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Application of the D_3-creatine muscle mass assessment tool to a geriatric weight loss trial: A pilot study

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Abstract

Background Traditionally, weight loss (WL) trials utilize dual energy X-ray absorptiometry (DXA) to measure lean mass. This method assumes lean mass, as the sum of all non-bone and non-fat tissue, is a reasonable proxy for muscle mass. In contrast, the D_3-creatine (D_3Cr) dilution method directly measures whole body skeletal muscle mass, although this method has yet to be applied in the context of a geriatric WL trial. The purpose of this project was to (1) describe estimates of change and variability in D_3Cr muscle mass in older adults participating in an intentional WL intervention and (2) relate its change to other measures of body composition as well as muscle function and strength.

Methods The INVEST in Bone Health trial (NCT04076618), used as a scaffold for this ancillary pilot project, is a three-armed, 12-month randomized, controlled trial designed to determine the effects of resistance training or weighted vest use during intentional WL on a battery of musculoskeletal health outcomes among 150 older adults living with obesity. A convenience sample of 24 participants (n = 8/arm) are included in this analysis. At baseline and 6 months, participants were weighed, ingested a 30 mg D_3Cr tracer dose, provided a fasted urine sample 3–6 days post-dosage, underwent DXA (total body fat and lean masses, appendicular lean mass) and computed tomography (mid-thigh and trunk muscle/intermuscular fat areas) scans, and performed 400-m walk, stair climb, knee extensor strength, and grip strength tests.

Results Participants were older (68.0 ± 4.4 years), mostly White (75.0%), predominantly female (66.7%), and living with obesity (body mass index: 33.8 ± 2.7 kg/m^2). Six month total body WL was −10.3 (95% confidence interval, CI: −12.7, −7.9) kg. All DXA and computed tomography-derived body composition measures were significantly decreased from baseline, yet D_3Cr muscle mass did not change [+0.5 (95% CI: −2.0, 3.0) kg]. Of muscle function and strength measures, only grip strength significantly changed [+2.5 (95% CI: 1.0, 4.0) kg] from baseline.

Conclusions Among 24 older adults, significant WL with or without weighted vest use or resistance training over a 6-month period was associated with significant declines in all bioimaging metrics, while D_3Cr muscle mass and muscle function and strength were preserved. Treatment assignment for the trial remains blinded; therefore, full interpretation of these findings is limited. Future work in this area will assess change in D_3Cr muscle mass by parent trial treatment group assignment in all study participants.

Keywords Aging; Deuterated creatine (D_3Cr) dilution; Muscle mass; Sarcopenia; Weight loss

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Introduction

The number of older adults living with obesity has steadily increased over the past four decades, with recent estimates indicating over 40% of adults aged 60 and older currently live with the condition.[1] The negative impact of obesity on health, independence, and quality of life in older adults is well described[2]; as is the ability of lifestyle-based weight loss (WL) interventions to mitigate adiposity-related illness in this population.[3] That said, clinical WL recommendation remains controversial for older adults,[4,5] due (in part) to concomitant loss of fat free mass and its potential to accelerate age-related functional decline. Paradoxically, randomized controlled trial data often show improvements in strength, physical performance, and mobility among older adults following intentional WL.[6–8] These observations may result from changes in complementary aspects of physical function (including augmented neural[9] and mitochondrial activity,[10] as well as reduced fat infiltration[11]); however, they may also reflect the indirect and imprecise methods conventionally used to measure muscle.

Historically, WL trials have often relied on dual energy X-ray absorptiometry (DXA) to approximate muscle mass.[12,13] However, DXA does not directly measure muscle mass, but rather provides an estimate of whole-body lean mass—including connective tissue, skin, and organs, in addition to muscle mass.[14] In contrast, the D_3-creatine (D_3Cr) dilution method takes advantage of known principles of creatine biology to directly measure whole body skeletal muscle mass via stable isotope tracer technology.[15–17] Whole body D_3Cr muscle mass has been shown to closely align with validated methods of measuring muscle volume, such as whole body magnetic resonance imaging ($r = 0.87$; $P < 0.01$),[18] and importantly, measures a muscle parameter (i.e., the creatine pool), which is associated with skeletal muscle function.[19] Indeed, an emerging body of observational data show significant associations between D_3Cr muscle mass—but not DXA-acquired lean mass—and strength, physical performance, and mobility in older adults.[20–23]

A salient application of the D_3Cr dilution method is in the context of geriatric weight management, where better understanding of the degree of muscle loss with WL would help inform clinical guidelines,[24,25] while also offering a model of robust weight change in a relatively short period of time. The INVEST in Bone Health trial (NCT04076618)[26] provides an ideal parent study to examine and interpret WL-related changes in D_3Cr muscle mass, as all participants in this study are following a similar dietary prescription (i.e., a higher protein, nutritionally complete, hypocaloric meal plan, designed to elicit 8–10% WL loss over a 6-month period[6]). Additionally, this study is acquiring a battery of longitudinal muscle-related outcomes, including bioimaging metrics obtained with DXA and computed tomography (CT) along with clinically meaningful muscle function and strength outcomes (400-m walk, stair climb, knee extensor strength, and grip strength). Therefore, the purpose of this ancillary pilot project is to describe estimates of change and variability in D_3Cr muscle mass in older adults participating in an intentional WL intervention, and relate its change to other measures of body composition as well as muscle function and strength to inform future work in this area.

Methods

Overview of INVEST in bone health trial

The purpose of the Incorporating Nutrition, Vests, Education, and Strength Training (INVEST) in Bone Health Trial is to evaluate the effects of WL with or without weighted vest use or resistance training (RT) on factors of bone health and risk of fracture in 150 older adults who are living with obesity or overweight. The trial is registered on ClinicalTrials.gov (NCT04076618), was approved by the Wake Forest School of Medicine Institutional Review Board (IRB No. 00058279), and was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments. Prior to completing any data collection, all participants provided written informed consent and completed a HIPAA authorization form. Full methodologic details can be found in the published design paper.[26] Briefly, the INVEST in Bone Health trial utilizes a three-arm randomized design in which one arm receives a higher protein, nutritionally complete, hypocaloric meal plan[6] targeting 8–10% WL over a 12-month period (WL only; $n = 50$); a second arm receives the same dietary WL intervention and is also asked to wear a weighted vest 8 h/day with weight replacement in the vest titrated up to 10% of an individual's lost weight (WL + VEST; $n = 50$); and a third arm receives the same dietary WL intervention and is also asked to participate in a structured RT programme 3 days/week consisting of three sets of 10–12 repetitions for eight different exercises at 70–75% 1 repetition maximum (WL + RT; $n = 50$). A convenience sample of 24 participants ($n = 8$/arm) with complete baseline and 6-month D_3Cr, DXA, CT, 400-m walk, stair climb, and grip strength data ascertained by 12 May 2022 was included in this ancillary pilot study, with $n = 20$ also presenting with completed knee extension strength data. Details relevant to the collection of exposure, outcome, and covariate measures are described below.

D_3-creatine whole body muscle mass

As previously described,[27] participants were instructed to ingest a 30 mg oral dose of deuterated creatine and provide a fasted urine sample (but not the first morning void) 3 to 6 days post-dose. This dosing/sampling time course allows

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for isotopic steady-state of urinary D_3-creatinine (D_3Cr) enrichment to be achieved.[18] Urine samples were frozen at −70°C and stored locally at the Wake Forest School of Medicine Biogerontology specimen repository until shipment to UC Berkeley on dry ice for batched analysis. Using stored urine samples, D_3Cr enrichment, as well as concentrations of unlabelled creatine and creatinine, were measured via liquid chromatography mass spectroscopy in accordance with previously published methodology.[14] Once obtained, the amount of D_3Cr estimated to have been excreted (mg) and mean steady state D_3Cr enrichment ratio was input into a published algorithm to determine total body creatine pool size.[27] Whole body muscle mass was then estimated by dividing the creatine pool size by 4.3 g/kg (which represents the average concentration of creatine in whole wet muscle).

Weight and dual energy X-ray absorptiometry-acquired body composition metrics

Weight was measured to the nearest 0.1 kg using a calibrated and certified balance beam scale (Tanita #WB-3000PLUS). Total body fat mass, total body lean mass, and appendicular lean mass were obtained from whole-body DXA scans using a GE iDXA (Medical Systems, Madison WI). Coefficients of variation from repeated scans at our institution are 1.3% for total body fat mass and 0.9% for total body lean mass. When appropriate (i.e., based on participant body size), a mirroring technique was applied to estimate mass scanned outside of the measuring field.[28] Daily quality control scans were performed with the manufacturer provided calibration phantom, and all DXA scans were completed and assessed by trained and blinded technicians.

Computed tomography-acquired body composition metrics

Participant CT scans were acquired by trained and blinded technicians using a 64-slice PET/CT GE Discovery (Medical Systems, Madison WI) scanner. The CT scan field of view extended from top of the first lumbar vertebrae through three centimetres below the mid-shaft of both femurs, and two 2-D scouts were acquired to capture the entire femoral length. Using the microdicom browser, the anterior–posterior 2-D scout was visualized and the entire femoral length was measured as the distance between the superior aspect of the greater trochanter and the inferior aspect of the lateral condyle. The midpoint of this length was used to identify a single CT slice corresponding to the mid-thigh region. Muscle and intermuscular adipose tissue (IMAT) were semi-automatically segmented from the dominant leg to derive cross-sectional area from this single CT slice using Mimics software (v23, Materialize, Leuven, Belgium) with standard thresholds of −29 to 150 Hounsfield Units (HU) and −190 and −30 HU for muscle and IMAT, respectively. Additionally, a single CT slice was selected from the middle of the third lumbar vertebrae (L3; half of the vertebral body height), and the same thresholds were used to segment muscle and IMAT cross-sectional area of the trunk using automatic methods in the Data Analysis Facilitation Suite (DAFS v3.7.1.) by Voroni Health Analytics, Inc.[29] All thresholding operations for both the mid-thigh and trunk regions were followed by manual tracing to ensure accuracy and exclude visceral content, skin, and bone, as done previously.[30]

Muscle function and strength assessments

To assess muscle function, a fast 400-m walk test (consisting of 10 laps on a 40-m course (20-m down and back) was performed, as described previously.[31] To assess stair climb time, the fastest time a participant climbed 12 steps was recorded. Likewise, to assess muscular strength, grip strength dynamometry was measured in both hands using a Jamar hydraulic hand dynamometer (Performance Health, Warrenville, IL), with the mean value from the two trials (unless an excess of 4 kg was observed between trials, in which case a third trial was completed) in the dominant hand was included for analysis.[32] Finally, knee extensor strength (in peak torque; Nm) was assessed via isokinetic dynamometry (Biodex, Shirley, NY) in the dominant leg at 60 degrees per second.

Covariate assessments

Self-reported demographic information (i.e., age, sex, and race/ethnicity) were assessed at baseline. Standing height without shoes was measured to the nearest 0.1 cm using a stadiometer and body mass index (BMI) was calculated as dividing weight in kg by height in m^2. Finally, the Community Healthy Activities Model Program for Seniors[33] physical activity questionnaire was administered to assess levels of caloric expenditure per week.

Statistical analyses

Baseline characteristics were summarized using descriptive statistics and presented as means and standard deviations (mean ± SD) or counts and percentages [*n* (%)]. Baseline and 6-month body weight, D_3Cr muscle mass, DXA/CT-acquired body composition, and muscle function/strength measures are presented as mean ± SD, with unadjusted 6-month change values presented as means and 95% confidence intervals (95% CI) obtained from paired *t*-tests. Per cent change over 6 months is also presented as means and 95% CIs, obtained from one-sample *t*-tests. Partial Pearson correlations (*r*), adjusted for age and sex, were used to deter-

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mine associations between change in D_3Cr muscle mass and change in body weight, DXA/CT-acquired body composition, and muscle function/strength variables. Partial Pearson's correlations, stratified by sex, were also conducted to examine potential sex differences in the relationship between change in D_3Cr muscle mass and DXA-acquired body composition measures. Linear regression models were used to confirm the presence of any interaction, and scatterplots were further presented to aid in visualization of potential sex differences. Analyses were performed using SAS v. 9.4 (SAS Institute Inc. Cary, NC) and R software, with *P*-values of 0.05 used to determine statistical significance.

Results

Baseline sample characteristics

Baseline characteristics of the convenience sample (n = 24) of participants included in this ancillary pilot project are summarized in Table 1. Overall, average age of the study sample was 68.0 ± 4.4 years, and the majority of participants were non-Hispanic, Caucasian/White, women (67% female, 75% Caucasian, 96% non-Hispanic/Latino). BMI was 33.8 ± 2.7 kg/m^2, and self-reported physical activity expenditure was 2805 ± 2022 calories per week, indicative of a moderately active sample, living with class 1 obesity.

Six month changes in body weight, composition, and muscle function and strength measures

Baseline and 6-month unadjusted values of total body weight (mass), body composition, and muscle strength and function are presented in Table 2. Total body weight was significantly reduced in all participants by −10.3 (95% CI: −12.7, −7.9) kg, with the majority of lost weight coming from fat mass [−8.4 (95% CI: −10.5, −6.3) kg]. Likewise, DXA-acquired total body lean mass [−0.7 (95% CI: −1.2, −0.1) kg] and appendicular lean mass [−0.9 (95% CI): −1.4, −0.4) kg] decreased significantly (though modestly); however, whole body muscle mass, as measured via the D_3Cr dilution method, did not significantly change [+0.5 (95% CI: −2.0, 3.0) kg]. CT-acquired muscle and IMAT areas obtained from the mid-thigh [muscle: −6.8 (−9.4, −4.3) cm^2; IMAT: −1.9 (−3.4, −0.5) cm^2] and trunk [muscle: −6.1 (−8.5, −3.6) cm^2; IMAT: −1.2 (−2.1, −0.2) cm^2] declined significantly from baseline; and, of muscle function and strength measures, only grip strength significantly changed (notably, increased) from baseline [+2.5 (95% CI: 1.0, 4.0) kg].

Table 1 Baseline descriptive characteristics of study sample (n = 24)

	N (%) or mean ± SD
Age, years	68.0 ± 4.4
Female	16 (66.7)
Race	
Caucasian/White	18 (75.0)
African American/Black	6 (25.0)
Ethnicity	
Hispanic/Latino	1 (4.2)
Non-Hispanic/Latino	23 (95.8)
Body mass (kg)	95.2 ± 11.1
Body mass index (kg/m^2)	33.8 ± 2.7
Physical activity (caloric expenditure/week)	2805.3 ± 2022.1

Correlations between change in D3-creatine muscle mass and change in body weight, body composition, and muscle function and strength

Partial Pearson correlations (adjusted for age and sex) between change in D_3Cr muscle mass and change in body weight and DXA and CT composition measures are presented in Table 3. No significant associations were observed between changes in D_3Cr muscle mass and changes in total body weight or DXA-acquired lean body mass or total body fat mass; however, change in D_3Cr muscle mass was modestly and directly correlated with change in appendicular lean mass (r = 0.46; P = 0.03). These data are displayed graphically by sex in Figure 1, with stronger correlations observed in women than men for both change in D_3Cr muscle mass and change in total body lean mass (women r = 0.35; men r = −0.04) and change in D_3Cr muscle mass and change in appendicular lean mass (women r = 0.65; men r = 0.13). That said, the interaction term for sex was non-significant; however, few men were included in these analyses (n = 8). No significant correlations were observed between change in D_3Cr muscle mass and change in any CT-acquired body composition variable.

Table 4 presents partial Pearson correlations (adjusted for age and sex) between change in all body weight and composition metrics with change in muscle function and strength. Only one significant association was observed between change in body composition and change in muscle function, with change in CT-derived IMAT fat area for the trunk inversely associated with gait speed (r = −0.44; P = 0.04). No significant associations were observed between any D_3Cr-, DXA- or CT-acquired muscle metric and muscle function or strength.

Discussion

The primary objective of this ancillary pilot project was to derive preliminary D_3Cr muscle mass estimates of change and variability in an older, largely female sample, participating in

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Table 2 Baseline and 6-month unadjusted values of relevant body weight, composition and muscle function/strength variables

	Baseline (mean ± SD)	6-month (mean ± SD)	6-month Δ (95% CI)	6-month % Δ (95% CI)
Total body weight (kg)	95.2 ± 11.1	84.9 ± 10.6	−10.3 (−12.7, −7.9)*	−10.7 (−13.1, −8.3)*
D_3Cr muscle mass (kg)	25.7 ± 8.3	26.2 ± 7.2	+0.5 (−2.0, 3.0)	6.1 (−6.9, 19.1)
DXA-acquired body composition				
Total body fat mass (kg)	41.7 ± 5.6	33.4 ± 8.1	−8.4 (−10.5, −6.3)*	−20.7 (−26.2, −15.2)*
Lean body mass (kg)	49.0 ± 10.0	48.3 ± 10.7	−0.7 (−1.2, −0.1)*	−1.6 (−2.7, −0.5)*
Appendicular lean mass (kg)	23.3 ± 5.6	22.4 ± 5.5	−0.9 (−1.4, −0.4)*	−3.7 (−6.3, −1.1)*
CT-acquired body composition				
Mid-thigh muscle area (cm^2)	130.2 ± 29.9	123.4 ± 26.8	−6.8 (−9.4, −4.3)*	−4.9 (−6.6, −3.2)*
Mid-thigh intermuscular fat area (cm^2)	11.8 ± 5.0	9.9 ± 3.4	−1.9 (−3.4, −0.5)*	−11.7 (−20.9, −2.6)*
Trunk muscle area (cm^2)	130.6 ± 35.4	124.5 ± 31.9	−6.1 (−8.5, −3.6)*	−4.2 (−5.8, −2.6)*
trunk Intermuscular fat area (cm^2)	17.9 ± 5.9	16.7 ± 6.2	−1.2 (−2.1, −0.2)*	−7.2 (−12.8, −1.6)*
Muscle function and strength				
Gait speed (m/s)	1.3 ± 0.2	1.3 ± 0.2	−0.0 (−0.1, 0.0)	−1.1 (−4.6, 2.4)
Stair climb (s)	6.5 ± 2.2	6.4 ± 2.1	−0.1 (−0.5, 0.3)	−0.1 (−6.1, 6.0)
Knee extension strength (nm)	127.9 ± 40.2	125.4 ± 41.2	−2.5 (−6.4, 1.4)	−2.2 (−5.0, 0.6)
Grip strength (kg)	26.5 ± 7.2	29.0 ± 7.3	+2.5 (1.0, 4.0)*	11.2 (4.7, 17.7)*

*Statistical significance at $P < 0.05$.

Table 3 Adjusted correlations (for age and sex), between 6-month change in D_3Cr muscle mass and changes in body weight and composition

	Δ D_3Cr muscle mass (kg)	
Body weight and composition	*r*	*P*
Δ Total body weight (kg)	0.29	0.19
DXA-acquired body composition		
Δ Total body fat mass (kg)	0.27	0.22
Δ Lean body mass (kg)	0.15	0.50
Δ Appendicular lean mass (kg)	0.46	0.03*
CT-acquired body composition		
Δ Mid-thigh muscle area (cm^2)	0.16	0.46
Δ Mid-thigh intermuscular fat area (cm^2)	0.18	0.42
Δ Trunk muscle area (cm^2)	0.17	0.45
Δ Trunk intermuscular fat area (cm^2)	−0.14	0.55

*Denotes statistical significance $P < 0.05$.

an intentional WL intervention with or without weighted vest use or RT. Secondarily, we sought to explore associations between change in D_3Cr muscle mass with WL-related changes in more traditional sarcopenia metrics—including bioimaging (DXA/CT-acquired body composition) and functional (including mobility and strength) measures. Herein, we report that ~11% loss of total body weight was accompanied by a ~1.5% reduction in DXA-acquired total body lean mass (coming predominantly from the appendicular region) and a ~4–5% loss in muscle area at the mid-thigh and trunk. However, whole body muscle mass as measured by the D_3Cr dilution method was preserved, as were measures of muscle function and strength. Change in D_3Cr muscle mass was moderately correlated with changes in DXA-acquired appendicular lean mass (r = 0.46), a finding which may be more evident in women than men. Finally, we did not observe significant associations between change in muscle mass, lean body mass, or muscle area (D_3Cr, DXA, and CT, respectively) and muscle function or strength. Collectively, results support the notion that prior literature, which reports discrepant findings between change in lean mass or regional changes in muscle area and function, may be driven by lack of an accurate measurement of muscle mass itself.

The baseline amount of D_3Cr muscle mass reported in our sample (25.7 ± 8.3 kg) is similar to DXA appendicular lean mass (23.3 ± 5.6 kg)—half of what is estimated with DXA total body lean mass (49.0 ± 10.0 kg) and approximately one quarter of total body mass, similar to what has been previously reported among older adults.[20,23] It is notable that the point estimate of change in D_3Cr muscle mass we observed was positive [+0.5 (−2.0, 3.0) kg], which is in contrast to both total body lean mass and appendicular lean mass estimates that were of equal magnitude, but in a negative direction. Discordant intervention-related changes in DXA lean mass and D_3Cr muscle mass were likewise reported in a RCT examining the effects of a hypocaloric diet and testosterone supplementation in young men.[34] Although stable or increasing muscle mass in older adults experiencing >10%

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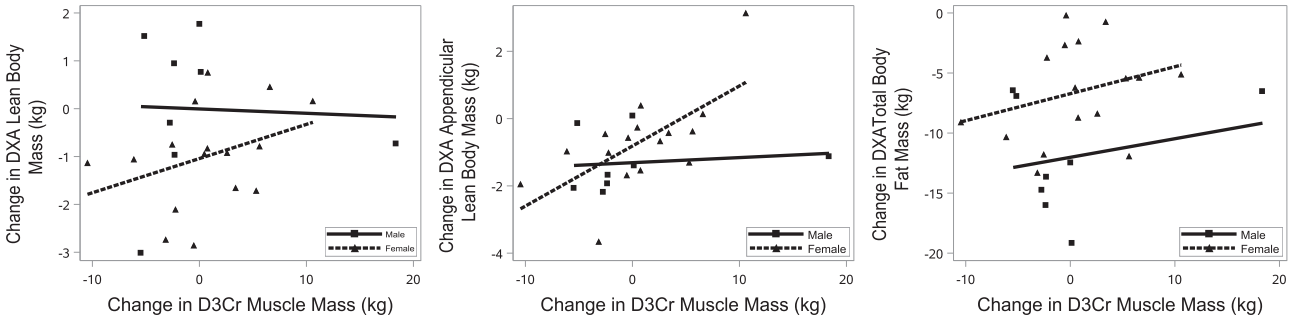

Figure 1 Scatterplots of 6-month change in D_3Cr muscle mass and change DXA-acquired measures of body composition by sex.

Table 4 Adjusted correlations (for age and sex), between 6-month change in D_3Cr muscle mass and body weight and composition measures with change in muscle function and strength

	Δ Gait speed (m/s)		Δ Stair climb (s)		Δ Knee extension strength (nm)		Δ Grip strength (kg)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Δ Total body mass (kg)	−0.18	0.43	−0.06	0.78	0.46	0.05	−0.01	0.96
Δ D_3Cr muscle mass (kg)	0.15	0.50	−0.25	0.26	0.35	0.16	0.19	0.39
DXA-acquired body composition								
Δ Total body fat mass (kg)	−0.27	0.23	−0.12	0.60	0.34	0.17	−0.03	0.89
Δ Lean body mass (kg)	−0.15	0.52	0.40	0.07	0.39	0.11	0.21	0.36
Δ Appendicular lean mass (kg)	−0.26	0.25	0.24	0.27	0.42	0.09	0.12	0.58
CT-acquired body composition								
Δ Mid-thigh muscle area (cm^2)	0.16	0.48	−0.19	0.40	0.27	0.29	−0.01	0.96
Δ Mid-thigh intermuscular fat area (cm^2)	−0.28	0.20	0.06	0.79	0.16	0.52	−0.39	0.07
Δ Trunk muscle area (cm^2)	−0.1	0.66	0.11	0.63	0.41	0.09	0.09	0.68
Δ Trunk intermuscular fat area (cm^2)	−0.44	0.04*	0.13	0.56	−0.22	0.39	−0.34	0.13

*Denotes statistical significance $P < 0.05$.

WL would generally be unexpected, because of the higher protein nature of our dietary prescription (which all participants received) and the fact that two-thirds of our sample was also engaged in some type of exercise programme (i.e., weighted vest use or structured RT), concomitant uptake of these anabolic stimuli may help to explain our findings. Indeed, a recent publication by Balachandran et al. demonstrated that the D_3Cr dilution method was sufficiently sensitive to detect increases in muscle mass due to resistance exercise training in frail older men and women.[35] As additional intervention studies utilizing the D_3Cr method are published, comparison of our findings—particularly once the blinding is lifted for the parent INVEST in Bone Health trial—to an emerging of body literature will be of significant interest to the sarcopenia research community.

Our findings compliment and extend prior work reporting moderate longitudinal correlations between D_3Cr muscle mass and DXA-acquired lean mass. Specifically, in a convenience sample (n = 40) of older men from the MrOS cohort who were followed for 1.6 years, Duchowny et al. reported correlations of 0.50 and 0.58 between changes in D_3Cr muscle mass and total body lean and appendicular lean mass, respectively.[21] Our observed correlations between change in D_3Cr muscle mass and DXA-acquired appendicular lean mass are in alignment; however, we did not observe a significant correlation with DXA-acquired total body lean mass. Although reasons for this discrepancy are not readily apparent, it may be due to potential error introduced as muscle mass is only about 50% of DXA lean mass (in older men[36]) with the remainder of lean mass consisting of body water and organ weights, which would more heavily influence total body lean mass, as compared with appendicular lean mass, measurement.

At the present time, only one other study has examined associations between D_3Cr muscle mass and CT-acquired measures of muscle.[37] Using data from over 1000 MrOS participants, Orwoll and colleagues examined associations between quartiles of high resolution peripheral CT (HRpQCT)-acquired muscle density at the tibia and D_3Cr mus-

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cle mass. Authors report that higher levels of tibial muscle density (Q4: 16.9 ± 1.3 mg/cm^3 vs. Q1: 5.8 ± 3.5 mg/cm^3) are associated with a higher amount of whole body D_3Cr muscle mass (Q4: 25.3 ± 3.5 kg vs. Q1: 22.5 ± 3.7 kg); however, measures were not found to be strongly correlated (r = −0.12). Unfortunately, as our study acquired axial CT scans, results are not directly comparable; however, it is notable that we also failed to observe significant correlations between change in D_3Cr muscle mass and change in CT-acquired muscle cross-sectional area at the mid-thigh and trunk. That said, given our small sample, this ancillary pilot study lacked power to detect all but the strongest correlations.

As noted previously, there is currently a dearth of D_3Cr dilution method data in women. This gap is particularly notable, given the well-described sex differences in body composition,[38] age-related functional decline,[39] and responsivity to WL.[40] While data presented in Figure 1 are certainly meant to be descriptive, it is interesting that associations between change in lean mass and muscle mass are seemingly more apparent in women than men. To date, only one other study applied the D_3Cr method to measure muscle mass in postmenopausal women and reported comparisons to DXA-acquired body composition and physical function measures, with similar associations reported between D_3Cr muscle mass and measures of DXA appendicular lean mass (r = 0.50 in Zhu et al.[23] vs. r = 0.46 observed in the present study).

In contrast to our hypothesis, and prior work reporting significant associations between D_3Cr muscle mass and muscle function[20,22] and strength,[37] we did not observe a significant association between change in D_3Cr muscle mass—or any bioimaging (DXA/CT) muscle metric—and muscle function or strength. Conversely, the lone significant association observed between change in body composition and change in muscle function was a modest (r = −0.44) inverse association between regional fat and gait speed, which has been shown before.[11] We speculate that our largely null findings may be attributed to the modest changes in muscle function and strength we observed in our very small, relatively well functioning sample. As interpretation of body composition changes—particularly in the context of geriatric WL—are of greatest clinical relevance when paired with changes in functional outcomes, further work in this area is warranted.

This ancillary pilot study has several strengths worth noting, including novel application of the D_3Cr dilution method in the context of a predominantly female, geriatric WL intervention. Additionally, utilizing the INVEST in Bone Health trial as a scaffold, we were able to compare the change in D_3Cr muscle mass to a battery of body composition and muscle function and strength metrics. Despite these strengths, however, our study is limited by its small, convenient sample, and findings are certainly not definitive. Moreover, it should be acknowledged that the equation used to estimate D_3Cr whole body muscle mass utilizes a constant, which may over or underestimate the actual concentration of creatine in whole wet muscle for any given individual.[27] Finally, as treatment assignment for the trial is blinded at this time, the impact of group intervention remains unknown and will be the focus of future work.

In sum, the primary objective of this pilot project was to generate estimates of change and variability in whole body muscle mass, as derived from the D_3Cr dilution method, in a predominately female sample undergoing intentional WL with and without weighted vest use or RT. We report that significant, clinically meaningful, loss in total body weight (11% of baseline weight) was accompanied by significant loss in several DXA and CT-derived body composition measures, yet preservation of D_3Cr muscle mass and muscle function. As the D_3Cr method is relatively new, our results add information from a mostly female sample and in the context of an intentional WL intervention, with and without exercise. Results from this project, and others, support the continued consideration of the D_3Cr dilution method as a scalable body composition assessment method.

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Conflict of interest

Drs. Evans holds two relevant patents related to the D_3Cr method and is the CEO of MyoCorps, Inc. Drs. Evans, Cawthon, and Shankaran hold stock or stock options in this company. All other authors declare no relevant conflicts of interest. Data that support the findings of this study are available from the corresponding author upon reasonable request. All authors certify that they comply with the ethical guidelines for authorship and publishing in the *Journal of Cachexia, Sarcopenia and Muscle*.

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