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CT muscle density, D_3Cr muscle mass and body fat associations with physical performance, mobility outcomes and mortality risk in older men

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Abstract

Background. Muscle mass declines with age, while body adiposity increases. Sarcopenic obesity has been proposed to be particularly deleterious. However, previous methods for estimating muscle mass have been inadequate, and the relative contributions of total body fat vs. muscle fat to adverse outcomes have been unclear.

Methods. In a large cohort of older men (N= 1017), we measured muscle mass (D_3 creatine dilution), muscle density (high resolution peripheral computed tomography in the diaphyseal tibia) as a proxy of muscle fat, and total body fat (dual energy x-ray absorptiometry). We examined their associations with physical performance (walking speed, grip strength, chair stand time), the risk of mobility outcomes (mobility limitations, mobility disability), and the risk of death over ~5 years.

Results. In combined models, lower muscle mass and muscle density were independently associated with worse physical performance and the risk of adverse outcomes, while total body fat was minimally related to physical performance and not related to mobility outcomes or mortality. For example, the relative risks for mortality per 1 standardized unit increase in muscle density, muscle mass, and total body fat were 0.84 (95% CI: 0.74, 0.70), 0.70 (0.57, 0.86), and 0.90 (0.64, 1.25), respectively.

Conclusions. Muscle mass and muscle density were associated with physical performance and adverse outcomes, and had independent, additive effects. There was little additional contribution of total body fat.

Key words: sarcopenia, obesity, physical performance, outcomes

Introduction

Sarcopenia, or reductions in the amount of muscle and in muscle quality (strength and endurance per unit of muscle mass) appears to be a component of the age-related decrement in physical performance[1] and is an important contributor to the decrease in physical function that occurs with aging[2]. Sarcopenia has major adverse public health implications including injurious falls, fractures, disability, and mortality[3-5].

Total body fat mass increases with age. The combination of decreased muscle mass/function and increased body fat (sarcopenic obesity) has been proposed as a particularly strong risk factor for decreased physical performance, increased fall risk, functional decline, disability and mortality[6-8], and has been postulated to be a major health problem[9]. The underlying mechanisms that contribute to reduced physical performance and adverse outcomes in sarcopenic obesity are likely complex. One possibility is that obesity is associated with an accumulation of muscle fat. Higher intermuscular adipose tissue and intramyocellular lipid content, or myosteatosis, is reflected in lower muscle density by computed tomography [10]. Myosteatosis increases with age and is consistently associated with lower muscle performance, mobility limitation and mortality in older adults[11-15]. Yet, total body fat may not be an adequate reflection of muscle lipid and the degree to which whole body fat is linked to myosteatosis is unclear. Moreover, whether myosteatosis and increased total body fat are similarly related to adverse outcomes has not been adequately described.

Moreover, most previous evaluations of sarcopenic obesity have been problematic as they relied on lean mass measures [9], which are only a gross approximation of muscle mass[16-18]. We recently reported that when muscle mass is accurately measured using D_3 creatine dilution, the additional contribution of total body fat to performance and adverse outcomes is minimal[19]. In view of this apparently limited effect of total body fat on physical function in older people, it is important to assess muscle lipid content more directly. For both research and clinical purposes, it is important to establish an understanding of the relative associations of muscle mass and muscle lipid, and total

body fat, with muscle dysfunction and physical performance. That knowledge would both enable focused studies of causation as well as improve the ability to predict those at risk of poor outcomes.

In the current analysis, we examined the relationships between muscle density assessed by high resolution peripheral computed tomography (HR-pQCT) in the diaphyseal tibia, muscle mass (using D_3 creatine dilution), and total body fat by dual energy x-ray absorptiometry (DXA), and evaluated their independent associations with several measures of physical performance and adverse outcomes in the Osteoporotic Fractures in Men Study (MrOS), a longitudinal study of musculoskeletal health in older men.

Methods

MrOS cohort and study sample

The MrOS cohort of 5994 men ≥65 years was initially recruited in 2000-2002. The study design and recruitment approaches have been described[20,21](https://mrosonline.ucsf.edu). In 2014–2016, 2,786 surviving MrOS participants were contacted; 1,841 participated in a clinic visit, and 1,641 agreed to the D_3Cr dilution protocol. Of these, 187 were excluded for incorrect completion of the protocol. As described in Cawthon 2019 et al.[22], the exclusions were due to incorrect timing of the dose or urine collection (either less than 72 hours or more than 144 hours between the dose and collection) or forgetting to take the dose or provide the specimen. Six samples were lost by the clinical center or laboratory and 23 men were excluded because of outlying values for D3Cr muscle mass/body mass more than 2 SD from the mean, most of which included values that exceeded 100% of body mass. Further, 408 were missing the computed tomography variables. Of the remaining 1017, all but one man had data for one or more of the cross-sectional outcomes (grip strength, walking speed, chair stands). The data for the mobility outcomes was gathered on an interim visit questionnaire 2.2 ± 0.3 years later; 894 men returned these questionnaires. Men with prevalent

outcomes at the initial timepoint were not included in the analyses of incident outcomes, leaving N=714 in the incident mobility limitation analyses and N=832 in the incident mobility disability analyses. All 1,107 men were included in the analysis of mortality. These groups are summarized in eFigure 1.

Muscle density by HR-pQCT

HR-pQCT images were acquired spanning a 10.2 mm section (168 slices with a 60.7 µm slice thickness) of the non-dominant tibia (XtremeCT II, Scanco Medical AG, Brüttisellen, Switzerland) (PMID: 29750848). The scan volume was centered at a distance 30% of tibial length, proximal to the distal end of the tibia. Muscle density was measured using Soft Tissue Analysis (Scanco Medical AG) adapted for second-generation HR-pQCT images. Briefly, this software automatically segments the muscle volume of interest, excluding the skin, subcutaneous fat, bone, extraosseal calcifications, and large fat deposits outside the muscle compartments[23]. The mean greyscale value for the muscle volume of interest (i.e., muscle density) was calculated in units of bone mineral density (mg/cm^3). A standardized bone mineral density phantom was scanned previously to derive fixed linear greyscale-to-density calibration coefficients[24]. The phantom was scanned on a daily basis to monitor stability of the density calibration. eFigure 2 shows the anatomical site of the HR-pQCT diaphyseal tibial scans used for the muscle density analyses. The cross-sectional images illustrate the muscle compartments identified, where muscle volume and muscle density are measured.

D_3Cr dilution method to estimate muscle mass

As described previously[25], the method involves the subject ingesting a 30-mg dose of stable isotope (deuterated) labeled creatine (D_3Cr), and providing a fasting, morning urine sample 3–6 days later. Urine D_3-creatinine, unlabeled creatinine and creatine are measured using HPLC and MS/MS; these measures are then included in an algorithm to determine total body creatine pool size and thus skeletal muscle mass[25]. The D3Cr dilution method has been extensively validated and is correlated with MRI-derived muscle volume at r=0.87, p<.001 in humans[26]. In a previously published

study[27], repeat measurements were made on urine samples from participants with wide range of ages revealing a coefficient of variation of 3.6%. Moreover, very small longitudinal changes in D3Cr muscle mass can be detected in infants[28], further demonstrating the precision of the technique.

Dual-energy X-ray absorptiometry

Total body lean mass, appendicular lean mass, fat, and bone mineral content were assessed by whole-body DXA scans (Hologic 4500 scanners, Waltham, MA)[29].

Physical performance, incident mobility problems, and mortality

During the clinic visit, walking speed at usual pace was measured over a 6-m course using the average of two trials (m/s), time to complete five repeated chair stands was assessed, and grip strength was measured using a Jamar dynamometer (Sammons Preston Rolyan, Bolingbrook, IL, USA[30]. Grip strength was tested twice with each hand and the maximum of the 4 values was used in these analyses. During follow-up, MrOS participants or their surrogates were regularly contacted by postcard questionnaire and/or phone contact. On the questionnaire administered 2.2 ± 0.3 years after the Visit 4 visit, participants answered questions about difficulty walking 2–3 blocks, climbing 10 stairs, or carrying or lifting 10 lbs. We defined mobility limitation as any new self-reported difficulty walking 2–3 blocks or climbing 10 steps or carrying or lifting 10 lb; mobility disability was defined as inability to do any of these tasks. Except in 55 cases in which documentation was pending, deaths were confirmed with death certificates and additionally with discharge summaries when available. The follow-up time for the mortality outcome was 4.9 ± 1.4 years (range 2 days to 6.6 years).

Statistical analysis

Characteristics of the participants were compared across quartile of tibial muscle density using chi-square tests for homogeneity categorical variables, ANOVA for normally distributed continuous variables, and Kruskal Wallis tests for continuous variables with skewed distributions.

Pearson partial correlations between measures of D_3Cr muscle mass, total body fat and muscle density were calculated, adjusting for age, clinic site, height and weight. In all statistical models, the independent variables used were standardized [(value-mean)/standard deviation] to allow for comparisons of effect sizes. Linear regression was used to assess the relationship of the 3 predictors (D_3Cr muscle mass, muscle density and total body fat) and the outcomes of walking speed, grip strength, and the number of chair stands completed in 10 seconds, with results presented as beta coefficients and their 95% CIs. Negative binominal regression was used to assess the relationship of measures of D_3Cr muscle mass, total body fat and muscle density with the risk of incident mobility limitations, and incident mobility disability. Results are presented as relative risks (RRs) and their 95% confidence intervals (CIs) per 1 standardized unit increase. Proportional hazards models were used to assess the relationship of measures of D_3Cr muscle mass, total body fat and muscle density with the risk of all-cause mortality, with results shown as hazard ratios and their 95% CIs per 1 standardized unit increase.

A series of models were constructed for each outcome. All models were adjusted for age, clinic site, height and weight. The first set of models (Model 1) examined the association of each of the 3 predictors with the outcomes, with each predictor in a separate model. Model 2 had two of the predictors in each model; with muscle density paired with D_3Cr muscle mass. Model 3 had D_3Cr muscle mass, muscle density and total body fat in the same model. In additional analyses, muscle area was added to model 3 to assess its independent effects. Finally, model 3 was further adjusted for race, self-reported health status, alcohol use, smoking status, comorbidity index, and oral

corticosteroid use. Variance inflation factors (VIFs) were examined to verify that the multicollinearity of all variables in the model was acceptable. When all variables were included in the same model (model 3) the highest VIF was 8.77 for the variable weight. VIF values are shown in eTable 1.

Because muscle density is the most novel element of these analyses, participant characteristics are described in Table 1 by quartiles of muscle density. Potential interactions between pairs of independent variables were examined by including a term for their interaction plus the main effects (all as continuous variables) in models adjusted by age, clinic site, height and weight. Interactions examined were muscle density with total body fat, muscle density with D_3Cr muscle mass, and D_3Cr muscle mass with total body fat. These potential interactions were also examined graphically by categorizing the independent variables into quartiles and examining the percentage of each event within each quartile combination. The continuous outcomes of grip strength, walking speed and number of chair stands completed in 10 seconds were categorized (lowest quartile vs the other quartiles) for these plots.

All significance levels were two-sided, and all analyses were conducted using SAS version 9.4 (SAS Institute Inc, Cary, NC).

Results

Cohort characteristics

The characteristics of the men included in these analyses are shown in Table 1, by quartiles of muscle density. The men were older (average age 84.2±4 years, range 77-101) and most reported good or excellent health. Characteristics of the MrOS cohort at this clinic visit by quartiles of D_3Cr muscle mass have been published elsewhere[22]. In the analyzed cohort there was a high proportion of men who were overweight or obese; there were 68% with %fat >25 (a common threshold for obesity in men), and 32% had %fat ≥30%. Fifty-

three % had BMI ≥26 and 13% had BMI ≥30. Approximately 22% had some mobility limitation, but few had mobility disability. However, during follow-up an additional 24% and 10% reported mobility limitation or disability, respectively. For the analysis of mortality, 273 had died and 744 were still living.

At baseline, men with lower muscle density were slightly older and more often white, were less often in good/excellent health, had higher body mass index (BMI) and body fat, and had lower D_3Cr muscle mass (but not lower DXA appendicular lean mass)(Table 1). In addition, they had lower PASE scores, performed less well on tests of physical performance and more often had prevalent mobility limitation.

Relationships between tibial muscle density, D_3Cr muscle mass and total body fat

Muscle density was not strongly related to total body fat or D_3 muscle mass (Figure 1). The correlations (adjusted for age, clinic site, height, and weight) were r= 0.34 and r= -0.12, respectively. D_3Cr muscle mass and total body fat were moderately correlated (r= -0.49) ($p<0.05$ for all).

Associations of muscle density and mass measures with physical performance

When examined individually (adjusted for age, clinic site, height and weight), higher muscle density and higher D_3Cr muscle mass were both associated with better performance in all three measures of physical performance (walking speed, grip strength and chair stands), and the standardized beta coefficients appeared to be of similar magnitude (Table 2, model 1). Moreover, when included in a model together, the associations of D_3Cr muscle mass and muscle density with physical performance each remained significant (Table 2, model 2) (Figure 2). There were no interactions between muscle density and D_3Cr muscle mass on grip strength and walk speed (interaction terms $p>0.05$) so their effects appeared to be additive. The significant interaction term ($p= 0.04$) between muscle density and D_3Cr muscle mass in their associations with chair stands

suggests that the effects of low muscle density were most marked in those men with low D_3Cr muscle mass and vice versa.

Associations of muscle and fat measures with physical performance

While higher muscle density and D_3Cr muscle mass were strongly associated with better physical performance measures, higher fat mass was associated with lower performance in all tests (Table 2, model 1). However, when included in a model together with muscle density and D_3Cr muscle mass, the associations of total body fat with performance were reduced and remained significant only for grip strength (Table 2, model 3). When total body fat was included in bivariate models with either muscle density or D_3Cr muscle mass, its associations with physical performance measures were attenuated; D_3Cr muscle mass appeared to attenuate the associations of total body fat to a greater degree than did muscle density (eTable 2).

There was a significant interaction between muscle density and total body fat in their associations with grip strength (p for interaction= 0.04, eFigures 3-5); a borderline interaction for the chair stands measure (p for interaction=0.10) but no significant interaction for the walking speed assessment. There was the suggestion of an interaction between D_3Cr muscle mass and total body fat for the chair stands measure (p for interaction=0.07), but no significant interaction for the grip strength or walking speed (p for interaction, >0.10 for both).

Associations of muscle density and mass measures with incident mobility outcomes

In models adjusted for age, clinic site, height and weight, higher muscle density and D_3Cr muscle mass were significantly associated with lower risks of incident mobility limitation and mobility disability (Table 2). When considered together (Table 2, model 2) the relative risks of muscle density and D_3Cr muscle mass with incident mobility outcomes were similar to model 1, indicating they had independent effects. The joint effects of muscle density and D_3Cr muscle mass on mobility

limitation and mobility disability are shown in Figure 3. Interaction terms were not significant. The risk of incident mobility limitation in men with the lowest D_3Cr muscle mass and lowest muscle density was ~10 fold higher than those with the highest muscle density and D_3Cr muscle mass. Similarly, the risk of incident mobility disability in those with the lowest D_3Cr muscle mass and lowest muscle density was ~4 fold higher than those with the highest muscle density and D_3Cr muscle mass.

Associations of muscle and fat measures with incident mobility outcomes

When considered independently (adjusted for age, clinic site, height and weight), higher total body fat was associated with a higher risk of incident mobility limitation and had a similar but nonsignificant association with mobility disability (Table 2, model 1). However, when included in a model together with muscle density and D_3Cr muscle mass (Table 2, model 3), total body fat was not significantly associated with either outcome. When total body fat was included in bivariate models with D_3Cr muscle mass, its associations with adverse mobility outcomes were attenuated, while in bivariate models with muscle density the associations were similar to those in univariate models (eTable 2).

There was an interaction between total body fat and muscle density in their associations with mobility limitation (p for interaction= 0.005) (eFigure 6); the men with the highest total body fat were at higher risk of mobility limitation regardless of their muscle density, while in men with the lowest total body fat, low muscle density was strongly associated with greater risk of mobility limitation. Although there was a suggestion of a similar interaction between D_3Cr muscle mass and total body fat, the interactions between these measures and incident mobility disability were of borderline significance (p for interaction= 0.07) (eFigure 7).

Associations of muscle and fat measures with mortality

The risk of all-cause mortality was significantly lower in men with higher muscle density or D_3Cr muscle mass (HR 0.76 (0.67, 0.86) and 0.67 (0.56, 0.81), respectively) (Table 2, model 1). Men with greater total body fat were at higher risk, albeit not significantly so. When considered together (Table 2, model 2) the hazard ratios of muscle density and D_3Cr muscle mass with mortality were similar and both remained significant, indicating they had independent effects (Figure 4). When muscle density, D_3Cr muscle mass and total body fat were included together (Table 2, model 3), higher muscle density and D_3Cr muscle mass remained significantly associated with lower mortality, but total body fat was not a statistically significant risk factor for mortality.

Other covariables of interest

Muscle volume was a non-significant contributor when considered in models with muscle density and D_3Cr muscle mass (data not shown). When models were further adjusted for race, self-reported health status, alcohol use, smoking status, comorbidity index, and oral corticosteroid use the associations were essentially unchanged (data not shown).

Analyses limited to men with increased body fat

To examine the validity of our results in men with increased body fat, we performed sensitivity analyses limited to those with %body fat $\geq$26 (a common definition of obesity in men). The associations were very similar to those in the entire cohort (eTable 3)

Discussion

In a large, well characterized cohort of older men, we found that muscle density (primarily an index of muscle adiposity) and D_3Cr muscle mass were not well correlated, indicating they reflect distinctly different characteristics of muscle. Each had strong, independent associations with better walking speed, grip strength and chair stands. The effects of these two measures were at least additive, such that men with the lowest muscle density and lowest D_3Cr muscle mass were more likely to have low physical performance than those with higher levels of each. These measures of physical performance have been linked to important downstream outcomes, and indeed in our cohort those with either lower muscle density or D_3Cr muscle mass tended to be at greater risk of mobility limitation and mobility disability during follow-up; those with both low D_3Cr muscle mass and low density were at highest risk. Finally, we previously reported that D_3Cr muscle mass is associated with increased mortality[16], and here show that lower muscle density and lower D_3Cr muscle mass were each independently associated with an increased risk of all-cause mortality. When muscle density and D_3Cr muscle mass were considered, total body fat was not associated with the risk of adverse mobility outcomes or mortality. These findings highlight the importance of assessing both muscle mass and muscle adiposity for the understanding of critical health outcomes.

A novel element of the current analysis is the inclusion of both CT measures of muscle density and an accurate measure of muscle mass (D_3Cr). Importantly, our epidemiologic data strongly suggest that men with the same amount of functional muscle mass (D_3Cr muscle mass) can have widely varying amounts of muscle lipid (muscle density) (Figure 1) and vice versa, suggesting they reflect unique and different aspects of muscle. The D_3 creatine dilution method is thought to be an index of functional muscle mass, as creatine (and creatine phosphate) in the sarcomere are found adjacent to the Z-disc and the A-band[31] and are not associated with non-contractile components of muscle such as lipid and fibrosis. Muscle density is thought to be a surrogate

for the amount of lipid in muscle (myosteatosis), as muscle biopsy studies have demonstrated a moderate, negative correlation between lipid deposition and CT derived muscle density[10]. While muscle density is strongly related to muscle lipids, it has also been considered an overall a marker of muscle quality. As assessed by computed tomography, muscle density has been repeatedly associated with poor physical performance and adverse outcomes in older people[12-15], and the results of the current analyses support that conclusion. Although our muscle density assessment is specific to the tibial muscles, it is associated with several measures of physical function (including grip strength, walking speed and chair stands) indicating that muscle lipid content of the tibial muscles is likely an indicator of whole-body skeletal muscle quality. Our results show that these adverse associations are independent of D_3Cr muscle mass and implicate muscle density as a valuable measure of muscle health.

These analyses have implications for the consideration of sarcopenic obesity. In this large cohort of older men, many of whom were overweight or obese, the correlation of total body fat with muscle density was minimal, indicating total body fat is not a good indicator of muscle lipid content. Furthermore, we show that muscle density, primarily a reflection of *muscle* lipid content, was independently associated with important outcomes, while the effects of total body fat were much less prominent. Hence, our data suggest myosteatosis is more critical for the health outcomes we assessed than is total body fat. The combination of higher muscle fat and low D_3Cr muscle mass appear to be particularly disadvantageous. We have previously reported that muscle mass is accurately measured by D_3Cr dilution, and is strongly related to poor physical performance (e.g., slower walking speed, slower chair stands performance) and a variety of deleterious outcomes, including mortality, mobility limitation, and incident disability in activities of daily living (ADL) and instrumental ADLs[16,17,22,32]. In addition, we recently reported that after D_3Cr muscle mass is considered, total body fat is less strongly associated with these conditions[19], and the current analyses again support those findings. Overall, these results suggest that higher muscle lipid and low

muscle mass, rather than increased total body fat, are most important in determining physical performance, mobility-related related adverse outcomes, and mortality. Thus, the concept of "sarcopenic obesity" might be replaced by a more sophisticated and specific consideration of the condition of low muscle mass and low muscle density (sarcopenia with myosteatosis).

The measurement of body composition, and how those measurements are used to evaluate age related declines in physical performance, has been of considerable interest in both clinical and research settings. Fat mass can be conveniently and accurately measured by dual energy x-ray absorptiometry (DXA). Muscle density, a reflection at least in part of lipid content[10], can be measured by computed tomography; lower muscle density has been related to reduced physical performance and disability in aging, to hospitalizations and to greater fall and fracture risk[33-35]. The measurement of muscle mass has been more challenging. Lean mass by DXA has been commonly used as a surrogate for muscle mass, and previous studies of the relative contributions of muscle mass, quality and adiposity have uniformly utilized lean mass measures as a substitute for actual determinations of muscle mass. However, lean mass is not a measurement of muscle mass[18] and is only weakly associated with physical performance or important health outcomes (e.g. falls, fractures, physical disability). Hence, previous studies of sarcopenia, and its relationship to adiposity, that relied on lean mass measures by DXA may be misleading.

This study has important strengths. It utilizes accurate state-of-the art measurements of muscle mass using D_3Cr, muscle density using CT and DXA total body fat. We examined a relatively large cohort of community dwelling, older men, a group at risk of impaired physical performance and adverse health outcomes, in a longitudinal, observational study design. The cohort included a wide range of muscle measures and fat mass, allowing adequate testing of the hypotheses proposed. Study sites were experienced and assessment methods were standardized. A major limitation of our work is that that the participants were older men who were primarily Caucasian; our findings may not pertain to other groups (e.g. women) and should be examined in larger, more

diverse populations. Only a single measurement of body composition and physical function were performed (i.e. at baseline) to predict adverse outcomes, and they may have changed over time. Also, we did not address other measures that have been proposed to reflect muscle quality (e.g. mitochondrial function). We assessed muscle density and volume in the distal calf; there are few data concerning the consistency of these measures across anatomical regions (e.g. calf vs. thigh vs. trunk). Although CT muscle density is heavily influenced by lipid content, there are likely other constituents of muscle tissue (e.g. fibrosis) that may affect CT density results and that we cannot quantify without muscle tissue. While our cohort included a relatively high proportion of obesity (68% with %fat >25) and our sensitivity analyses indication that our results were the same in them as in the entire cohort, there were relatively few with severe obesity. The relationships we describe could be different in that group. Finally, we purposefully addressed the relationship of muscle density, muscle mass and total body fat in the context of physical performance, mobility problems and mortality, primarily because sarcopenia and reduced muscle quality are most robustly associated with these outcomes. We did not consider other issues (e.g. metabolic disturbances) that have been linked to sarcopenia and adiposity.

In summary, in a longitudinal study of a large cohort of older men we found that D_3Cr muscle mass and CT muscle density were both independently, and additively, associated with physical performance, the risk of mobility limitation and mobility disability, and the risk of mortality. Total body fat had inconsistent associations with physical performance and no independent effect on incident outcomes. These results emphasize the value of assessing both muscle mass and muscle density in the study of sarcopenia. They also suggest the concept of sarcopenic obesity should be reconsidered to include low D_3Cr muscle mass and muscle density (myosteatosis), rather than total body fat.

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Author contributions

Study concept and design: E.S.O., W.J.E. and P.M.C. Acquisition of subjects and/or data: E.S.O., S.R.C., J.A.C., W.R.H, and P.M.C. Analysis and interpretation of data: All the authors. Preparation of initial manuscript: E.S.O.

Conflict of Interest

None of the authors have relationships or activities that could appear to have influenced the submitted work. W.J.E. is listed as a coinventor on the granted patents for the D_3Cr dilution method. However, he does not derive any income or royalties or own the intellectual property for the method.

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Table and figure legends

Table 1. Characteristics [mean ± SD or N (%)] of the MrOS participants by quartile of tibial muscle density, mg/cm^3.

Table 2. Associations of muscle and fat measures with physical performance and incident outcomes. Beta coefficients, relative risks, or hazard ratios and their 95% confidence intervals, per 1 unit standardized increase.

Figure 1. The correlations of A) tibial muscle density and D_3Cr muscle mass, B) tibial muscle density and total body fat. Pearson correlations with age, clinic site, height and weight as the partial variables.

Figure 2. The percent of men with A) the lowest quartile of walking speed as a function of tibial muscle density and D_3Cr muscle mass, B) the lowest quartile of grip strength as a function of tibial muscle density and D_3Cr muscle mass, C) the lowest quartile of chair stands as a function of tibial muscle density and D_3Cr muscle mass.

Figure 3. A) the percent of men with incident mobility limitation as a function of tibial muscle density and D_3Cr muscle mass, B) the percent of men with incident mobility disability as a function of tibial muscle density and D_3Cr muscle mass.

Figure 4A. The percent of men who died during follow-up as a function of A) tibial muscle density and total body fat, B) D_3Cr muscle mass and total body fat, C) tibial muscle density and D_3Cr muscle mass.

Tables

Table 1. Characteristics [mean ± SD or N (%)] of the MrOS participants by quartile of tibial muscle density, mg/cm^3.

Characteristics	Quartile 1 <9.585 (N= 254)	Quartile 2 9.585 to <12.845 (N= 254)	Quartile 3 12.845 to <15.190 (N= 254)	Quartile 4 ≥15.190 (N= 255)	*p*-Value
Age, y	85.7 ± 4.4	84.7 ± 4.0	83.6 ± 3.9	82.6 ± 3.1	<.001
Race, nonwhite	15 (5.9)	20 (7.9)	40 (15.7)	37 (14.5)	<.001
Alcohol intake, drinks/wk					.610
<1 drink/wk	121 (47.6)	114 (45.1)	121 (47.6)	115 (45.5)	
1-13 drinks/wk	117 (46.1)	127 (50.2)	116 (45.7)	129 (51.0)	
14+ drinks/wk	16 (6.3)	12 (4.7)	17 (6.7)	9 (3.6)	
Smoking status					.252
Never smoker	90 (35.4)	98 (38.6)	105 (41.3)	101 (39.6)	
Past or current smoker	149 (58.7)	144 (56.7)	145 (57.1)	141 (55.3)	
Missing smoking data	15 (5.9)	12 (4.7)	4 (1.6)	13 (5.1)	
Number of comorbidities					.002
0	147 (57.9)	169 (66.5)	170 (66.9)	188 (73.7)	
1	72 (28.3)	57 (22.4)	65 (25.6)	55 (21.6)	
2+	35 (13.8)	28 (11.0)	19 (7.5)	12 (4.7)	
Oral corticosteroid use	5 (2.0)	9 (3.5)	8 (3.1)	5 (2.0)	.582
Physical activity score (PASE)	105.4 ±	110.7 ± 60.1	130.0 ± 65.0	131.9 ±	<.001

	67.0			56.7	
Walking speed, m/s	0.97 ± 0.23	1.07 ± 0.23	1.15 ± 0.21	1.18 ± 0.21	<.001
Number of chair stands in 10 s	3.0 ± 1.8	3.6 ± 1.6	4.2 ± 1.5	4.7 ± 1.5	<.001
Maximum grip strength, kg	33.0 ± 7.6	34.5 ± 7.8	36.6 ± 7.7	38.4 ± 7.2	<.001
Weight, kg	80.9 ± 12.7	79.1 ± 11.2	77.5 ± 11.5	75.3 ± 9.2	<.001
Height, cm	171.8 ± 6.6	171.6 ± 6.3	171.5 ± 6.6	171.9 ± 6.1	.894
Body mass index, kg/m^2	27.4 ± 4.0	26.8 ± 3.5	26.3 ± 3.4	25.5 ± 2.6	<.001
Excellent/good self-rated health	214 (84.3)	227 (89.4)	235 (92.5)	240 (94.5)	<.001
D_3Cr muscle mass/weight	0.28 ± 0.04	0.30 ± 0.04	0.32 ± 0.04	0.34 ± 0.04	<.001
D_3Cr muscle mass, kg	22.5 ± 3.7	23.6 ± 4.0	24.5 ± 3.9	25.3 ± 3.5	<.001
Total body fat, kg	23.5 ± 7.7	22.4 ± 6.6	21.1 ± 6.4	18.8 ± 5.5	<.001
Percent total body fat	28.9 ± 5.9	28.3 ± 5.7	27.3 ± 5.3	25.0 ± 5.3	<.001
Appendicular lean mass (kg)/height(m)^2	7.6 ± 0.9	7.5 ± 0.9	7.5 ± 0.8	7.5 ± 0.8	0.617
Tibial muscle density, mg/cm^3	5.8 ± 3.5	11.2 ± 0.9	14.0 ± 0.6	16.9 ± 1.3	<.001
Prevalent mobility limitation	105 (41.3)	58 (22.9)	38 (15.0)	18 (7.1)	<.001
Prevalent dismobility	46 (18.1)	21 (8.3)	12 (4.7)	2 (0.8)	<.001
Incident mobility limitation**	48 (37.8)	51 (29.1)	48 (24.4)	26 (12.1)	<.001
Incident dismobility**	31 (17.3)	29 (14.0)	14 (6.5)	9 (3.9)	<.001
Follow-up for mobility outcomes, y	2.2 ± 0.3	2.2 ± 0.3	2.2 ± 0.3	2.2 ± 0.3	.994
Mortality	101 (39.8)	69 (27.2)	60 (23.6)	43 (16.9)	<.001
Follow-up for mortality, y	4.5 ± 1.6	4.8 ± 1.4	5.0 ± 1.3	5.1 ± 1.2	<.001

p-Values for continuous variables are from ANOVA for normally distributed variables or from a Kruskal-Wallis test for skewed data.

p-Values for categorical data are from a chi-square test for homogeneity.

*Comorbidities include congestive heart failure, chronic obstructive pulmonary disease, diabetes mellitus, and myocardial infarction.

** Those with prevalent mobility disability were excluded from the incident mobility disability variable. Similar for mobility limitation.

***Falls within 1 year of follow-up.

Abbreviations: PASE=Physical Activity Scale for the Elderly

Table 2. Associations of muscle and fat measures with physical performance and incident outcomes. Beta coefficients, relative risks, or hazard ratios and their 95% confidence intervals, per 1 unit standardized increase.

	Model 1: Independent associations*			Model 2: Bivariate associations**		Model 3: Associations including three measures***		
	Tibial muscle density (mg/cm^3)	**D_3Cr muscle mass (kg)**	**Total body fat (kg)**	**Tibial muscle density (mg/cm^3)**	**D_3Cr muscle mass (kg)**	**Tibial muscle density (mg/cm^3)**	**D_3Cr muscle mass (kg)**	**Total body fat (kg)**
Physical Performance, Beta Coefficient (95% CI)								
Walking speed (m/sec)	0.06 (0.05, 0.08) †	0.08 (0.06, 0.09) †	-0.06 (-0.09, -0.03) †	0.05 (0.03, 0.06) †	0.06 (0.04, 0.07)†	0.05 (0.03, 0.06) †	0.06 (0.04, 0.08) †	0.01 (-0.026, 0.04)
Maximum grip strength (kg)	1.61 (1.17, 2.06) †	2.28 (1.77, 2.79) †	-3.22 (-4.15, -2.29) †	1.05 (0.59, 1.52) †	1.85 (1.31, 2.39) †	1.10 (0.63, 1.57) †	1.37 (0.75, 1.99) †	-1.73 (-2.77, -0.68) †
Chair stands (N/10 sec)	0.61 (0.50, 0.71) †	0.72 (0.60, 0.84) †	-0.76 (-0.98, -0.54) †	0.45 (0.34, 0.55) †	0.54 (0.42, 0.66) †	0.43 (0.33, 0.54) †	0.49 (0.35, 0.63) †	-0.23 (-0.46, 0.005)
Incident Outcomes, Relative Risk (95% CI)								
Mobility limitation	0.75 (0.64, 0.89) †	0.76 (0.62, 0.93) ‡	1.43 (1.01,	0.79 (0.66, 0.94) ‡	0.82 (0.66, 1.01)	0.78 (0.66, 0.93) ‡	0.86 (0.67, 1.11)	1.20 (0.80, 1.81)

			2.02) #					
Mobility disability	0.71 (0.57, 0.87) ‡	0.54 (0.40, 0.72) †	1.36 (0.83, 2.22)	0.80 (0.64, 1.01)	0.59 (0.43, 0.80) †	0.82 (0.65, 1.04)	0.53 (0.36, 0.78) ‡	0.76 (0.42, 1.34)
All-cause mortality, Hazard ratio (95% CI)	0.76 (0.68, 0.86) †	0.68 (0.58, 0.80) †	1.27 (0.95, 1.70)	0.82 (0.72, 0.93) ‡	0.74 (0.62, 0.88) †	0.84 (0.74, 0.96) ‡	0.70 (0.57, 0.86) †	0.90 (0.64, 1.25)

* Each association adjusted for age, clinic site, height, weight

** Adjusted for age, clinic site, height, weight and including tibial muscle density and D_3Cr muscle mass

** Adjusted for age, clinic site, height, weight and including D_3Cr muscle mass, tibial muscle density and total body fat measures

†P≤0.001, ‡p<0.01, #p<0.05

Figure 1

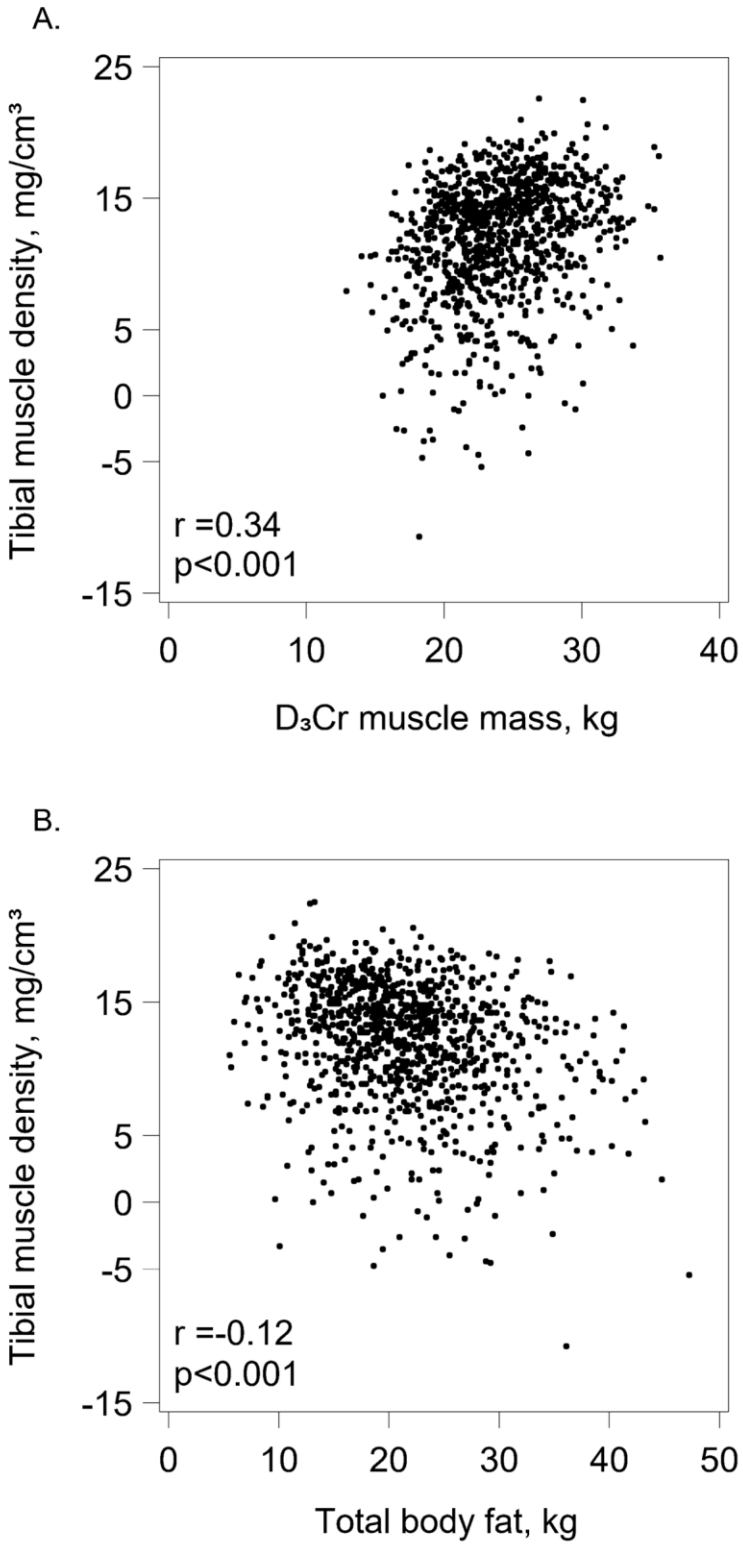

Figure 2

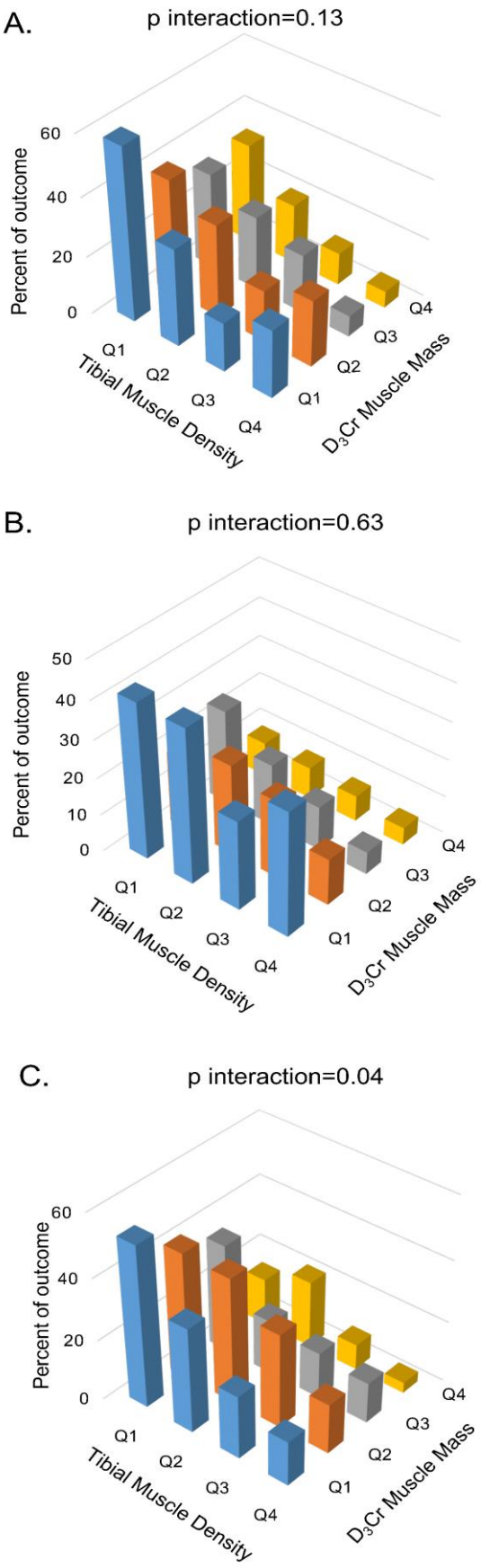

Figure 3

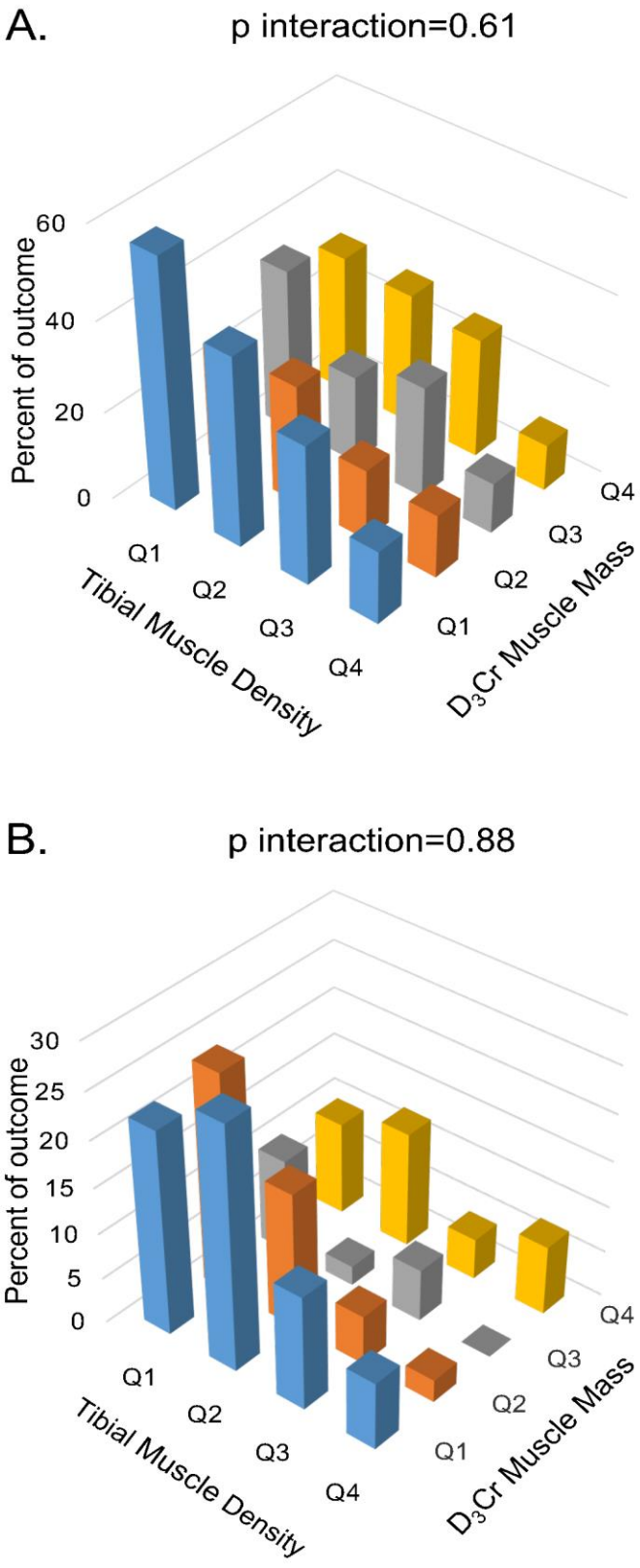

Figure 4