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# ASSESSMENT OF ANALYTICAL METHODS USED TO MEASURE CHANGES IN BODY COMPOSITION IN THE ELDERLY AND RECOMMENDATIONS FOR THEIR USE IN PHASE II CLINICAL TRIALS

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**Abstract:** It is estimated that in the next 20 years, the amount of people greater than 65 years of age will rise from 40 to 70 million, and will account for 19% of the total population. Age-related decreases in muscle mass and function, known as sarcopenia, have been shown to be related to functional limitation, frailty and an increased risk of morbidity and mortality. Therefore, with an increasing elderly population, interventions that can improve muscle mass content and/or function are essential. However, analytical techniques used for measurement of muscle mass in young subjects may not be valid for use in the elderly. Therefore, the purpose of this review is to examine the applied specificity and accuracy of methods that are commonly used for measurement of muscle mass in aged subjects, and, to propose specific recommendations for the use of body composition measures in phase II clinical trials of function-promoting anabolic therapies.

**Key words:** Sarcopenia, bioelectrical impedance, dual-x-ray absorptiometry, computed tomography, magnetic resonance imaging.

## Introduction

The age-related decline in skeletal muscle mass and function are collectively referred to as sarcopenia (45). Sarcopenia is a risk factor for functional limitation (7, 43) and frailty (8), and added an excess cost to the US health care system of $18.5 billion, in the year 2000 (44). Elderly subjects within the age range 70-80 in the lowest quartile of muscle density were found to have a 51% higher risk of hospitalizations than those in the highest quartile (16), and may explain the sarcopenia-associated increased risk of morbidity and mortality (6). Low levels of skeletal muscle cross-sectional area, density and mass have been shown to be directly related to the strength deficits (61) and mobility limitation (92) characteristic of sarcopenia. Therefore, use of analytic methods with adequate validity, precision and accuracy are necessary to identify high-risk groups for age-related muscle mass loss, and, to monitor potential intervention efficacy. However, the changes in body composition that occur during the aging process have been shown to not occur uniformly. For example, decreases in total body water, bone and muscle mass (82), and an increase in body adiposity (25) have each been found during aging.

At the atomic level, body composition consists of 11 elements: oxygen, hydrogen, carbon, nitrogen, calcium, phosphorus, sulfur, sodium, chlorine, magnesium and potassium, that in total account for greater than 99.8% of body weight (81). Woodward et al. (1956) were among the first investigators to suggest the existence of a direct relationship between total body potassium, measured as $^{40}$K, and body cell mass. Body cell mass consists of muscle, organs, intracellular and extracellular water, and bone, and has been quantified via by multiplying [$^{40}$K] by 0.0083 (59). $^{40}$K content is used to calculate body cell mass because the intracellular potassium concentration is a constant value, at 140-160 meq per liter of cell water (15). Lean body mass (not including the fat and bone compartments) can then be calculated as the sum of body cell mass, extracellular fluid, and extracellular solids (22, 84), so measurements of $^{40}$K would be expected to closely predict muscle mass, assuming constant values for extracellular fluid and extracellular solids. Indeed, levels of $^{40}$K have been shown to be highly correlated (r = .98) with muscle mass content, with MRI used as the reference standard (94).

$^{40}$K content has been shown to decline with in subjects with an age range of 19-80 years (66). Cohn et al. (1983) found significant decreases in males (10%) and females (18%) for both $^{40}$K and fat-free mass in an older group (age range, 50-79 years) when compared with a younger group (20-49 years), data that suggests body cell mass declines at a similar rate as fat free mass during aging. Fat-free mass was calculated as the sum of total body water, total body protein and mineral, and was estimated from $^{3}$H dilution volume, total body nitrogen, and total body calcium, respectively. Based on these data, it can be concluded that measurement of $^{40}$K content during aging can be used to accurately monitor age-related changes in fat-free mass. However, use of this technique is expensive, requires extensive technical experience, does not provide for a rapid result, and is impractical because of background radiation-shielding issues. Other techniques capable of accurate, non-invasive, and rapid measurement of fat-free mass in elderly subjects are necessary.

Pace et al. (1945) found that fat-free mass can be approximated by measurement of total body water and use of the ratio, total body water/fat-free mass = 0.73. Although the total body water/fat-free mass ratio is regarded as a constant, excess fluid may accumulate in conditions such as aging or

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disease. Excess fluid may be reflected by a rise in extracellular water (extracellular fluid; Gutyon and Manning, 1980) or in total body water.

Whether total body water content changes with age has shown conflicting results. Virgili et al. (1992) found that hydration steadily decreases with age in men within the age range 70-100 years. However, multiple investigators have found that fat-free mass hydration does not change during aging (9, 20, 21, 32, 53, 74, 91). Mazariegos et al. (1994) compared values of fat-free mass hydration in a young (average age 30) and older (average age 74) group, and although absolute values for total body water and fat-free mass were reduced in the older group, the total body water/fat-free mass ratio was found to be similar in the young and old groups. Conversely, fat-free mass hydration has been shown to be higher in older subjects, when compared with the values obtained in their younger counterparts by both Hewitt et al. (1993) and Bergsma-Kadijk et al. (1996). Unfortunately, because of the potential for age-related fluctuations in total body water, analytical methods that rely on this technique for the prediction of fat-free mass (i.e. DXA and BIA) may be unreliable (17).

Hydrostatic weighing has been suggested (13) as the gold standard for 2-compartment (fat-free and fat-mass) models of body composition, primarily because the density of fat and fat-free mass (0.9 kg/L and 1.1 kg/L, respectively) are known, constant values. In addition, the relative amounts of the three major components of fat-free mass (aqueous, mineral, and protein) are known and constant in all individuals (13). However, changes in bone mineralization or fat-free mass hydration, as evidenced during aging, may confound hydrostatic weighing results (9, 57). Therefore, use of hydrostatic weighing is insufficient in terms of measuring age-related changes in bone mass, or, for measuring fat infiltration into skeletal muscle. Furthermore, the technical constraints inherent in performing the hydrostatic weighing measurement in older individuals makes this technique impractical. Other techniques are required for accurate measurement of these parameters.

Four main techniques are commonly used to measure skeletal muscle mass and/or quality: bioelectric impedance (BIA), dual energy X-ray absorptiometry (DXA), computed tomography (CT) and magnetic resonance imaging (MRI).

***Dual energy X-ray absorptiometry (DXA)***

The principle of using DXA for measurements of body composition is based on the notion that when a beam of X-rays is passed through a complex material, the beam is attenuated in proportion to the composition and thickness of the material. The DXA scanner emits two X-ray beams comprised of photons at two differing energy levels (40 keV and 70 keV), and as a result of the interaction within the human body, the incident X-ray photon energy is exponentially attenuated. By knowing how many photons are transmitted with respect to the number detected, the amount of bone mineral and soft tissue (fat and fat-free mass) can be determined. Skeletal muscle and adipose tissue contain primarily water and organic compounds and each restrict the flux of X-rays less than bone (55). Notable advantages of DXA include low cost, speed of measurement (whole-body scans require less than 20 min), exposure to low levels of radiation (<1 mrem), and the ability to perform fast serial-section measurements (Table 1). DXA has been reported to be the new gold standard for measurement of body composition (77; for review see ref. 3).

**Table 1**
Summary of Methodologies Used for Assessment of Human Body Composition

| Method | Principle | Reproducibility | Time of Measurement | Radiation (mrem) | Cost |
|---|---|---|---|---|---|
| DXA | Based on X-Ray attenuation of bone mineral-free tissue | 3% | 10 – 20 min. | 0.5 | High |
| BIA | Impedance-Electric current is maximally impeded by adipose rich tissue | 3% (62) | 1 min. | 0 | Low |
| CT | X-Ray exit transmission intensity | < 2% | 10 sec. | 200 | High |
| MRI | Magnetic Field emits radio frequency in proportion to different body composition compartments | 3% | 1-10 min. | 0 | Very High |

Table 1 adapted from ref. 68.

DXA-calculated lean-soft tissue (including skeletal muscle, skin, organs, and connective tissue) has been shown to closely approximate limb skeletal muscle mass when total body nitrogen (40) and CT (96) were used as the reference standards. The primary basis for this measurement is the uniform fat-free mass hydration value of 0.73, and, electrolyte constancy (67).

DXA-obtained skeletal muscle values have been validated for use in elderly subjects (age range 51-84), when measurements of total body $^{40}K$ (35, 96) and nitrogen (35) were used as the reference standard, and provides evidence that body cell and skeletal muscle mass decline at the same rate during aging.

However, as mentioned in the previous section, the possibility exists that during aging the body cell mass fraction disproportionately declines relative to total body water, thereby limiting the accuracy of DXA-based estimations of fat-free mass. If body cell mass, extracellular fluid or extracellular solids each are equally reduced in proportion to fat-free mass, then no changes in the fat-free mass normalized ratio would be expected. However, if body cell mass decreases at a greater rate than DXA-meausured fat-free mass, or, if extracellular fluid increases while body cell mass decreases, then these changes will have to be accounted for when making a prediction of fat-free mass content.

Several studies have demonstrated cross-sectional alterations in body cell mass during aging. Wang et al. (2004) found that body cell mass (measured as $^{40}K$) and lean-soft tissue mass (via DXA) were each reduced with greater age but the relative

reduction was greater for body cell mass. Thus, the amount of body cell mass relative to total lean-soft tissue mass is smaller in older adults, data that was verified by St-Onge et al. (2004). Gallagher et al. (1996) found that with increasing age (20-94 years) there is a reduction in the total body $^{40}K$/fat-free mass ratio (fat-free mass measured by DXA), data that suggests an age-related DXA-based overestimation of fat-free mass. Furthermore, the DXA measured fat-free mass change in > 75 year old subjects was found to be decreased by ~10% when compared to the corresponding value in an 18-34 year old group, as reported by Kyle et al. (2001). However, body cell mass was found to decreased by 25% in the older group, suggesting an altered composition of fat-free mass in older subjects. These studies suggest that the lean-soft tissue mass compartment has a smaller fraction of body cell mass and a larger fraction of extracellular fluid in old subjects when compared with their younger counterparts. Since DXA does not differentiate between water and bone free-lean issue, DXA-measured lean body mass may be overestimated in the elderly, who have been shown to have extracellular fluid accumulation (69).

The data are conflicting with respect to changes in extracellular fluid or extracellular solids during aging. Mazariegos et al. (1994) did not find an age-related (19-35 year old group compared with > 65 years) change in extracellular fluid content, but body cell mass, intracellular fluid and extracellular solids were each found to be decreased. When compared as ratios, an increase in both the extracellular fluid/intracellular fluid and extracellular fluid /fat-free mass ratio was found, but, body cell mass/fat-free mass was found to be decreased (57). The age-related decrease in the body cell mass/fat-free mass ratio suggests an altered fat-free mass composition-i.e. relative increases in connective tissue or structural protein. Conversely, Pierson et al. (1982) found an increased extracellular fluid/total body water ratio in subjects over age 80 years when compared with subjects younger than 30 years, suggesting an age-related expansion of the extracellular fluid compartment. Because intracellular volume is defined as total body water minus extracellular fluid, this data is indicative of an age-related decrease in intracellular fluid content (4).

Extracellular solids represents total bone mineral mass (93), with ~85% of extracellular solids being accounted for by bone wet weight (59). Cohn et al. (1983) did not find differences in the extracellular solids/fat-free mass ratio in old (50-79 years) when compared with younger subjects (20-49 years). However, Mazariegos et al. (1994) found a significant decrease in the extracellular solids/fat-free mass ratio in elderly women (> 65 years) when compared with their younger counterparts (19-35 years old).

It is important to note that the choice of either first (DXApencil) or second (DXAfan) generation DXA models may affect measurements of body composition. The principle underlying use of DXApencil (Hologic model QDR 2000) is that the X-ray photon beam is tightly collimated with the photon-emitting source and the detector, which then move together in a rectilinear manner to create an image. DXAfan (Hologic model QDR 4500), the second generation X-ray densitometer uses an X-ray source which fans out in the short axis plane of the patient-an array of detectors then performs measurements in the same plane. DXAfan has the advantage of increased resolution and scan speed over DXApencil. DXAfan has been shown to produce higher fat-free mass and lower fat-mass estimates when compared with DXApencil in younger subjects (26) and in the elderly (87).

***Bioelectric Impedance (BIA)***

The principle of using BIA for measurements of body composition is based on the notion that tissues rich in water and electrolytes are less resistant to the passage of an electrical current than lipid-rich adipose tissue. In theory, an individual with no adipose tissue would have minimum impedance, and impedance would increase to a maximum when all lean tissue was replaced by lipid-filled adipose tissue. The two main determinants of impedance, resistance and reactance, respond differently at any given frequency to intracellular and extracellular fluids. The reciprocal of the impedance is proportional to total body water for a current frequency ≥ 50 kHz or, to extracellular fluid, for frequencies below 5 kHz (54). Impedance values are then converted into values specific for total body water or extracellular fluid and then, into fat-free mass by means of equations that are population specific. Once fat-free mass is known, total body fat is calculated as the difference between body weight and fat-free mass.

However, BIA results have been shown to be confounded by fluid retention, as found in patients with COPD (75). Hydrostatic disturbances, peripheral oedema and the use of diuretic medication may affect the validity of BIA measurements in older age groups (34). Changes in the level of hydration have been attributed to the aging process, i.e., the reduction of fat-free mass and total body water that occurs with age (85). Unfortunately, BIA methods that have been validated for the prediction of fat-free mass in young individuals have been found to be inadequate when used in elderly populations (34). Visser et al. (1995) found that existing prediction equations for BIA described in the literature (23) that were based on young and middle-aged subjects overestimated lean mass and underestimated the percentage of body fat in an elderly (aged 60-87 years) population.

In contrast, several studies suggest that BIA-related measurement errors are not related to age, but to the specific population under investigation. Roubenoff et al. (1997) compared a published BIA equation designed to predict fat-free mass that had been derived in a young population (mean age 27), with equations that were developed for the elderly by using data from participants in the Framingham Heart Study (mean age 78 years). When the young-population equation was applied to subjects from the Framingham study it caused an overestimation of fat-free mass in heavier subjects that was eliminated by use of the age-specific equation. However, when

the two equations were tested in the New Mexico Aging Process Study (mean age 76 years), the published equation gave estimates of fat-free mass that were closer to the DXA-based reference than use of the Framingham equations. It was concluded from these data that the accuracy of a given BIA equation was not age-dependent, but, body-composition dependent. Roubenoff et al. (1997) suggested that use of BIA in elderly populations requires uniform validation procedures in the actual study population, rather than reliance on age-specific equations. In support of the notion that BIA related fat-free mass measurement errors are not age, but population-specific, Rech et al. (2008) found agreement for the fat-free mass measurement in elderly males (age range of 60-81) with use of the BIA equations of Kyle et al. (age range, 20-94; ref. 52), Dey et al. (75 year olds; ref. 25) and Sun et al. (age range 12-94; ref. 85). Among women the equations of Kyle et al. (2001) and Dey et al. (2003) were found to be valid when compared with Rech et al. (2008). In contrast, Genton et al. (2001) compared four equations specific for BIA analysis in elderly subjects and found only the equations derived by Kyle et al. (2001) to accurately predict fat-free mass in a group of subjects with an age range of 65-94 years. The BIA formulas developed by Deurenberg et al. (age range 60-83; ref. 24), Roubenoff et al. (average age 78; ref. 73) and Dey et al. (average age 75; ref. 25) were not found to be valid by Genton et al. (2001). From these data it can be concluded that BIA equations are subject to errors that cannot be determined a priori unless they are validated in the specific population in which they are to be applied (25, 65, 73, 83). Furthermore, the BIA equations of Segal et al. (1988) have been shown to be generalizable across sex, ethnicity, age, and degrees of adiposity, but these fatness-specific equations require an a priori determination of percentage body fat by using a skinfold equation or densitometry to categorize subjects into obese or nonobese groups. Use of BIA for predicting LBM is enhanced by sex and fatness-specific equations (76). Unfortunately, these procedures negate the use of BIA as a fast and simple method.

The previous sections have investigated using a molecular-based approach to measure body composition. Body composition can also be assessed at the tissue level with use of computed tomography (CT) and magnetic resonance imaging (MRI). Cadaver validation studies have shown excellent accuracy for CT and MRI in measuring skeletal muscle mass (both with r = 0.99) (58).

***Computed Tomography (CT)***

The principle of using CT for measurements of body composition is based on the use of a scanning (as produced from a rotating source) X-ray beam that passes through the patient. The X-ray exit transmission intensity is monitored by a series of detectors, which results in the visual production of cross-sectional slices about 10 mm thick. The exit transmission (at any angle) is then used to calculate the average attenuation coefficient along the length of the X-ray beam. Attenuation coefficients are reported in terms of Hounsfield units (HU), in which bone and other dense materials are equal to +1000, water is equal to zero, and air is equal to -1000 (47). Normal-density muscle is defined as having attenuation values in the 40–100 HU range (46). One of the major advantages of using CT over other analytical techniques used to measure body composition is the ability to measure fat infiltration into skeletal muscle. Adipose tissue area within a traced region of skeletal muscle can be estimated by selecting the pixels that range between -190 and -30 HU (80). Ross (2003) determined that normal-density muscle can be differentiated from low-density muscle by identification of attenuation values in the 0–30 HU range (72). CT has been shown to be able to identify fat infiltration into skeletal muscle in young obese women (78).

CT was first used to quantify arm skeletal muscle cross sectional area (39) and abdominal fat content (11) in 1979 and 1982, respectively. CT has been shown to be highly reliable in the evaluation of both adipose tissue (27) and fat-free mass (79). One advantage of CT use is the ability to discriminate total fat content into subcutaneous and visceral components (79).

CT measurements on individuals within the age range of 10-59 years (14) and 10-89 years (42) have shown increases in muscle density up to ~40 years of age, with a progressive decline following. CT has been shown to be capable of measuring not only changes in muscle mass, but muscle quality, during aging. An age-related decline in muscle quality was shown by Borkan et al. (1983), who reported the presence of fat infiltration within and between old muscle in subjects with a mean age of 69 years, when compared with a younger group (mean age, 46). CT was used by Rice et al. (1989) to identify a decrease in muscle mass (28-36%) and an 81% increase in non-muscle tissue in the plantar flexors of an elderly group, when compared with the corresponding values in young subjects. Furthermore, elderly arms were found to have a greater amount of skin plus subcutaneous tissue than in the young, but no significant difference was found in the legs (71). It is important to note that a 38% increase in skin plus subcutaneous fat was also found in elderly men, when compared with a younger group by Overend et al. (1992). The results of Rice et al. (1989) and Overend et al. (1992) further demonstrate that DXA and BIA-related measurements of muscle mass in the elderly may be overestimated, because of the increased amounts of skin and subcutaneous tissue found in elderly subjects. Use of CT has identified age-related decreases in quadricep (26%) and hamstring (18%) muscle mass and increases in non-muscle tissue located within both the quadriceps (59%) and hamstrings (127%), when comparing the values in an elderly group with younger counterparts (63).

***Magnetic Resonance Imaging (MRI)***

The principle underlying use of MRI involves a cylindrical magnet with an internal diameter large enough to enclose the human body, thereby allowing for the production of an external

magnetic field. The presence of gradient coils creates a smaller identification field, known as a gradient field. The presence of the external gravitational field in combination with the gradient field produces a net external magnetic field. The radio frequency coil generated by these magnetic fields provides the force necessary to rotate nuclear spin away from the direction of the external magnetic field. As the nuclear spins precess back toward the direction of the external magnetic field, they emit radio frequency signals (T1 and T2), which are combined to form an image. Variations in the radio frequency pulse sequence are then used to make determinations about adipose tissue or fat-free mass. For example, a short T1 and a long T2 proton relaxation time has been shown to be indicative of adipose tissue (47). Furthermore, use of MRI has been shown to identify the relative percentage of Type I skeletal muscle fibers in human vastus lateralis (41, 50, 51).

The relative accuracy of the MRI measurement is obtained by using multislice techniques. Typically, 10 or more slices are acquired at a time, covering an area of the body of 40 cm or more. Conventional indirect techniques such as underwater weighing, body water dilution, impedance, and anthropometry measure body fat content by using empirically determined relationships on the basis of population averaging. A critical difference between MRI and these techniques is that the MRI volume measurement is capable of absolute calibration. Thus, tissue distribution and tissue content are the only possible sources of MRI-based measurement errors, with the result of decreased individual variability, and, a higher statistical power for a given sample size (86). Furthermore, unlike DXA (0.5 mrem) and CT (200 mrem), MRI does not expose the patient to ionizing-radiation (68).

MRI was first used with the goal of discriminating fat from muscle tissues by Foster et al. (1984). Subcutaneous adipose tissue (37, 72), visceral fat (30) and total body fat (86) have each been quantified by MRI. MRI has been shown to accurately measure adipose tissue in vivo, showing good agreement with values produced by dissection and chemical analysis (1, 29). The error inherent in the MRI-based accuracy and precision measurements of abdominal adipose tissue mass was shown to be less than 3% when compared with data obtained by direct weighing of adipose tissue after dissection from human cadavers (2). Conversely, Thomas et al. (1998) showed significant variation when whole body MRI was used to determine the percentage of visceral, adipose and total internal fat tissue in subjects with a range of adiposity. Furthermore, the amount of total, subcutaneous, and visceral adipose tissue was found to not be related to standard anthropometric measurements such as skinfold measurements, body mass index, and waist-to-hip ratio.

Unfortunately, few studies have used MRI to examine age-related changes in skeletal muscle mass and/or quality. Gray et al. (2010) found a 37% decrease in quadricep muscle mass in an elderly group (age range, 76-82 years), when compared with their younger counterparts (age range, 19-30 years). Unlike BIA and DXA, MRI is capable of measuring the amount of non-contractile tissue found within skeletal muscle. Kent Braun et al. (2000) found a decrease in leg skeletal muscle cross sectional area in both women (11%) and men (19%) when comparing young (age range, 26-44 years) with an older group (age range, 65-83). Despite the age-related decrease in muscle cross sectional area, an increase of approximately 11% in non-contractile tissue within skeletal muscle was found for both elderly men and women, when compared with the amount of non-contractile tissue found within muscle in the younger group. Macaluso et al. (2002) found muscle contractile volume in both the quadriceps and hamstrings to be decreased by approximately 20%, when comparing an older group (mean age 70) with their younger counterparts (mean age 23). In addition, the amount of non-contractile tissue found within each of these muscle groups was significantly increased by approximately 6%.

### *Combined methods*

#### *BIA and DXA*

Several studies have used multiple analytical techniques in conjunction with the goal of measuring age-related changes in body composition. Augustemak de Lima et al. (2008) did not find differences in the skeletal muscle values obtained from both BIA and DXA in an elderly group (age range, 61-80 years). Conversely, when compared with DXA measurements, BIA has been shown to underestimate total fat content in a young obese group (60), leaving open the possibility that BIA would underestimate (relative to DXA) age-related changes in adiposity.

#### *BIA and MRI*

In subjects with a BMI range of 19 ≥ 40 kg/m$^2$ and an age range of 18-45 years, measurement of percent body fat by BIA was shown to be significantly correlated with MRI-based measurements (r = .93). The strongest association for MRI and BIA-based measurements of percent body fat were found at a BMI above 25 kg/m$^2$ (r = .84 or greater). Although the association between BIA and MRI-based measurements for the lean group (BMI, 19-24.9 kg/m$^2$) was statistically significant, the correlation was not as strong (r = .54) as for the higher BMI groups (86). Chien et al. (2008) compared measurements of skeletal muscle by both MRI and BIA across the age range of 22-90 years and found a significant correlation ($R^2$ = .95) between the two techniques. No significant difference was found for the BIA prediction equation, when compared with the MRI-based measurement of skeletal muscle mass, thereby verifying the potential use of BIA in older populations for the determination of skeletal muscle mass (19).

#### *DXA and MRI*

A high correlation (r = .97) between DXA-predicted appendicular lean soft tissue mass and MRI-measured total body skeletal muscle mass was reported for men and women, within the age range 18-92 by Kim et al. (2002). Similarly,

DXA-derived lean soft tissue mass was found to be significantly correlated with MRI-measured skeletal muscle mass for the whole body (r = 0.94) and leg region (r = 0.91) in an elderly group with an average age of 71 (18). However, DXA-derived fat mass has been shown to be lower than MRI-measured adipose tissue mass (18).

*DXA and CT*

Hansen et al. (2007) examined the ability of DXA to measure mid-thigh skeletal muscle mass in comparision with CT in an elderly group with an average age of 81 years, and found that DXA was able to predict CT-derived muscle cross sectional area with an error of ~12% of the mean CT-derived value. In addition, DXA was able to accurately predict the fat-to-lean ratio, as measured by CT. Based on these data, Hansen et al. (2007) concluded that assessment of sarcopenia by DXA is a potential low-radiation, accessible alternative to CT scanning of older patients. These data were confirmed by Visser et al. (1999), who found significant agreement for DXA (DXAfan), in comparison with CT, for measurement of calf ($R^2$ =.86), midthigh ($R^2$ = .94), total thigh ($R^2$ = .96) and total leg muscle mass ($R^2$ = .96) in an elderly group of subjects (age range, 70-79). However, DXA was found to overestimate muscle mass for each leg region (with the exception of calf muscle mass, in which no significant difference was found) by approximately 7-10%, when comparing DXA and CT derived values.

## Conclusions

The purpose of this review was to examine the analytic methods that are commonly used for measurement of muscle mass in aged subjects. We find that the technical constraints inherent in performing hydrostatic weighing in older individuals makes this technique impractical. Athough measurement of $^{40}K$ has been shown to be closely related to changes in body cell mass during aging, the technical cost, background radiation-shielding issues, and limited access to a suitable facility make this technique impractical for older populations, particularly in multi-center clinical trials. Measurement of total body water (i.e., BIA) for the purpose of predicting skeletal muscle mass has shown conflicting results during aging. DXA has been shown to be a reliable method for measurement of fat-free mass during aging. Unfortunately, DXA is unable to measure fat-infiltration into skeletal muscle. CT and MRI have been well documented to detect within-skeletal muscle changes in fat content or connective tissue. However, the high cost of operation for both CT and MRI can be a limiting factor for their use. Based on the existing literature, we propose that DXA in conjunction with either CT or MRI is a valid approach for measuring changes in both muscle mass and quality in older populations, or to determine the efficacy of selected agents in phase II clinical trials of function-promoting anabolic therapies.

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## References

1. Abate N, Burns D, Peshock RM, Garg A, and Grundy SM. Estimation of adipose tissue mass by magnetic resonance imaging: validation against dissection in human cadavers. J Lipid Res. 1994; 35: 1490–1496.
2. Abate N, Garg A. Heterogeneity in adipose tissue metabolism: causes, implications and management of regional adiposity. Prog Lipid Res 1995:34:53-70.
3. Albanese CV, Diessel E, Genant HK. Clinical applications of body composition measurements using DXA. J Clin Densitom 2003;6:75–85.
4. Armstrong LE, Kenefick RW, Castellani JW, Riebe D, Kavouras SA, Kuznicki JT, Maresh CM. Bioimpedance spectroscopy technique: intra-, extracellular, and total body water. Med Sci Sports Exerc. 1997;29:1657–1663.
5. Augustemak de Lima LR, Rech CR, Petroski EL. Use of bioelectrical impedance for the estimation of skeletal muscle mass in elderly men. Arch Latinoam Nutr. 2008;58(4):386-91.
6. Bales CW, Ritchie CS. Sarcopenia, weight loss, and nutritional frailty in the elderly. Ann Rev Nutr. 2002;22:309–323.
7. Basu R, Basu A, Nair KS. Muscle changes in aging. J Nutr Health Aging. 2002;6:336–341.
8. Bauer JM, Sieber CC. Sarcopenia and frailty: a clinician's controversial point of view. Exp Gerontol 2008;43:674–678.
9. Baumgartner RN, Heymsfield SB, Lichtman S., Wang J, and Pierson RN. Body composition in elderly people: effect of criterion estimates on predictive equations. Am J Clin. Nutr. 1991;53: 1345–1353.
10. Bergsma-Kadijk JA, Baumeister B, Deurenberg P. Measurement of body fat in young and elderly women: comparison between a four-compartment model and widely used reference methods. Br J Nutr 1996;75:649–57.
11. Borkan GA, Gerzof SG, Robbins AH, Hults DE, Silbert CK, Silbert JE. Assessment of abdominal fat content by computed tomography. Am J Clin Nutr 1982;36:172–177.
12. Borkan G, Hults D, GersofS, Robbins A, Silbert C. Age changes in body composition revealed by computed tomography. J Gerontol I 1983;38:673-677.
13. Brozek J, Grande F, Anderson J, and Keys A. Densitometric analysis of body composition: revision of some quantitative assumptions. Ann. NY Acad. Sci 1963;110:113–140.
14. Bulcke JA, Termote JL, Palmers Y, & Crolla D. Computed tomography of the human skeletal muscular system. Neuroradiology 1979;17:127-136.
15. Burmeister W. Potassium-40 Content as a basis for the calculation of body cell mass in man. Science 1965;148:1336-1337.
16. Cawthon PM, Fox KM, Gandra SR, Delmonico MJ, Chiou CF, Anthony MS, Sewall A, Goodpaster B, Satterfield S, Cummings SR, Harris TB; Health, Aging and Body Composition Study. Do muscle mass, muscle density, strength, and physical function similarly influence risk of hospitalization in older adults? J Am Geriatr Soc. 2009;57(8):1411-1419.
17. Chamney PW, Wabel P, Moissl UM, Müller MJ, Bosy-Westphal A, Korth O, Fuller NJ. A whole-body model to distinguish excess fluid from the hydration of major body tissues. Am J Clin Nutr 2007;85:80–89.
18. Chen Z, Wang Z, Lohman T, Heymsfield SB, Outwater E, Nicholas JS, Bassford T, LaCroix A, Sherrill D, Punyanitya M, Wu G, Going S. Dual-energy X-ray absorptiometry is a valid tool for assessing skeletal muscle mass in older women. J Nutr. 2007;137(12):2775-2780.
19. Chien MY, Huang TY, Wu YT. Prevalence of sarcopenia estimated using a bioelectrical impedance analysis prediction equation in community-dwelling elderly people in Taiwan. J Am Geriatr Soc. 2008;56(9):1710-1715.
20. Chumlea WC, Guo SS, Zeller CM, Reo NV, Siervogel RM. Total body water data for white adults 18 to 64 years of age: the Fels Longitudinal Study. Kidney Int. 1999;56:244–252.
21. Cohn SH, Vartsky D, Yasumura S. Compartmental body composition based on total-body nitrogen, potassium, and calcium. Am J Physiol 1980;239:E524–30.
22. Cohn SH, Vartsky D, Yasumura S, Vaswani AN, Ellis KJ. Indexes of body cell mass: Nitrogen versus potassium. Am J Physiol 1983;244:E305-E310.
23. Deurenberg P, van der Kooij K, Evers P, Hulshof T. Assessment of body composition by bioelectrical impedance in a population aged > 60 y. Am J Clin Nutr. 1990;51:3-6.
24. Deurenberg P, Westerterp KR; Velthuis-te Wierik EJM. Between-laboratory comparison of densitometry and bio-electrical impedance measurements. Br J Nutr. 1994;71:309-316.
25. Dey DK, Bosaeus I. Comparison of bioelectrical impedance prediction equations for fat-free mass in a population-based sample of 75 y olds: the Nora study. Nutrition. 2003;19(10):858-864.
26. Ellis KJ and Shypailo RJ. Bone mineral and body composition measurements: cross-calibration of pencil-beam and fan-beam dual-energy X-ray absorptiometers. J Bone

Miner Res 1998;13:1613-1618.
27. Ferland M, Després JP, Tremblay A, Pinault S, Nadeau A, Moorjani S, Lupien PJ, Thériault G, Bouchard C. Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. Br J Nutr 1989;61:139-148.
28. Foster MA, Hutchison JMS, Mallard JR, Fuller M. Nuclear magnetic resonance pulse sequence and discrimination of high- and low-fat tissues. Magn Res Imaging 1984;2:187–192.
29. Fowler PA, Fuller MF, Glasbey CA, Foster MA, Cameron GG, McNeill G, and Maughan RJ. Total and subcutaneous adipose tissue in women: the measurement of distribution and accurate prediction of quantity by using magnetic resonance imaging. Am J Clin Nutr. 1991;54:18–25.
30. Gallagher D, Visser M, Wang Z, Harris T, Pierson RN Jr, Heymsfield SB. Metabolically active component of fat-free body mass: influences of age, adiposity, and gender. Metabolism. 1996;45(8):992-997.
31. Genton L, Karsegard VL, Kyle UG, Hans DB, Michel JP, Pichard C. Comparison of four bioelectrical impedance analysis formulas in healthy elderly subjects. Gerontology. 2001;47(6):315-323.
32. Goran MI, Poehlman ET, Danforth E Jr, Nair KS. Comparison of body fat estimates derived from underwater weight and total body water. Int J Obes Relat Metab Disord 1994;18:622–626.
33. Gray C, Macgillivray TJ, Eeley C, Stephens NA, Beggs I, Fearon KC, Greig CA. Magnetic resonance imaging with k-means clustering objectively measures whole muscle volume compartments in sarcopenia/cancer cachexia. Clin Nutr. 2010 Aug 18. [Epub ahead of print]
34. Haapala I, Hirvonen A, Niskanen L, Uusitupa M, Kröger H, Alhava E, Nissinen A. Anthropometry, bioelectrical impedance and dual-energy X-ray absorptiometry in the assessment of body composition in elderly Finnish women. Clin Physiol Funct Imaging. 2002;22(6):383-391.
35. Hansen RD, Raja C, Aslani A, Smith RC, Allen BJ. Determination of skeletal muscle mass and fat-free mass by nuclear and dual-energy X-ray absorptiometry methods in men and women aged 51 to 84. Am J Clin Nutr 1999;70:228-233.
36. Hansen RD, Williamson DA, Finnegan TP, Lloyd BD, Grady JN, Diamond TH, Smith EU, Stavrinos TM, Thompson MW, Gwinn TH, Allen BJ, Smerdely PI, Diwan AD, Singh NA, Singh MA. Estimation of thigh muscle cross-sectional area by dual-energy X-ray absorptiometry in frail elderly patients. Am J Clin Nutr. 2007;86(4):952-958.
37. Hayes PA, Sowood PS, Belyanin A, Smith FW. Subcutaneous fat thickness measured by magnetic resonance imaging, ultrasound and calipers. Med Sci Sports Exercise 1998;20:303–309.
38. Hewitt MJ, Going SB, Williams DP, Lohman TG. Hydration of the fat free body mass in children and adults: implications for body composition assessment. Am J Physiol 1993;265:E88–95.
39. Heymsfield SB, Olafson RP, Kutner MN, Nixon DW. A radiographic method of quantifying protein-calorie undernourishment. Am J Clin Nutr 1979;32:693–702.
40. Heymsfield SB, Smith R, Aulet M, Bensen B, Lichtman S, Wang J, Pierson RN Jr.Appendicular skeletal muscle mass: measurement by dual-photon absorptiometry. Am J Clin Nutr 1990;52:214 -218.
41. Houmard JA, Smith R, Jendrasiak GL. Relationship between MRI relaxation time and muscle fiber composition. J Appl Physiol 1995;78(3):807-809.
42. Imamura K, Ashida H, Ishikawa T, Fujii M. Human major psoas muscle and sacrospinalis muscle in relation to age: a study by computed tomography J Gerontol. 1983;38(6):678-681.
43. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. J Am Geriatr Soc. 2002;50:889–896.
44. Janssen I, Shepard DS, Katzmarzyk PT, Roubenoff R. The healthcare costs of sarcopenia in the United States. J Am Geriatr Soc. 2004;52(1):80-85.
45. Kamel HK. Sarcopenia and aging. Nutr Rev. 2003;61:157–167.
46. Kelley DE, Slasky BS, and Janosky J. Skeletal muscle density: effects of obesity and non-insulin-dependent diabetes mellitus. Am J Clin. Nutr. 1991;54:509–515.
47. Kelsey CA (1993). Introduction. In: Juhl JH, Crummy AB, eds. Paul and Juhl's essentials of radiologic imaging, 6th ed. Philadelphia: JB Lippincott Company, pp l-18.
48. Kent Braun JA, Ng AV, Young K. Skeletal muscle contractile and non contractile components in young and older women and men. J Appl Physiol 2000;88:662e8.
49. Kim J, Wang Z, Heymsfield SB, Baumgartner RN, Gallagher D. Total body skeletal muscle mass: estimation by a new dual-energy X-ray absorptiometry method. Am J Clin Nutr. 2002;76:378–383.
50. Kuno S, Katsuta S, Akisada M, Anno I, and Matsumoto K. Effect of strength training on the relationship between magnetic resonance relaxation time and muscle fiber composition. Eur J Appl Physiol Occup Physiol. 1990; 61:33-36.
51. Kuno S, Katsuta S, Inouye T, Anno I, Matsumoto K, and Akisada M. Relationship between MR relaxation time and muscle fiber composition. Radiology 1988;169:567-568.
52. Kyle UG, Genton L, Hans D, Karsegard L, Slosman DO, Pichard C. Age-related differences in fat-free mass, skeletal muscle, body cell mass and fat mass between 18 and 94 years. Eur J Clin Nutr. 2001;55(8):663-672.
53. Lesser GT, Markofky J. Body water compartments with human aging using fat-free mass as the reference standard. Am J Physiol. 1979;236:R215–220.
54. Lukaski HC. Methods for the assessment of human body composition: traditional and new. Am J Clin Nutr. 1987;46:537–556.
55. Lukaski HC. Soft tissue composition and bone mineral status: evaluation by dual-energy X-ray absorptiometry. J Nutr. 1993 Feb;123(2 Suppl):438-443.
56. Macaluso A, Nimmo M, Foster J, Cockburn M, McMillan N, De Vito G. Contractile muscle volume and agonist-antagonist co activation account for differences in torque between young and older women. Muscle and Nerve 2002;25:858e63.
57. Mazariegos M, Wang ZM, Gallagher D, Baumgartner RN, Allison DB, Wang J, Pierson RN Jr, Heymsfield SB. Differences between young and old females in the five levels of body composition and their relevance to the two-compartment chemical model. J Gerontol 1994;49:M201-M208.
58. Mitsiopoulos N, Baumgartner RN, Heymsfield SB, Lyons W, Gallagher D, Ross R. Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. J Appl Physiol. 1998;85:115–122.
59. Moore FD & Boyden CM. Body cell mass and limits of hydration of the fat-free body: their relation to estimated skeletal weight. Ann NY Acad Sci. 1963;110:62-71.
60. Neovius M, Hemmingsson E, Freyschuss B, Udden J. Bioelectrical impedance underestimates total and truncal fatness in abdominally obese women. Obesity (Silver Spring) 2006;14:1731–1738.
61. Newman AB, Haggerty CL, Goodpaster B, Harris T, Kritchevsky S, Nevitt M, Miles TP, Visser M; Health Aging And Body Composition Research Group. Strength and muscle quality in a well functioning cohort of older adults: The Health, Aging and Body Composition study. J Am Geriatr Soc 2003;51:323–330.
62. Olde Rikkert M, Deurenberg P, Jansen R, van't Hof , Hoefnagels W. Validation of multifrequency bioelectrical impedance analysis in monitoring fluid balance in healthy elderly subjects. J Gerontol Med Sci. 1997;52A:M137–M141.
63. Overend TJ, Cunningham DA, Paterson DH, Lefcoe MS. Thigh composition in young and elderly men determined by computed tomography. Clin Physiol. 1992 Nov;12(6):629-640.
64. Pace N, Rathbun EN. The body water and chemically combined nitrogen content in relation to fat content. J Biol Chem 1945;158:685–691.
65. Pichard C, Kyle U, Gremion G, Gerbase M, Slosman D. Body composition by X-ray absorptiometry and bioelectrical impedance in elite female runners. Med Sci Sports Exerc 1997;29:1527-1534.
66. Pierson RN Jr, Wang J, Colt EW, Neumann P. Body composition measurements in normal man: The potassium, sodium, sulphate and tritium spaces in 58 adults. J Chronic Dis 1982;35:419-428.
67. Pietrobelli A, Formica C, Wang ZM, Heymsfield SB. Dual-energy X-ray absorptiometry body composition model: review of physical concepts. Am J Physiol. 1996;271:E941–951.
68. Plourde G. The role of radiologic methods in assessing body composition and related metabolic parameters. Nutr Rev. 1997;55(8):289-296.
69. Proctor DN, O'Brien PC, Atkinson EJ, Nair KS. Comparison of techniques to estimate total body skeletal muscle mass in people of different age groups. Am J Physiol. 1999;277(3 Pt 1):E489-E495.
70. Rech CR, Cordeiro BA, Petroski EL, Vasconcelos FA. Validation of bioelectrical impedance for the prediction of fat-free mass in Brazilian elderly subjects. Arq Bras Endocrinol Metabol. 2008 Oct;52(7):1163-1171.
71. Rice C, Cunningham D. Paterson D, Lefcoe M. Arm and leg composition determined by computed tomography in young and elderly men. Clin Physiol 1989;9:207-220.
72. Ross R. Advances in the application of imaging methods in applied and clinical physiology. Acta Diabetol. 2003;40 Suppl 1:45–50.
73. Roubenoff R, Baumgartner RN, Harris TB, Dallal GE, Hannan MT, Economos CD, Stauber PM, Wilson PW, Kiel DP. Application of bioelectrical impedance analysis to elderly populations. J Gerontol. 1997; 52:M129–M136.
74. Schoeller DA. Changes in total body water with age. Am J Clin Nutr 1989;50(suppl):1176–1181.
75. Schols AM, Wouters EF, Soeters PB, Westerterp KR. Body composition by bioelectrical-impedance analysis compared with deuterium dilution and skinfold anthropometry in patients with chronic obstructive pulmonary disease. Am J Clin Nutr 1991;53:421–424.
76. Segal KR, Van Loan M, Fitzgerald PI, Hodgdon JA, Van Itallie TB. Lean body mass estimation by bioelectrical impedance analysis: a four-site cross-validation study. Am J Clin Nutr. 1988;47(1):7-14.
77. Shaw KA, Srikanth VK, Fryer JL, Blizzard L, Dwyer T, Venn AJ. Dual energy X-ray absorptiometry body composition and aging in a population-based older cohort. Int J Obes (Lond) 2007;31:279–284.
78. Simoneau JA, Colberg SR, Leland Thaete F, Kelley DE. Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. FASEB J. 1995;9:273-278
79. Sjohstrom L. A computer-tomography used multicompartment body composition technique and anthropometric predictions of lean body mass, total and subcutaneous adipose tissue. Int J Obes Relat Metab Disord 1991;115:19-30
80. Sjohstrom L, Kvist H, Cederblad A, Tylen U. Determination of total adipose tissue and body fat in women by computed tomography, 40K, and tritium. Am J Physiol 1986;250:736-745.

81. Snyder WS, Cook MJ, Nasser ES, Karhausen LR, Howells GP, Tipton IH (1984). Report of the task group on reference man. Oxford, UK: Pergamon Press.
82. Steen B. Body composition and aging. Nutr Res. 1988;46(2):45-51.
83. Stolarczyk LM, Heyward VH, Van Loan MD, Hicks VL, Wilson WL, Reano LM. The fatness-specific bioelectrical impedance analysis equations of Segal et al:are they generalizable and practical? Am J Clin Nutr 1997;66(1):8-17.
84. St-Onge MP, Wang Z, Horlick M, Wang J, Heymsfield SB.Dual-energy X-ray absorptiometry lean soft tissue hydration: independent contributions of intra and extracellular water. Am J Physiol Endocrinol Metab. 2004;287(5):E842-847.
85. Sun SS, Chumlea WC, Heymsfield SB, Lukaski HC, Schoeller D, Friedl K, Kuczmarski RJ, Flegal KM, Johnson CL, Hubbard VS. Development of bioelectrical impedance analysis prediction equations for body composition with use of a multicomponent model for use in epidemiologic surveys. Am J Clin Nutr. 2003;77(2):331-40.
86. Thomas EL, Saeed N, Hajnal JV, Brynes A, Goldstone AP, Frost G, Bell JD.Magnetic resonance imaging of total body fat. J Appl Physiol. 1998;85:1778–1785.
87. Tylavsky F, Lohman T, Blunt BA, Schoeller DA, Fuerst T, Cauley JA, Nevitt MC, Visser M, Harris TB. QDR 4500A DXA overestimates fat-free mass compared with criterion methods. J Appl Physiol. 2003;94(3):959-965.
88. Virgili F, D'Amicis A, Ferro-Luzzi A. Body composition and body hydration in old age estimated by means of skinfold thickness and deuterium dilution. Ann Hum Biol 1992;19:57–66.
89. Visser M, Deurenberg P, van Staveren WA. Multi-frequency bioelectrical impedance for assessing total body water and extracellular water in elderly subjects. Eur J Clin Nutr. 1995;49: 256–266.
90. Visser M, Fuerst T, Lang T, Salamone L, Harris TB. Validity of fanbeam dual-energy X-ray absorptiometry for measuring fat-free mass and leg muscle mass. Health, Aging, and Body Composition Study–Dual-Energy X-ray Absorptiometry and Body Composition Working Group. J Appl Physiol. 1999;87:1513–1520.
91. Visser M, Gallagher D, Deurenberg P, Wang J, Pierson RN Jr, Heymsfield SB. Density of fat-free body mass: relationship with race, age, and level of body fatness. Am J Physiol. 1997;272:E781–787.
92. Visser M, Goodpaster BH, Kritchevsky SB, Newman AB, Nevitt M, Rubin SM, Simonsick EM, Harris TB. Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well functioning older persons. J Gerontol A Biol Sci Med Sci 2005;60A:324–333.
93. Wang J, Pierson RN Jr, Heymsfield SB: The five level model:A new approach to organizing body composition research. Am J Clin Nutr 1992;56:19-28.
94. Wang Z, Zhu S, Wang J, Pierson RN Jr, Heymsfield SB. Whole-body skeletal muscle mass: development and validation of total-body potassium prediction models. Am J Clin Nutr. 2003;77(1):76-82.
95. Wang Z, St-Onge MP, Lecumberri B, Pi-Sunyer FX, Heshka S, Wang J, Kotler DP, Gallagher D, Wielopolski L, Pierson RN Jr, Heymsfield SB Body cell mass: model development and validation at the cellular level of body composition. Am J Physiol Endocrinol Metab. 2004;286:E123–E128.
96. Wang ZM, Visser M, Ma R, Baumgartner RN, Kotler D, Gallagher D, Heymsfield SB. Skeletal muscle mass: evaluation of neutron activation and dual-energy X-ray absorptiometry methods. J Appl Physiol 1996;80(3):824-831.
97. Woodward KT, Trujillo TT, Schuch RL, Anderson EC. Correlation of total body potassium with body-water. Nature. 1956;178(4524):97-98.