	Predictors	R^2	Adjusted R^2	RMSE	p-value model†	p-value QMLT‡
CT muscle CSA (cm^2)	QMLT	0.20	0.19	39.1	<0.001	<0.001
CT muscle CSA (cm^2)	covariates	0.56	0.55	29.3	<0.001	NA
CT muscle CSA (cm^2)	covariates+QMLT	0.61	0.59	27.8	<0.001	<0.001

Covariates are: age (linear), sex (binary), BMI (linear), Charlson Comorbidity Index (linear) and admission type (binary). All regression models are based on 149 patients with complete QMLT within 72 hours of CT. Variance inflation factor for QMLT in model with all covariates is 1.2.†P-value of the overall model by the global F-test. ‡P-value of the QMLT variable. CSA, cross-sectional area; CT, computed tomography; NA, not applicable; QMLT, quadriceps muscle layer thickness; RMSE-root mean square error.

Table 5. 2: Ability of QMLT to predict low CT skeletal muscle CSA by logistic regression

Outcome	Low MM/n	Predictors	c	p-value model†	p-value QMLT‡
Low muscle CSA	86/149	QMLT	0.67	<0.002	<0.002
Low muscle CSA	86/149	covariates	0.72	<0.001	NA
Low muscle CSA	86/149	covariates+QMLT	0.77	<0.001	<0.005

Covariates are: age (linear), sex (binary), BMI (linear), Charlson Comorbidity Index (linear) and admission type (binary). All regression models are based on 149 patients with complete QMLT within 72 hours of CT. Low muscle CSA defined as <170 cm^2 for males and <110 cm^2 for females.[97] Variance inflation factor for QMLT in model with all covariates is 1.2. †P-value of the overall model by the global F-test. ‡P-value of the QMLT variable. CSA, cross-sectional area; CT, computed tomography; c, concordance index; NA, not applicable; QMLT, quadriceps muscle layer thickness; RMSE-root mean square error.

CONCLUSIONS

Using BMI classifications, 68% of the patients in this study were overweight or obese and only 2.7% were underweight; yet, 58% patients had lower than normal

muscularity based on CT imaging. While CT imaging has been an important measure in identifying patients with low muscularity, a more practical modality is needed to measure muscularity in the ICU. This study was the first to compare muscle quantifications of ultrasound with the precise CSA measures of CT analysis. Here, our results suggest that with our current protocol, QMLT alone may not accurately predict muscle CSA or identify patients with low muscularity. However, ultrasound based QMLT with additional predictors including age, BMI, sex, Charlson Comorbidity Index and admission type may be valuable in predicting low muscularity in this group of ICU patients, but may not be practical or useful in a clinical setting.

CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS

CONCLUSIONS

Malnutrition with subsequent muscle loss is devastating to the well-being and meaningful longevity of clinical populations. The ability to accurately and objectively assess muscle mass throughout treatment course and hospitalization has, however, eluded clinicians for centuries. It is imperative to create objective bedside methods that can longitudinally evaluate muscle quantity and quality in order to direct nutritional interventions and improve patient outcomes. Conjointly to muscle characterization, the application of sophisticated tracer methods are sorely needed to more accurately define protein and amino acid requirements needed for muscle maintenance.

Previous investigators have shown the promise of various imaging modalities in assessing muscle mass at the bedside including, bioimpedance analysis and ultrasound imaging. There is still, however, much that is unknown about the usefulness of these techniques at the individual patient level. Differences between technique method, equipment type, and measured outcomes continues to complicate progress in this area of study and will continue to do so until consensus can be established.

In order to mitigate lean tissue and muscle loss and to improve patient outcomes, many researchers have investigated the need for more advanced methods that can accurately determine protein needs during illness and disease. Whole body protein synthesis and breakdown are important endpoints that can be measured using new stable isotope tracer methodologies. Such physiologically relevant endpoints paint an objective picture of protein turnover during times of stress and quantification is needed to appropriately dose protein for improved patient outcomes. Investigators are currently showing the usefulness of tracer methodology, but more data is needed in order to refine

such methods for broader application across clinical populations in order to develop strong, evidence-based protein recommendations for all populations.

The primary objective of this dissertation was two-fold. First, it was to investigate various objective imaging modalities that can be used at the bedside of hospitalized patients to quantify loss of muscle in order to inform appropriate nutritional interventions. Specifically, raw bioimpedance parameters PA and IR showed an improved ability to predict low muscle as determined by CT compared to previous subjective methods and correlate with ICU length of stay. Also, it was determined that US measures of muscle thickness at the mid-thigh alone does not adequately predict low muscle as determined by CT. More research is needed with improved technologies and refined protocols supported by global consensus before US can be promoted as an adequate modality to measure longitudinal changes in muscle.

The second objective was to investigate new methods capable of measuring whole body protein synthesis and breakdown in order to propose new protein recommendations for clinical populations. This objective is currently under investigation in a population of stage III and IV HNC undergoing chemoradiation therapy. Further study details can be found in the "Future Directions" section of this dissertation.

In conclusion, the overall goal of this dissertation was to improve the way we provide nutrition within the hospital to better care for patients. In order to accomplish this goal, muscle imaging and assessment was used as a surrogate of nutritional status. If we can accurately monitor changes in muscle quantity and quality, we can then start to tailor nutritional intervention by way of optimal amino acid and protein dosing. This will

greatly improve functional status and quality of life, while decreasing mortality and healthcare costs. Further investigations into these areas remain of paramount importance.

FUTURE DIRECTIONS: CURRENT STUDY

Current Project Summary

Malnutrition and lean tissue loss in individuals with head and neck cancer (HNC) is a major problem that adversely impacts quality of life, functional status, survival, and health-related costs. Protein requirements in HNC, however, are not well defined. A better understanding of whole-body protein kinetics in individuals with HNC is essential to developing meaningful protein recommendations capable of preserving lean mass and preventing or slowing the progression of malnutrition. The ability to accurately identify the development of malnutrition and monitor changes in lean tissue at the bedside are essential to optimizing nutrition interventions throughout cancer treatment; however, there is currently a lack of valid bedside tools.

Novel Study Components

Use of Stable Isotope Tracers for Understanding Protein Turnover

The provision of adequate protein intake is essential to the preservation of lean mass, which is core to the maintenance of nutritional status. In the setting of cancer, multiple factors including inflammation and tumor-derived cytokines, drive an increased rate of protein breakdown and thus increase dietary protein needs.[15, 33,297] Furthermore, many of these factors lead to development of cachexia, which is associated with even greater protein breakdown, resulting in accelerated muscle loss and reduced anabolic capacity.[298,299] Protein requirements to promote lean tissue maintenance and anabolism in

cancer patients are not well-defined. Current protein recommendations for non-surgical oncology patients range from 1-2 gm/kg/day, but are based on limited evidence and are difficult to apply across various cancer populations.[13]

Emerging research supports the use of whole body protein kinetics as the best choice for defining protein requirements.[252,300] This has been demonstrated to be an effective method for determining anabolic threshold (i.e. protein intake level required to prevent net protein loss and maintain lean tissue mass) and anabolic capacity (i.e. the relationship between protein intake and net protein synthesis) in a number of different populations.[252, 266, 270,301,302]

Whole body protein kinetic studies utilize stable isotope labeled amino acid tracers given as a continuous intravenous infusion and/or enteral bolus feeds.[303,304] Infusion of stable isotopically labeled amino acids, termed "tracers", have the same chemical properties as their natural amino acids, termed "tracees."[302,303] Since atomic mass is the only differing component between tracers and tracees, they can be safely incorporated into the exchangeable body amino acid pool and measured with mass spectroscopy.[303,305] This relationship is expressed as the tracer-tracee-ratio (TTR).[302] Changes in TTR can provide objective information regarding protein synthesis and breakdown and allow for determination of anabolic capacity and anabolic threshold (i.e. the protein intake level where protein breakdown equals synthesis).[302,303 304] The most ideal amino acid tracer combination is an infusion of labeled PHE and TYR.[300,303] PHE is an essential amino acid that undergoes one of two fates in the body; it is either incorporated into protein (synthesis) or oxidized to tyrosine (degradation).[300] Therefore, whole-body protein turnover can be assessed by measuring changes in the TTR of PHE

when a constant infusion of isotopically labeled PHE is provided - as long as oxidation to TYR is measured.[303]

Ultrasound for the Longitudinal Assessment of Muscle Quantity and Quality

Recently, US has received much attention by clinician researchers for its potential application for bedside body composition assessment, due to its ability to quantify muscle thickness.[51,177] US shares many of the benefits of BIS over CT and DXA, including the noninvasive, portable nature of the measurements and ability to be used at the bedside.[11] US devices are widely available in inpatient and outpatient clinical settings, and thus, US is an attractive option as a lean tissue assessment method for clinicians.[51,306] The primary anatomical sites shown to have good capability to reflect whole body muscle mass are the quadriceps femoris and biceps brachii.[307–310] There is a growing body of validation literature of US compared to other body composition techniques[171, 174,311]; however, it is not clear how well US muscle thickness values can be extrapolated to whole body lean tissue estimates and technical errors limit the use of US for measuring longitudinal changes in lean mass. Furthermore, there is not yet a consensus on the optimal protocol to follow when measuring lean tissue, with regard to muscle compressibility, the patient's resting state and hydration status, and lean tissue adipose stranding (i.e. fatty infiltration of muscle tissue).[11,72] For these reasons, we will evaluate a newly developed force-measuring US device for measurement of muscle thickness compared to DXA-derived LST and FFM and to detect changes in muscle thickness after CRT in individuals with HNC.

Specific Aims, Hypotheses, and Study Objectives

Specific Aim 1: To determine the protein intake required to prevent net protein loss (anabolic threshold) and to evaluate the relationship between protein intake and net protein synthesis (anabolic capacity) of individuals with head and neck cancer, before and after chemoradiation therapy, using a novel stable isotope amino tracer multi-step feeding protocol.

Hypothesis 1: Individuals with HNC will have a higher anabolic threshold than has been observed previously in healthy older adults (~0.72 gm/kg FFM/day).[312]

Hypothesis 2: The anabolic threshold will be higher after CRT compared to pre-treatment.

Hypothesis 3: Anabolic capacity in individuals with HNC will be negatively associated with inflammation, as indicated by C-reactive protein and inflammatory cytokines.

Outcome Measures for Hypotheses 1 - 3:

- Whole body protein breakdown (WbPB) and synthesis (WbPS)
- Net protein synthesis (i.e. WbPS – WbPB)
- Anabolic threshold will be determined from linear regression of net protein synthesis and protein intake, will be used to identify the anabolic threshold.

Specific Aim 2: To examine several clinically-accessible tools, including force-measuring ultrasound (US) and bioimpedance for lean tissue assessment at the bedside in individuals with HNC.

Hypothesis 4: A novel force-measuring ultrasound device can be used as a surrogate marker of lean tissue and detect changes in lean tissue over time.

Hypothesis 5: Bioimpedance-derived phase angle, impedance ratio, NHLT, and FFM can be used as surrogate markers of lean tissue.

Outcome Measures for Hypothesis 4:

- Quadriceps femoris and biceps brachii muscle thickness measured by US
- Whole body and regional LST measured by DXA
- Changes in above measurements between Visit 1 and Visit 4

Outcome Measures for Hypothesis 5:

- Whole body NHLT by BIS
- Whole body FFM by BIS
- 50 kHz PA by MF-BIA
- 200/5 kHz IR by MF-BIA
- Whole body LST by DXA
- Whole body FFM by DXA
- Changes in whole body measurements as above between Visit 1 and Visit 4

Specific Aim 3: To evaluate the validity of bioimpedance to measure changes in fluid status compared to dilution techniques

Hypothesis 6: Bioimpedance analysis will accurately reflect changes in fluid status as measured by dilution methods with acceptable sensitivity (i.e. sensitivity will be > 90%).

Outcome Measures:

- Total body water
- Intra- and extra-cellular fluid

Specific Aim 4: To evaluate the nutrition-focused physical examination approach for assessing muscle loss

Hypothesis 7:

Presence of muscle loss detected by subjective nutrition-focused physical examination will not provide acceptable sensitivity (i.e. sensitivity will be < 90%[313]) to identify individuals with low muscle mass as defined by appendicular skeletal muscle index by DXA, according to published cutpoints.[11,76]

Outcome Measures:

- Appendicular skeletal muscle index (sum of arm and leg LST mass divided by height in meters squared)
- Subjective determination of muscle loss (multiple sites including clavicle and acromion bone region, temple region, scapular bone region, dorsal hand, calf and thigh)[36]

Study Procedures

In this observational study, 20 individuals with Stage 3-4 HNC will undergo testing at four time points:

- Visit 1: within 1 – 4 weeks prior to beginning CRT – after placement of a central venous catheter (CVC) for chemotherapy administration and gastric feeding tube
- Visit 2: ~3.5 weeks (±1 week) into treatment
- Visit 3: within 4 weeks (±2 weeks) post final CRT
- Visit 4: within 3 months (±1 month) post final CRT

Comprehensive testing including the **stable isotope multi-step feeding protocol** to establish the anabolic threshold will occur at Visit 1 and Visit 3 (see **Figure 6.1**). On these two testing days, participants will report to the CTSI Masonic Cancer Research Unit at the University of Minnesota at 0700 hours on study day following an overnight fast of 8-10 hours. Prior to initiating the stable isotope multi-step feeding protocol, participants will undergo height and weight measurements, a DXA scan, and will subsequently have necessary peripheral catheters placed. A catheter will be placed in a superficial dorsal vein of the hand or lower arm of the contralateral arm to the CVC port and serve as the site for arterialized-venous blood sampling using a heating pad as previously described.[314] The CVC port will serve as a catheter for a 10 ml venous blood draw (for subsequent biochemical analysis) before initiation of the stable isotope protocol, and will provide the route of continuous tracer infusion throughout the protocol. If the participant's CVC port is removed prior to visit 4, per discretion of physicians, a

second peripheral catheter will be placed into an antecubital vein in the opposite arm of the peripheral catheter used for blood draws.

A baseline arterialized-venous blood draw (5 ml) from the peripheral IV will occur immediately prior to the start of the continuous tracer infusion, followed by 16 incremental blood draws (5 ml each) throughout the duration of the tracer infusion period. The participant will be placed into a relaxed position based on individual preference and the stable isotope multi-step feeding protocol will begin. After 30 minutes in the relaxed position, the participant will undergo resting metabolic rate (RMR), bioimpedance, and US measurements. Two hours after initiation of the IV tracer infusion, the **enteral feeding** will begin and continue for six hours, in conjunction with the continuous IV tracer infusion, until completion of the protocol. During this period, dietary intake data, handgrip strength, PG-SGA, and psychosocial measures will be completed.

At Visit 2 and Visit 4, visits will be coordinated with outpatients at Masonic Cancer Clinic and Radiation Oncology Clinic. At these visits PG-SGA will be conducted, dietary intake data will be collected, and body weight, US, bioimpedance, and handgrip strength measurements will be taken.

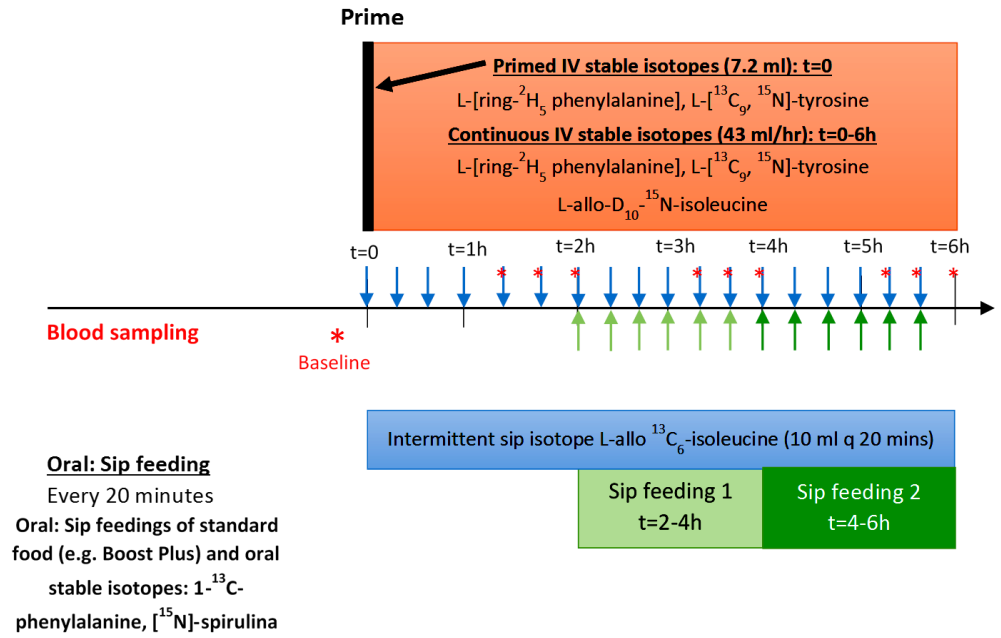

Figure 6. 1: Study Protocol

Stable Isotope Multi-Step Feeding Protocol

Following the baseline arterialized-venous blood draw, which will be used to correct for background enrichment, participants will receive a 7.2 ml IV priming infusion of L-[ring-$^{2}H_5$]-phenylalanine and L-[$^{13}C_9$,^{15}N]-tyrosine. Simultaneously, a priming dose of 10 – 20 mls of L-[$^{13}C_6$]-allo-isoleucine will be provided enterally via a gastric tube. Immediately following the priming doses, an IV continuous stable isotope infusion (containing L-[ring-$^{2}H_5$]-phenylalanine, L-[$^{13}C_9$,^{15}N]-tyrosine, and L-[D_{10}-^{15}N]-allo-isoleucine) and an oral continuous stable isotope infusion (containing L-[$^{13}C_6$]-allo-isoleucine) (Figure 1) will be initiated (T0) and continued over 6 hours to measure protein kinetics.

Incremental arterialized-venous blood draws will occur every 20 minutes during the second hour of each two-hour time block during the continuous tracer infusion.

The **enteral feeding** sip regimen will begin at hour 2 (t=2h) and will be provided in 20 minute increments until the end of the 6-hour study period. The feedings that occur every 20 minutes will be provided via syringe through the participant's gastric tube. If the participant does not have a gastric feeding tube, the participant will consume the enteral feedings orally throughout the study period. Each 2 hour volume will be divided by six and provided in 20 minute increments, consisting of a Boost nutritional supplement mixed with 120 mg of L-1-[^{13}C]-phenylalanine tracer. Protein intake levels will increase incrementally every 2 hours starting at hour 2, and until the end of the 6-hour infusion period. The volume of the prepared liquid meal will be adjusted accordingly to match required protein levels. The protein levels to be used in this study are based on the recently completed study by Dr. Deutz' team which indicated that the anabolic threshold in healthy subjects occurs at 0.03 gm/kg FFM/hr (representing approximately 0.72 gm/kg FFM/day).[312]

<u>The two protein levels will be</u>:

Feeding 1, 0.036 gm/kg FFM/hr (approximately 0.87 gm/kg FFM/day or 0.65 gm/kg/day total body weight);

Feeding 2, 0.091 gm/kg FFM/hr (approximately 2.17gm/kg FFM/day or 1.63 gm/kg/day total body weight).

The FFM value used to calculate protein intake will be obtained from the DXA scan that will occur shortly after arrival on the morning of the study day. Blood samples collected during the enteral feeding period will be taken immediately before the administration of each 20-minute incremental feeding.

Force-Measuring Ultrasound (US)

Although previous lean tissue assessment studies support the preliminary use of US to detect tissue changes, there is not yet a consensus on the optimal measurement protocol to follow when measuring lean tissue, particularly in individuals with acute and critical illness, and in individuals with edema and obesity. We will be testing a new US device which takes advantage of spatial and force-related variations in the tissue that we believe will remedy the current direction of standard US in the diagnosis of muscle loss, and thus, malnutrition. The muscle layer thickness (MLT) of the quadriceps femoris and biceps brachii muscles will be measured, using the same force of compression for all subsequent measurements. At each testing visit, subjects will have multiple ultrasound measurements taken on the arm and leg (2 on each leg; one at the midpoint between the patella and anterior superior iliac spine, and one at the 2/3rd point between the patella and anterior superior iliac spine and 1 on each arm; the anterior surface of the arm, 60% distal the acromion process and the lateral epicondyle of the humerus).[174]

This device will be used to accurately detect longitudinal changes in lean tissue thickness. Intra-rater variability will be evaluated by multiple measurements. Inter-rater variability of the device will be tested by having 2 different researchers independently measuring the same sites; researchers will be blinded from each others' evaluations and differences will be compared statistically after completion of all data collection. The procedure is painless and involves no risk to the patient. If the patient were to have a lower/upper extremity injury or pre-existing skeletal deformity to the quadriceps or biceps regions, those limbs would not be used for US examination. US measurements will be repeated at each study visit.

Significance

This project is innovative in that it addresses two critically important issues faced by clinicians caring for individuals with HNC, lack of evidenced based protein recommendations and lack of validated bedside tools for assessing lean tissue. Importantly, our study will yield insights that are easily transferable to other clinical populations susceptible to malnutrition. Specifically, our unique stable isotope multi-step feeding protocol will allow us to determine the anabolic threshold and capacity in our HNC population, and as such, can be considered a new diagnostic tool for determining protein requirements in HNC and other clinical populations. This innovative approach has never before been applied to a clinical population and has the potential to improve nutritional care by laying the groundwork to ultimately establish both the minimum (for lean tissue maintenance) and optimal (for lean tissue anabolism) protein requirements in this and other vulnerable populations. Data from the current project will be used to support the development of an NIH application to fund a larger, long-term feeding study, in order to clearly define the protein requirements necessary to maintain and/or replete lean tissue mass in a HNC population. Furthermore, building from the foundation laid by the current research project, future research efforts using stable isotope tracers will focus on determining protein requirements in other clinical populations. This line of research offers to provide a significant breakthrough in clinical nutrition that can benefit all clinical populations in the future.

This project also offers an exceptional opportunity to address the lack of clinically accessible tools for lean tissue assessment by evaluating two novel bedside technologies for their ability to measure cross-sectional and longitudinal changes throughout treatment

course in individuals with HNC. The innovative force-measuring US probe used in this study will remedy significant problems associated with standard US and provide the foundation for establishing standardized measurement protocols. Furthermore, the evaluation of bioimpedance for its ability to measure and monitor changes in lean tissue is also highly relevant and will advance our understanding of the optimal utility of this technology in the clinical setting. Both US and MF-BIA and BIS devices carry the potential to identify malnutrition and serve as effective lean tissue monitoring tools during nutritional and other interventions, thus helping clinicians to target lean tissue maintenance and/or repletion with resulting reductions in patient morbidity/mortality, hospital length of stay, readmissions, and healthcare costs. Ultimately, this study will meaningfully address several gaps in clinical care, through the establishment of a framework for future translational research aimed at determining protein needs in clinical populations and by providing data to support the implementation of improved bedside techniques to objectively assess lean tissue changes and further the work aimed at defining malnutrition in the clinical setting.[315]

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