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Research Article

Greater Skeletal Muscle Fat Infiltration Is Associated With Higher All-Cause and Cardiovascular Mortality in Older Men

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Abstract

Background. Skeletal muscle fat infiltration (myosteatosis) increases with aging, and has been associated with poor metabolic and musculoskeletal health, independent of overall adiposity. Studies examining the relationship of myosteatosis and mortality among older individuals recruited without regard to their health status are sparse.

Methods. We evaluated the association of peripheral computed tomography measured calf myosteatosis (intermuscular fat and muscle density as a measure of intramuscular fat) with mortality in 1,063 community-dwelling older men. Cox proportional hazards models were used to estimate the risk of mortality independent of potential confounders.

Results. During a mean follow-up of 7.2 years, 317 participants died. After adjustment for potential covariates and additional adjustment for whole body fat, lower skeletal muscle density was associated with increased all-cause mortality and cardiovascular disease mortality (hazard ratio [95% confidence interval] per standard deviation lower skeletal muscle density: 1.24 [1.09–1.41] and 1.46 [1.15–1.86], respectively), and to some extent with noncardiovascular disease mortality (1.18 [1.0–1.38], $p = .053$). After adjusting for trunk fat in a separate multivariable model, the association between skeletal muscle density and all-cause and cardiovascular disease mortality remained significant (both $p < .01$), while its association with noncardiovascular disease mortality became of borderline significance ($p = .085$). No other measures of adiposity, including calf intermuscular fat, were associated with mortality.

Conclusion. Our study reveals an independent association between skeletal muscle density and mortality in a community-based sample of older, predominantly Caucasian men. Further studies are needed to establish if this association is independent of other ectopic fat depots, and to identify the biological mechanisms underlying this relationship.

Key Words: Skeletal muscle fat infiltration—Muscle density—Mortality—Aging.

Increased accumulation of fat around and within nonadipose tissue organs that normally contain only small amounts of fat, such as in liver, heart, and skeletal muscle, can impair the normal physiological function of those organs (1). This "ectopic fat" storage is now recognized as a risk factor for several chronic diseases, in particular type 2 diabetes (T2D) and cardiovascular disease (CVD), independent of general obesity (2–9). Some propose that the tendency to accumulate adipose tissue in ectopic depots may be explained by inadequate function and capacity of subcutaneous depot to store excess fat (10,11), increased fatty acid transport, uptake and storage, and reduced fatty acid oxidation (2,12,13), and/or increased macrophage infiltration which inhibits adipocyte differentiation (14).

Ectopic fat within and around skeletal muscle (known as myosteatosis) is a unique ectopic fat depot that is associated with poor metabolic and musculoskeletal health, and with accelerated aging (1,5,9,15–22). Specifically, myosteatosis has been identified as a risk factor for insulin resistance and T2D (16,22), increased risk of osteoporotic fractures (18,21), decreased muscle strength and mobility loss (15,19), reduced physical performance (23), and impaired longevity (20). Previous studies showed that skeletal muscle fat infiltration increases with advancing age (24), and that elderly individuals have greater myosteatosis (25,26) compared to younger individuals. Despite the emerging role of myosteatosis as an independent risk factor for metabolic dysfunction and other aging-related disorders, no previous studies have examined its association with mortality among older populations recruited without regard to their health status. Thus, in the current study, we evaluated the relationship between myosteatosis and mortality in a cohort of community-dwelling older men. We tested if this relationship was independent of total body and trunk fat, muscle size, lifestyle factors, comorbidities, frailty, and medications that may influence skeletal muscle metabolism. Given its important role in cardio-metabolic health, we hypothesized that increased myosteatosis would be associated with a greater risk of all-cause and CVD mortality.

Methods

Study Population

The Osteoporotic Fractures in Men (MrOS) Study is a prospective multicenter study that enrolled 5,994 men aged 65 years and older from six geographic regions (Birmingham, AL; Minneapolis, MN; Palo Alto, CA; Pittsburgh, PA; Portland, OR; and San Diego, CA) in the United States between March 2000 and April 2002. Details of the study have been described (27). The parent study was designed to determine risk factors for bone loss, fractures, and other conditions of aging among elderly men. To participate in MrOS, men had to be able to walk without assistance from another person, and could not have had a bilateral hip replacement surgery. Men were recruited primarily through community-based mailing lists. Advertisements in local and senior newspapers and presentations to community groups were used to supplement recruitment efforts. The study was approved by the Institutional Review Boards at all study sites, and written informed consent was obtained from participants prior to data collection.

After an average of 4.5 years, participants at the Minneapolis and Pittsburgh Study Centers returned for a second visit for assessments of body composition with dual-energy X-ray absorptiometry (DXA) and calf skeletal muscle composition with peripheral quantitative computed tomography (CT). Participants also completed questionnaires regarding medical history, medications, physical activity, alcohol intake, and smoking. The present analysis is thus limited to MrOS participants from the Pittsburgh and Minneapolis study sites, and who have complete data on general obesity, whole body and trunk fat, and skeletal muscle composition at Visit 2 ($N = 1,063$ MrOS men: 489 from Minneapolis and 583 from Pittsburgh).

CT Quantification of Myosteatosis

Studies that utilize CT scans for measures of myosteatosis examine either intermuscular fat (visible fat beneath the fascia lata) and/or muscle density (fat between muscle fibers and fat within muscle cell). Lower skeletal muscle density is indicative of greater intramuscular fat content (28,29). In our study, measures of myosteatosis were obtained by peripheral quantitative CT (pQCT) scan of the calf, which was performed using the Stratec XCT-2000 (Pittsburgh site) and the XCT-3000 (Minneapolis site) scanners (Orthometrix, Inc., White Plains, NY). The only difference between the 2000 and 3000 scanners is the gantry size. The 3000 model has twice the gantry size opening (15 vs 30 cm), which permits the upper limbs to be scanned. Both scanners are calibrated to the same factory set standard. This calibration is for a single energy (~58 kVp) and to hydoxyapatite. Specifically, each Stratec XCT 2000 and 3000 scanner is calibrated to the European Forearm Phantom which contains four volumetric density bone mineral inserts of 0, 50, 100, and 200 mg/cc. Each XCT 2000 and 3000 scanner operated at a fixed kVp, and were calibrated to the European Forearm Phantom daily. Quality assurance phantom, supplied by the manufacturer, provided an accurate check of machine stability. This quality assurance phantom contains density inserts of a known density and cross-sectional area that must be correctly determined before clinical scans can be acquired. The same acquisition and analysis software was used to analyze scans at both sites.

At a second study visit, conducted after an average of 4.5 years from the baseline visit, 2.2-mm cross-sectional images of the calf skeletal muscle composition were obtained at 66% of the tibia length, proximal to the terminal end of the tibia, because this is the region of the calf with the largest circumference of the calf with less variability between individuals (30). The length of each tibia was measured (in millimeters) and men were seated comfortably with their lower leg positioned in the pQCT gantry such that the tibia was parallel to the floor with the limb supported at the distal thigh and foot. Standardized procedures for participant positioning and data analysis are used for all scans (31). A scout view was obtained prior to the tomographic scan to define an anatomic reference line, to which the relative location of the subsequent tomographic slice is automatically adjusted. The reference line was set according to the recommendations outlined in the manual supplied by the manufacturer (31). It is recommended that the reference line is placed through the flat portion of the distal tibia endplate. The reference line location was used between the two study sites and confirmed for each image before analysis. Different tissues in the analyses were separated according to different density thresholds, appropriate for soft tissue segmentation, using the manufacturer's suggested analysis parameters (31). Based on calibration of the XCT scanners (2000 and 3000), fat, muscle or lean tissue, and cortical bone were measured with mineral equivalent densities of 0, 80, and 1,200 mg/cm³, respectively. Therefore, muscle, fat, and bone with an image can be separated using appropriate fixed and gradient thresholds to separate the tissue compartments. Changes in muscle tissue to fat tissue were detected as a shift in mineral equivalent density of the muscle from 80 to 0 mg/cm³. Automatic threshold-based iterative edge

detection-guided segmentation of muscle from bone was performed using a density threshold of $280\,mg/cm^3$ with contour mode 1 and peel mode 2 (bone area and mass). Segmentation of muscle from subcutaneous fat followed a threshold of $40\,mg/cm^3$ with contour mode 3 and peel mode 1 (total muscle + bone mass/content). The threshold of $40\,mg/cm^3$ was selected as it is halfway between the densities for fat and water or muscle-like tissues and represents a standard image processing threshold selection for defining the gradient (or edge) between tissues. The units of bone mass/content (or bone mineral content) are mg/cm. To determine muscle cross-sectional area, bone area was subtracted from total bone mass/content + muscle area. Similarly, bone mass/content was subtracted from total bone + muscle mass/content to derive muscle mass/content. After subtraction of intermuscular adipose tissue cross-sectional area, muscle density was computed by dividing total muscle mass (mg/cm) by muscle cross-sectional area (cm^2).

Image processing and quality control procedures were completed by a single investigator blinded to outcome status using the Stratec analysis software (Version 5.5). In addition to measures of myosteatosis (intermuscular adipose tissue cross-sectional area [mm^2] and skeletal muscle density [mg/cm^3]), we also obtained measures of total adipose tissue cross-sectional area (mm^2) and subcutaneous adipose tissue cross-sectional area (mm^2). The coefficients of variation were determined by repeat pQCT scanning in 15 individuals. The coefficients of variations for total, subcutaneous, and intermuscular fat, and muscle density were 0.98%, 1.5%, 7.6%, and 1.1%, respectively.

DXA Measured Total Body and Trunk Fat

Percent whole body fat and trunk fat (calculated as [trunk fat/total fat] × 100) were measured from DXA scans using Hologic QDR 4500 scanners (Hologic, Inc., Bedford, MA). Scans were analyzed with QDR software version 8.26a. Reproducibility of DXA measurements was ensured by use of a central quality control laboratory (San Francisco Coordinating Center, San Francisco, CA), standardized scanning procedures, and certification of DXA operators.

Other Measures

Height was measured with wall-mounted Harpenden stadiometers, and body weight was measured on standard balance beam using standard protocols. Body mass index was calculated from height and weight (kg/m^2). Information on lifestyle habits (smoking, alcohol use, sedentary lifestyle, and physical activity), demographic information, and medical conditions were assessed using self-administered questionnaires. Self-reported smoking behavior was recorded as never smoked, past smoker, and current smoker. Alcohol drinking frequency was self-reported in predefined categories. Physical activity was measured by the Physical Activity Scale for the Elderly (PASE) (a unit less measure of relative physical activity) which measures total, occupational, household, and leisure physical activities over the previous 7 days. Sedentary activity was described as > 4 hours sitting per day. Participants were instructed to bring in all prescription medications taken in the past 30 days to their clinic visit, and study coordinators recorded the medications and data were stored in an electronic medications inventory database (San Francisco Coordinating Center, San Francisco, CA). The Iowa Drug Information Service Drug Vocabulary (College of Pharmacy, University of Iowa, Iowa City, IA) was used to identify ingredient(s) in the medications [32]. Antidiabetic drugs were defined as hypoglycemic medications and insulin. Antihypertensive drugs were defined as thiazide

diuretics, potassium-sparing diuretics, loop diuretics, angiotensin-converting-enzyme (ACE) inhibitors, α-adrenergic blockers, hypotensive agents-angiotensin II, calcium channel blockers, β-blockers, and nitrates. Lipid-lowering drugs were defined as gemfibrozil and HMG-CoA reductase inhibitors (statin).

Frailty is a clinical syndrome in older adults characterized by decreased physiologic reserve and weakness that causes an increased vulnerability to stressors, and was defined using criteria similar to those initially proposed in the Cardiovascular Health Study [33]. A man was considered frail if three or more of the following five criteria were present: (i) Shrinking or sarcopenia [34]: appendicular lean mass (adjusted for height and whole body fat) in the lowest quintile; (ii) Weakness: grip strength in the lowest quintile stratified according to BMI (quartiles); (iii) Exhaustion: answer of "a little or none" to the question "How much of the time during the past four weeks did you have a lot of energy?"; (iv) Slowness: walk speed in the lowest quintile stratified according to standing height (median); and (v) Low physical activity: PASE score in the lowest quintile.

Mortality Assessment

During a mean follow-up of 7.2 years, 317 participants died (Table 1). A single physician adjudicator at the Coordinating Center verified all deaths using death certificates and hospital discharge summaries when available, and a prespecified adjudication protocol. The International Classification of Diseases, Ninth Revision(ICD9) code was used for the underlying cause of death. The primary outcomes of interest were all-cause, cardiovascular, and non-CVD mortality, which included all causes of mortality except for CVD. Codes 394.9, 396.9, 398.9, 401.1, 401.9–442.0, 443.9, 785.51, and 996.71 were classified as CVD deaths.

Statistical Methods

We described the participant characteristics at the visit when pQCT measures were obtained (MrOS Visit 2) using means (standard deviation [*SD*]) or prevalence. We first inspected the distributions of the skeletal muscle composition measures for outliers using histograms and scatter plots. An outlier was defined as any observation outside the interval ($Q_1 - 3 \times IQR$, $Q_3 + 3 \times IQR$), IQR = Interquartile range, and was excluded from subsequent analyses. We used two-sample *t*-tests and/or Wilcoxon rank sum tests (continuous variables) or chi-square tests (categorical variables) to compare characteristics between survivors and deceased participants. We assessed the association between continuous measures of myosteatosis (per 1 *SD* greater intermuscular fat and per 1 *SD* lower muscle density) and all-cause, CVD, and non-CVD mortality using semiparametric Cox proportional hazard models. We also calculated hazard ratios and 95% risk limits for all-cause, CVD, and non-CVD mortality across quartiles of muscle density.

Covariates of interest included age, race, study site, height, weight change since baseline visit, lifestyle variables (smoking status, alcohol intake, PASE score, sedentary activity ≥4 hours sitting activity, excellent/good health status), lipids and lipoproteins (high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, total cholesterol), comorbidities (T2D, coronary heart disease, hypertension, stroke, cancer, renal disease), frailty status, and medications that influence metabolism (antidiabetic, antihypertensive, and lipid lowering). T2D was defined by self-report, use of hypoglycemic medications at Visit 2, or having a baseline fasting glucose level ≥126 mg/dL. All multivariable

Table 1. Characteristics by Vital Status in 1,063 MrOS Men (Follow-up March 2005–Feb 2014)

Characteristics	All (n = 1,063)	Survivors (n = 746)	Deceased (n = 317)	p Value*
Socio-demographic				
Age (years)	77.2 ± 5.2	76.2 ± 4.7	79.4 ± 5.4	<.0001
Site, n (%)				<.0001
Minneapolis	480 (45.2)	369 (49.5)	111 (35.0)	
Pittsburgh	583 (54.8)	377 (50.5)	206 (65.0)	
Race, n (%)				.3058
White	1045 (98.3)	730 (97.9)	315 (99.4)	
Non-white	18 (1.7)	16 (2.1)	2 (0.6)	
Physical measures				
Height (cm)	173.0 ± 6.8	173.4 ± 6.6	171.9 ± 7.0	.0006
Weight (kg)	83.7 ± 13.3	84.4 ± 12.8	82.0 ± 14.3	.0021
Weight change since baseline visit (kg)	-1.5 ± 4.9	-1.0 ± 4.6	-2.6 ± 5.4	<.0001
BMI (kg/m²)	27.9 ± 3.8	28.0 ± 3.6	27.7 ± 4.3	.0616
Lifestyle, physical activity, and health status				
Smoker at Visit 2, n (%)	32 (3.0)	19 (2.6)	13 (4.1)	.1749
Drinking frequency at Visit 2, n (%)				.2599
None	417 (39.2)	276 (37.0)	141 (44.5)	
<1 drink/wk	167 (15.7)	121 (16.2)	26 (14.5)	
1–2 drinks/wk	121 (11.4)	86 (11.5)	35 (11.0)	
3–5 drinks/wk	152 (14.3)	116 (15.6)	36 (11.4)	
6–13 drinks/wk	157 (14.8)	112 (15.0)	45 (14.2)	
≥14 drinks/wk	49 (4.6)	35 (4.7)	14 (4.2)	
Physical Activity Scale for the Elderly score	141.1 ± 65.0	145.7 ± 63.2	130.1 ± 68.1	<.0001
Sedentary lifestyle (> 4 hours sitting activity per day), n (%)	331 (31.1)	222 (29.8)	109 (34.4)	.1362
Excellent/good health status, n (%)	921 (86.6)	674 (90.4)	247 (77.9)	<.0001
Frailty status†, n (%)				< 0.0001
Robust	318 (29.9)	258 (34.6)	60 (18.9)	
Intermediate	573 (53.9)	414 (55.5)	159 (50.2)	
Frail	172 (16.2)	74 (9.9)	98 (30.9)	
Obesity ‡, n (%)	272 (25.6)	193 (25.9)	79 (24.9)	.7453
Comorbidities				
Prevalent diabetes at Visit 2, n (%)	199 (18.7)	131 (17.6)	68 (21.5)	.1368
Hypertension, n (%)	557 (52.4)	380 (50.9)	177 (55.5)	.1436
Myocardial infarction, n (%)	178 (16.8)	112 (15.0)	66 (20.8)	.0204
Stroke, n (%)	72 (6.9)	37 (5.0)	36 (11.4)	.0002
Cancer, n (%)	319 (30.0)	199 (26.7)	120 (37.9)	.0003
Renal disease, n (%)	16 (1.5)	6 (0.8)	10 (3.2)	.0040
Medication use				
Antihypertensive drugs, n (%)	733 (69.0)	497 (66.6)	236 (74.5)	.0116
Lipid lowering drugs, n (%)	493 (46.4)	358 (48.0)	135 (42.6)	.1061
Anti-diabetic drugs, n (%)	127 (12.0)	81 (10.9)	46 (14.5)	.0930
Biochemical measures				
HDL-C (mg/dL)	46.7 ± 12.5	47.0 ± 12.6	46.2 ± 12.3	.2491
LDL-C (mg/dL)	116.1 ± 31.3	116.8 ± 30.9	114.3 ± 32.3	.2429
Triglycerides (mg/dL)	153.2 ± 79.0	151.5 ± 77.5	157.1 ± 82.6	.3671
Total Cholesterol (mg/dL)	193.4 ± 35.2	194.1 ± 34.8	191.9 ± 36.2	.3654

Notes: Values are unadjusted mean ± *SD*, unless indicated otherwise. BMI = body mass index; CHS = Cardiovascular Health Study; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.

*p Values for comparisons between survivors and decedents from two-sample *t*-tests, Wilcoxon two-sample test, or chi-square tests.

†Frailty status: Robust (none of 5 CHS frailties), Intermediate (one or two of five CHS frailties), frail (at least three of five CHS frailties).

‡Obesity = BMI ≥ 30.

models were adjusted for the covariates listed above regardless of statistical significance within the model. Models of calf skeletal muscle fat measures also included adjustment for calf muscle cross sectional area. The proportional hazards assumption was confirmed graphically and formally using Schoenfeld residuals. The model fit was verified by plotting the Nelson-Aalen cumulative hazard of the Cox-Snell residuals. All statistical analyses were performed using the Statistical Analysis System (SAS, version 9.3; SAS Institute, Cary, NC).

Results

Baseline Characteristics

Characteristics of the 1,063 study participants are presented in Table 1. The mean age of the study sample was 77.2 years. Most participants were Caucasian (98%), only 3.0% were current smokers, and the majority reported excellent or good health (86.6%). Participants who died during the follow-up (N = 317) were older, more likely from the Pittsburgh site, lost more weight

since baseline, were less physically active, less likely to report excellent or good health status, and more likely to use antihypertensive drugs. Furthermore, men who died during the follow-up also had a higher prevalence of several chronic diseases (myocardial infarction, stroke, cancer, and renal disease) and were more likely to be frail. Participants who died during the follow-up also had lower calf muscle area, lower trunk fat percent, greater intermuscular fat, and lower muscle density (indicative of greater intramuscular fat) compared with those who survived (Table 2).

Myosteatosis and All-Cause Mortality

Cox proportional hazard models exploring the association of intermuscular fat with mortality revealed a significant association of intermuscular fat with all-cause mortality in unadjusted analyses (hazard ratio [HR] [95% confidence interval (CI)] per *SD* greater intermuscular fat: 1.23 [1.11–1.35]). However, after multivariable adjustments for potential confounders (HR [95% CI]: 1.03, [0.93–1.16]), or after the additional adjustment for whole body fat percent (HR [95% CI]: 1.04, [0.93–1.17]) or trunk fat percent (HR [95% CI]: 1.03, [0.92–1.15]), we found no significant association between intermuscular fat and all-cause mortality.

In addition, we found an 18% increase in the multivariable-adjusted risk of all-cause mortality per *SD* lower muscle density (HR [95% CI]: 1.18, 1.06–1.33; Table 3). This association remained statistically significant after additional adjustments for whole body fat or trunk fat percent (results presented in Table 3).

No other adiposity measures studied were associated with mortality, with the exception of trunk fat percent, which was inversely associated with the risk of all-cause mortality in unadjusted analysis (HR [95% CI] per *SD* greater percent trunk fat: 0.85 [0.76–0.95]), but not after the inclusion of age, race, and study site or after the inclusion of other potential confounders (Supplementary Table 1).

Myosteatosis and Cause-Specific Mortality

There were 100 CVD deaths during 7.2 years of follow-up. We found a significant association between intermuscular fat and CVD mortality in an unadjusted model (HR [95% CI] per *SD* greater intermuscular fat: 1.44 [1.22–1.68]), which was no longer significant after adjustment for potential confounders (HR [95% CI]: 1.17 [0.97–1.41]), after the additional adjustment for whole body fat percent (HR [95% CI]: 1.18 [0.98–1.42]), or after the additional adjustment for trunk fat percent (HR [95% CI]: 1.15 [0.95–1.38]). Lower skeletal muscle density was associated with an increase in the multivariable-adjusted risk of CVD mortality (HR [95% CI] per *SD* lower muscle density 1.34, [1.08–1.66]) and remained significant after additional adjustment for whole body fat or trunk fat percent (Table 3).

There were 199 non-CVD deaths during 7.2 years of follow-up. We found a significant association between intermuscular fat and non-CVD mortality in an unadjusted model (HR [95% CI] per *SD* greater intermuscular fat: 1.14 [1.00–1.30]), which was no longer significant after adjustment for potential confounders (HR [95% CI]: 1.00 [0.86–1.16]). One *SD* lower muscle density (Table 3) was associated with non-CVD death in an unadjusted model (HR [95% CI] 1.29 [1.12–1.49]), but this association became weaker and borderline significant (Table 3) after adjusting for potentially important confounders or whole body fat and trunk fat percent ($p = .079$ in multivariable-adjusted model, $p = .053$ in multivariable-adjusted model with whole body fat percent, and $p = .085$ in multivariable-adjusted model with trunk fat percent).

Quartiles of Myosteatosis and Mortality Risk

There was a significant trend for decreasing risk of all-cause mortality with lowering muscle density quartile in an unadjusted model (p trend < .0001, data not shown) and after adjustment for significant covariates and whole body fat percent (Figure 1, p trend = .0072). Similarly, in unadjusted models, the hazard ratios for CVD (p trend = .0001, data not shown) and for non-CVD mortality decreased from lowest to highest quartile (p trend = .0043, data not shown). The trend for decreasing risk of CVD mortality with greater muscle density quartile remained significant after adjustment for significant covariates as well as whole body fat (Figure 1, p trend = .0048), but was not significant for non-CVD mortality (Figure 1, p trend = .2262). Moreover, there appeared to be a threshold at a muscle density value of approximately 71.99 mg/cm^3 below which a lower muscle density was associated with a greater risk of CVD mortality.

Discussion

The current study found an independent association between calf skeletal muscle density and increased all-cause and CVD mortality risk among older men, which persisted even after adjustment for potentially important lifestyle and medical covariates, and general and central adiposity. With the exception of trunk fat percent, which was inversely associated with the risk of all-cause mortality in unadjusted analysis, no other measures of adiposity were associated with mortality. This is not surprising as increasing evidence suggests that the association between general adiposity and mortality may be attenuated with aging (35–37). Despite the growing appreciation of a potential role for ectopic fat in metabolic health and aging independent from overall adiposity (9,18–23), few studies have examined the association between ectopic fat and mortality, particularly for skeletal muscle fat infiltration. Existing studies

Table 2. Body Composition and Skeletal Muscle Characteristics by Vital Status in 1,063 MrOS Men (follow-up March 2005–Feb 2014)

Characteristics	All (N = 1,063)	Survivors (n = 746)	Deceased (n = 317)	p value[†]
DXA whole body fat %	27.3 ± 5.2	27.3 ± 5.1	27.2 ± 5.5	.6913
DXA trunk fat %	55.1 ± 5.3	55.4 ± 5.3	54.4 ± 5.3	.0046
Calf total fat (mm^2)	2,269.5 ± 901.1	2,264.9 ± 876.0	2,280.4 ± 959.3	.7708
Calf muscle area (mm^2)	7,502.3 ± 1,197.9	7,642.9 ± 1,194.1	7,171.3 ± 1,142.0	<.0001
Calf subcutaneous fat (mm^2)	1,772.5 ± 755.4	1,788.8 ± 731.3	1,734.1 ± 809.4	.0863
Calf intermuscular fat (mm^2)	304.6 ± 271.6	280.8 ± 256.7	361.5 ± 296.9	<.0001
Calf skeletal muscle density (mg/cm^3)	74.1 ± 3.5	74.5 ± 3.4	73.1 ± 3.6	<.0001

Notes: Values are unadjusted mean ± *SD*.

[†]*p* Values for comparisons between survivors and decedents from two-sample *t*-tests or Wilcoxon two-sample test.

Table 3. Hazard Ratios (95% CI) for Mortality Per *SD* Increase in Skeletal Muscle Density in 1,063 MrOS Men

	Unadjusted model	Adjusted for age, race, study site	Multivariable model	MV model* + DXA whole body fat (%)	MV model* + DXA % trunk fat
All-cause mortality	1.39 (1.25–1.55)‡	1.20 (1.07–1.35)†	1.18 (1.06, 1.33)†	1.24 (1.09, 1.41)†	1.18 (1.05, 1.33)†
CVD mortality	1.66 (1.37–2.01)‡	1.34 (1.09–1.65)†	1.34 (1.08, 1.66)†	1.46 (1.15, 1.86)†	1.34 (1.09, 1.66)†
Non-CVD mortality	1.29 (1.12–1.49)‡	1.15 (0.99–1.33)§	1.14 (0.99, 1.33)‖	1.18 (1.00, 1.38)¶	1.14 (0.98, 1.32)#

Notes: *All multivariable (MV) models were adjusted for age, race, study site, height, weight change since baseline, smoking, drinking, sedentary lifestyle, Physical Activity Scale for the Elderly score, health status, frailty status, diabetes, hypertension, myocardial infarction, stroke, cancer, renal disease, antihypertensive drugs use, lipid-lowering drugs, antidiabetic drugs use, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, and calf muscle cross sectional area. CVD = cardiovascular disease.

†$p < .01$, ‡$p < .001$, §$p = .073$, ‖$p = .079$, ¶$p = .053$, #$p = .085$.
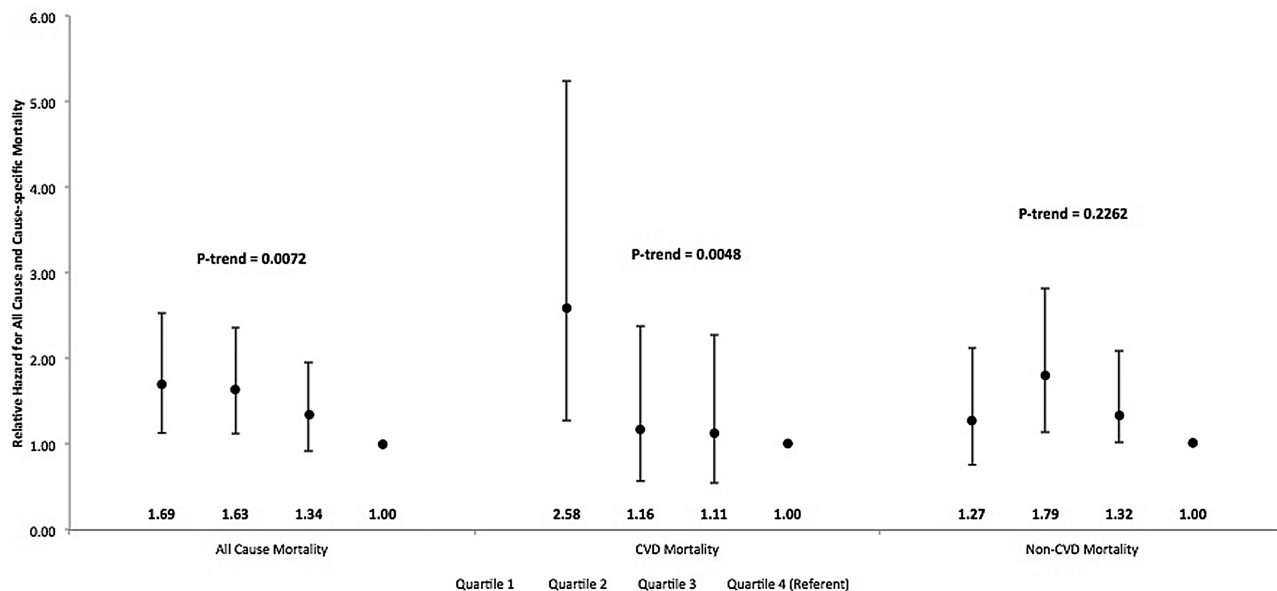

Figure 1. Adjusted hazard ratios for mortality across quartiles of skeletal muscle density in 1,063 MrOS men. All multivariable models were adjusted for age, race, study site, height, weight change since baseline, smoking, drinking, sedentary lifestyle, Physical Activity Scale for the Elderly score, health status, frailty status, diabetes, hypertension, myocardial infarction, stroke, cancer, renal disease, antihypertensive drugs use, lipid-lowering drugs, antidiabetic drugs use, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, calf muscle cross sectional area, and dual-energy X-ray absorptiometry total body fat.

of ectopic fat have focused exclusively on visceral, liver, and pericardial fat (9), or on highly selected individuals rather than the general population (24,25). Visceral fat has been the most extensively studied ectopic fat depot and is associated with mortality in some (38–40), but not all studies (41). Likewise, despite a strong link between increased liver fat infiltration and cardio-metabolic disturbances (42), few studies have examined the association of liver fat with mortality. In the Third National Health and Nutrition Examination Survey, no association was observed between fatty liver and mortality among 1,371 participants aged 20–74 (43). Although, pericardial fat has been associated with both prevalent (8) and incident (44) CVD, the Framingham Heart Study (41) found no association between pericardial fat and mortality. Moreover, very few data exist on the potential relationship between skeletal muscle fat infiltration and mortality in community dwelling populations, and if the associations are independent of general adiposity and other potentially important covariates. The InCHIANTI study reported no significant association between calf muscle density and mortality over a mean of 5.1 years among 934 community-dwelling women and men aged 65+ years (45). Although in this study lower calf muscle density

was associated with higher mortality in unadjusted analyses, none of the associations remained statistically significant after additional adjustment for age and sex. Thus, the current study reports new results in a larger sample of older men and if the associations of muscle density and mortality are independent of general and central adiposity. Similar to our findings, the Walking and Leg Circulation Study (46) reported that lower calf skeletal muscle density is associated with greater all-cause and CVD mortality among 434 older women and men with peripheral arterial disease. Our study extends these observations to the general population who were recruited without regard to their health status.

The mechanisms linking increased skeletal muscle fat infiltration and mortality are not clear. A possible mechanism by which inter- and intramuscular fat may influence risk of mortality may be through an association with cardio-metabolic risk factor levels, and particularly through effects on insulin sensitivity (9). However, the mechanisms linking inter- and intramuscular fat with insulin resistance and T2D and other metabolic diseases may differ, which could explain the observed differences in their association with mortality. Increased accumulation of intermuscular fat may induce changes in

muscle metabolism and insulin sensitivity via increasing local secretion of proinflammatory adipokines from adipocytes surrounding muscle fibers (3). Intermuscular fat may also impair nutritive blood flow to muscle and thus contribute to insulin resistance by impairing insulin action and insulin diffusion capacity (47). As for intramuscular fat, it is still controversial whether it is a marker or a mediator of insulin resistance. Some propose that accumulation of intramuscular fat may impair the insulin receptor substrate 1 (IRS1)/phosphatidylinositol 3-kinase (PI3K) pathway and growth-factor–regulated protein kinase B (Akt/PKB) pathway of insulin signaling (48). In our study, an association between muscle density, a measure of intramuscular fat, and mortality persisted even after adjusting for a wide range of cardio-metabolic comorbidities, including diabetes, suggesting that this fat depot might be an independent marker of aging. In fact, middle-aged individuals predisposed to increased familial longevity have less intramuscular fat compared to matched controls with similar body composition (20). The authors postulated that lower intramuscular fat found in the offspring of long-lived individuals might be associated with better mitochondrial capacity as greater accumulation of intramuscular fat can cause mitochondrial dysfunction (49). Indeed, mitochondrial dysfunction has been previously linked to greater intramuscular fat, and may play a role in increased mortality risk for adults with higher amounts of intramuscular fat (49). Another possible link between intramuscular fat and increased risk of mortality may be via increased oxidative stress, as visceral fat has been associated with increased oxidative stress (50). Increased lipid content in skeletal muscle cells affects their metabolism, thereby causing an increased expression in oxidative metabolism genes (51). Finally, recent evidence in animal models shows that intramuscular fatty-acid metabolism plays a critical role in mediating the protective effects of dietary restriction in flies (52). Thus, insulin resistance, increased inflammation, greater oxidative stress, and poorer mitochondrial function are potential mechanisms underlying an association between greater intramuscular fat and mortality.

The present study has several potential limitations. First, our study is observational, and causality cannot be definitely determined. Second, our sample consists of predominately Caucasian males, and thus, our findings may not apply to women or to other ethnicities. Third, although the observed association between intramuscular fat and mortality was independent of whole body and trunk body fat, ectopic depots in other nonadipose tissues and organs, such as the visceral compartment, liver and heart, were not assessed, and thus, we were not able to test if the observed associations are independent of these other ectopic fat depots. Fourth, due to the lack of data on oxidative stress, mitochondrial function, and markers of insulin resistance, we were unable to test for mechanisms that may link intramuscular fat with an increased risk of mortality. Thus, the potential mechanisms underlying an association between greater intramuscular fat and mortality remain to be determined. However, our study also has notable strengths including its longitudinal design, community-based sampling design, relatively long follow-up period, and extensive measures of potential confounding lifestyle and medical variables.

In conclusion, our study reveals a previously unreported, independent association between skeletal muscle density, a measure of intramuscular fat, and mortality in a community-based sample of older, predominantly Caucasian men. Further studies are needed to establish if the association of intramuscular fat with mortality is independent of other ectopic fat depots, and to identify the possible biological mechanisms underlying this relationship.

Supplementary Material

Funding

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