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Original Article

Associations of calf inter- and intra-muscular adipose tissue with cardiometabolic health and physical function in community-dwelling older adults

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Abstract

Objectives: To determine associations of inter- and intra-muscular adipose tissue (IMAT) with cardiometabolic health and physical function in older adults. **Methods:** 48 community-dwelling older adults aged ≥65 years (mean 71.6±4.8 years; 52% women) underwent whole-body dual-energy X-ray absorptiometry, to assess appendicular lean mass (ALM), and peripheral quantitative computed tomography (pQCT; 66% tibia), to assess calf IMAT cross-sectional area ([CSA]; cm^2) and muscle density (mg/cm^3; higher values indicate lower fat infiltration). Fasting glucose, lipids, triglycerides and C-reactive protein (CRP) were analysed. Physical function was assessed by postural sway (computerised posturography; N=41), and gait analysis (GAITRite Electronic Walkway; N=40). **Results:** Higher IMAT CSA and muscle density were associated with significantly higher (B=0.85 95%CI [0.34, 1.36]) and lower (-2.14 [-4.20, -0.08]) CRP and higher (0.93 [0.56, 1.30]) and lower postural sway (-3.12 [-4.74, -1.50]), respectively, after adjustment for age, sex and ALM/BMI. Higher IMAT CSA was associated with slower gait speed and cadence, and greater step time and step width (all $P<0.03$), while higher muscle density was associated with smaller step width ($P<0.01$) only. **Conclusions:** Older adults with higher calf IMAT have poorer balance, mobility and inflammatory status. Interventions aimed at improving physical function in older adults should incorporate strategies to reduce IMAT.

Keywords: Intermuscular Adipose Tissue, Sarcopenia, Balance, Mobility, Ageing

Introduction

Sarcopenia, the age-related decline in muscle mass and function, is substantially influenced by changes in neurological processes and skeletal muscle composition[1]. Muscle composition deteriorates with age in large part due to increases in inter- and intra-muscular adipose tissue (IMAT), adipose tissue located between muscle groups, beneath muscle fascia and within individual muscles[2]. Higher amounts of IMAT are thought to increase insulin resistance and the risk of type 2 diabetes, independently of overall adiposity[3]. This association is potentially related to the IMAT-associated release of inflammatory cytokines from within skeletal muscle, the primary site for glucose metabolism in the body[4].

High levels of IMAT in the lower-limbs have also been associated with mobility limitations in older adults, even after adjustment for muscle size, possibly because altered muscle fibre orientation or inflammation compromise muscle function[5,6]. IMAT may also compromise physical performance by increasing insulin resistance, which is associated with increased risk for mobility limitation in older adults[7]. IMAT accumulation in

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different lower-limb muscle groups may have varying effects on components of physical performance; hip (gluteal), but not thigh, muscle IMAT assessed by computed tomography (CT) has been associated with poor balance and gait performance[8], while IMAT reductions in the calf, but not thigh, muscles assessed by magnetic resonance imaging (MRI) are associated with improved walking speed[9].

Peripheral quantitative CT (pQCT) is a non-invasive technique for quantifying calf muscle composition, with benefits including its smaller size and cost relative to MRI and CT, and lower radiation dose compared with CT[10]. Thus, pQCT may be a convenient tool for investigating effects of calf IMAT on cardiometabolic health (risk for cardiovascular disease and diabetes) and physical function, but few data on these associations are available in healthy community-dwelling older men and women. The aim of the present study was to examine how pQCT-assessed calf IMAT is associated with indicators of cardiometabolic health, physical function and gait performance in healthy Australian community-dwelling older adults, independent of muscle mass.

Materials and methods

Study design and participants

Fifty community-dwelling older adults residing in Melbourne (Victoria, Australia), aged ≥65 years, and who responded to advertisements at local hospitals, general practices, community groups, and sporting and recreation clubs, were recruited for this study. Participants were English speaking, capable of walking across a room unaided, and had no self-reported diagnosis of progressive neurological or psychotic disorders, severe arthritis (awaiting a joint replacement), or life expectancy <12 months. The study was approved by the Melbourne Health Human Research Ethics Committee (HREC 2013.079) and was performed in accordance with the National Statement on Ethical Conduct in Human Research (2007)[11]. All participants provided written informed consent.

All testing was conducted at Sunshine Hospital, located in the suburb of St Albans in north-west Melbourne, between March and July 2014. Participants completed a questionnaire including questions on employment status (retired/pensioner or working full/part-time). Presence of chronic conditions was assessed by responses to the question "Have you ever been told by a doctor or other medical professional that you have any of the following physical conditions?" A comorbidity score was calculated as the total number of health conditions reported from a specified list including: Coronary heart disease, hypertension, hypercholesterolaemia, thrombosis, diabetes, asthma, bronchitis or emphysema, any form of cancer, osteoporosis, osteoarthritis, rheumatoid arthritis, hyperthyroidism, and hypothyroidism. We have previously demonstrated that the comorbidity score is significantly higher for older adults with low muscle mass and high fat mass compared to those with normal muscle and fat mass[12].

Anthropometrics and body composition

Weight (Seca 804 electronic scales, Seca, Hamburg, Germany) and height (Seca 222 wall-mounted stadiometer, Seca, Hamburg, Germany) were measured with footwear, headwear, and heavy items of clothing removed. Body mass index (BMI) was calculated as weight (kg)/ height (m^2).

Dual Energy X-ray Absorptiometry (DXA; Hologic Discovery W, Hologic, Bedford MA, USA) estimated body composition. The DXA was calibrated daily using the manufacturer's phantom. Short-term inter-individual coefficients of variation (CV) for total lean mass and appendicular lean mass (ALM) in our laboratory were 0.7% and 1.0%, respectively. This is acceptable precision for lean mass according to the International Society for Clinical Densitometry[13]. Measurements including total (minus head) and regional lean mass and fat mass were derived using computer algorithms provided by the manufacturer. ALM normalised to BMI (ALM/BMI), rather than height, was calculated as an estimate of relative muscle mass given that body size influences the relationship between ALM and physical performance[14].

A Stratec XCT3000 (Stratec Medizintechnik GmbH, Pforzheim, Germany) pQCT device determined calf muscle composition. The device was calibrated daily using the manufacturer's phantom and CV for the duration of this study for phantom density was 0.2%. Participants were seated with their dominant leg positioned inside the pQCT gantry. The dominant leg was preferentially selected for this assessment in order to allow comparability of muscle composition measures with knee extension strength assessed in the same limb. Single 2.5-mm transverse scans were obtained at 66% of tibial length measured proximally beginning from the tibiotarsal joint with a voxel size of 0.8mm and scan speed of 20 mm/sec. All pQCT scans were acquired and analysed by one observer (DS). Calf muscle cross-sectional area (mm^2) and density (mg/cm^3) were determined using manufacturer's algorithms and software (version 6.2). Muscle density is an indirect measure of muscle fat infiltration, with higher density values representing lower fat content[15]. Calf IMAT cross-sectional area (CSA; cm^2) was quantified using outer (40 mg/mm^3) and inner (15 mg/mm^3) thresholds16 to isolate fat tissue within the muscle compartment, and outer (180 mg/cm^3) and inner threshold (15 mg/cm^3) to subtract bone marrow fat from this area. The short-term intra-individual CVs for IMAT CSA and muscle density using these protocols were 4.8% and 1.0%, respectively.

Cardiometabolic risk factors

A blood sample was collected at the hospital pathology centre after an overnight fast of at least 10 hours. Serum glucose, total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, and triglycerides concentrations were analysed using the automated ADVIA 1650 Chemistry System (Siemens Healthcare Diagnostics Incorporation, Australia). A latex enhanced immunoturbidimetric assay determined serum wide range C-reactive protein (CRP) concentration (ADVIA 1650; CV=3.5%). Pathology reported low CRP levels as <1 mg/L and an arbitrary value of 0.01 mg/L was assigned to these cases.

Physical function

Hand grip strength of the dominant hand was assessed using a Jamar Plus Digital hydraulic hand grip dynamometer (Patterson

Medical, Bolingbrook, IL, USA). Participants were seated with their elbow fully extended in front of them at shoulder height and gripped the dynamometer with maximal force for three seconds. The test was completed three times with a 30-second rest between trials and the maximal value was recorded. Knee extension strength was assessed using a hand-held dynamometer (HHD; Lafayette Manual Muscle Tester Model 01165, Lafayette Instrument Company, Lafayette, IN, USA). The participant was seated with their arms folded across their chest, hip and knee joint angles at 90 degrees, and feet above the floor. The participant exerted maximal force for three seconds to the HHD, which was held stationary by the tester about 10cm above the ankle joint[17]. Mean peak force for three trials was calculated. The same tester assessed all participants and the intra-class correlation coefficient for trials two and three was 0.98 (95% CI 0.96-0.99). Participants also performed a timed sit-to-stand test[18]. From a seated position with arms folded across their chest and feet flat on the floor, the participant rose from the chair to a standing position and returned to a seated position five times. Time to complete this task was recorded.

Bipedal standing balance with feet apart and eyes open was assessed in N=41 participants using a Nintendo Wii Balance Board (RVL-021; Nintendo, Kyoto, Japan) and custom software. The test has been demonstrated to measure movements in centre of pressure with acceptable reliability and validity compared to a laboratory-grade force platform[19,20]. Measurements including path length (total distance travelled by the centre of pressure) and anteroposterior and mediolateral amplitude (the maximum range of the centre of pressure in each axis) were recorded by the software using the calibration and data analysis techniques described previously[21]. The mean values from two 30 second trials with 30 second inter-trial rest periods were calculated. Participants also completed the Balance Outcome Measure for Elder Rehabilitation (BOOMER), a validated measure of standing balance impairment consisting of four tests[22]. An overall BOOMER score (range 0-16; higher score is better) was calculated based on performance in the following:

Step test: The participant attempted to place their foot on a 7.5cm high step and then return it to the ground as many times as possible in 15 seconds. A 30-second rest period was provided, and the test was repeated using the opposite leg. Step test number was calculated as the mean score for the left and right legs.

Timed Up-and-Go: The participant rose from a chair, walked three metres and then turned around and returned to a seated position on the chair. The mean time taken time to complete two tests (with a 30-second rest period in between) was recorded as the criterion value for timed up-and-go (TUG).

Functional Reach Test: Participants stood next to a wall and positioned the right arm (closest to the wall) at 90 degrees of shoulder flexion anteriorly. The arm length (cm) was measured using a measuring tape affixed to a wall at shoulder height. Participants then attempted to reach as far forward as possible without losing balance or taking a step. The functional reach test (FRT) score was the difference between the initial position and the maximal reach distance.

Static Timed Standing with Eyes Closed Test: Participants stood with feet together, arms folded, and with eyes closed for 30 seconds. The trial was terminated if the participant opened their eyes, flexed their knees, or moved their arms or feet, and the total number of seconds elapsed was calculated as the score for the trial. If the participant successfully completed a trial, they were not required to complete the subsequent trial/s. Rather, a score of 30 was attributed to the completed and remaining trial(s) as recommended in the BOOMER protocol[22]. Therefore, a maximum score of 90 could be obtained for this test. If a participant failed trial one and/or two, they were required to complete subsequent trial/s with a 30-second rest period between trials.

Gait was evaluated using the GAITRite® Electronic Walkway System (CIR Systems Inc., Clifton, NJ, USA). Participants walked barefoot along a 10m walkway at a self-selected comfortable pace. Three-metre 'lead in' and 'lead off' distances were provided to achieve steady state walking. Six trials were performed with a rest period between each trial. Mean values for the following gait parameters were calculated: speed (cm/s; normalised to leg length), cadence (steps/min), step time (s), step length (cm), step width (cm), swing time (%) and double support time (%).

Statistical analyses

Continuous data were assessed for normality and non-parametric tests were used when transformation did not achieve normality. Spearman correlations were performed to assess associations of IMAT CSA and muscle density with cardiometabolic risk markers, and multivariable linear regression investigated these associations controlling for potential confounders age, sex and ALM/BMI. Multivariable regression analysis adjusting for age, sex and ALM/BMI was also performed to examine associations of IMAT CSA and muscle density with physical function parameters. P-values <0.05 or 95% confidence intervals (CI) not including the null point were considered statistically significant. All analyses were performed in SPSS Statistics 22 (IBM, USA).

Results

Of the 50 participants recruited, review of whole-body DXA scans revealed two had total knee arthroplasty with prostheses extending to beyond 66% tibial length, precluding pQCT measurement. Hence, 48 participants (mean age 71.8±4.8 yr; range 65.0 – 84.9) were included in the analyses. Table 1 presents descriptive characteristics of participants. There were similar numbers of men and women, and the majority of participants were retired. The average BMI was 29.6 and almost half (48%) of participants were obese (BMI ≥30). Participants generally demonstrated good performance in BOOMER (72% achieved the maximum score of 16) and gait speed assessments (90% had mean gait speed ≥100 cm/s). Mean total cholesterol and trigylcerides levels for this cohort were also in the healthy range. Mean fasting glucose level was consistent with pre-diabetes however only eight (17%) participants had fasting glucose levels in the diabetic range (≥7.0 mmol/L).

Table 2 reports Spearman correlation coefficients for the relationship of each of calf IMAT CSA, and muscle density with cardiometabolic risk markers. Participants with higher IMAT CSA had lower HDL cholesterol, but higher glucose and CRP. In

Age (y), mean ± SD	71.6 ± 4.8
Women, N (%)	25 (52)
Retired, N (%)	33 (69)
Number of comorbidities, mean ± SD	2.3 ± 1.6
Total cholesterol (mmol/L), median [IQR]	4.4 [3.8, 5.4]
HDL cholesterol (mmol/L), median [IQR]	1.7 [1.2, 1.8]
LDL cholesterol (mmol/L), median [IQR]	2.2 [1.8, 3.0]
Triglycerides (mmol/L), median [IQR]	1.1 [0.9, 1.5]
Glucose (mmol/L), median [IQR]	5.8 [5.3, 6.4]
CRP (mg/L), median [IQR]	1.0 [0.01, 3.0]
BMI (kg/m^2), mean ± SD	29.6 ± 5.1
Total body fat (kg), mean ± SD	27.1 ± 9.4
Appendicular lean mass (kg), mean ± SD	20.9 ± 5.2
Calf muscle CSA (cm^2), mean ± SD	699.0 ± 160.9
Calf muscle IMAT (cm^2), median [IQR]	16.9 [11.1, 35.2]
Calf muscle density (mg/cm^3), mean ± SD	72.1 ± 3.9
Hand grip strength (kg), mean ± SD	30.7 ± 1.0
Total BOOMER score (out of 16), median [IQR]	15.0 [14.0, 16.0]
Gait speed (cm/s; N=40), mean ± SD	125.9 ± 24.4

Abbreviations: IQR; inter-quartile range, HDL; high-density lipoprotein, LDL; low-density lipoprotein, CRP; C-reactive protein, BMI; body mass index, CSA; cross-sectional area, IMAT; inter- and intra-muscular adipose tissue, BOOMER, Balance Outcome Measure for Elder Rehabilitation. All serum variables were assessed after fasting for at least 10 hours.

Table 1. Descriptive characteristics of study participants.

	IMAT	**Muscle density**
	Spearman correlation (P-value)	
Total cholesterol	-0.109 (0.460)	0.121 (0.411)
HDL cholesterol	**-0.423 (0.003)**	0.231 (0.114)
LDL cholesterol	0.060 (0.685)	0.062 (0.673)
Triglycerides	0.132 (0.373)	-0.067 (0.649)
Glucose	**0.331 (0.022)**	-0.216 (0.140)
CRP	**0.438 (0.002)**	-0.181 (0.219)
	B-coefficient (95% CI)*	
Total cholesterol	-0.005 (-0.020, 0.011)	-0.008 (-0.068, 0.052)
HDL cholesterol	**-0.009 (-0.017, -0.001)**	0.009 (-0.023, 0.040)
LDL cholesterol	0.002 (-0.011, 0.016)	-0.012 (-0.064, 0.039)
Triglycerides	0.004 (-0.004, 0.011)	-0.010 (-0.040, 0.020)
Glucose	0.009 (-0.032, 0.051)	0.027 (-0.131, 0.185)
CRP	**0.847 (0.339, 1.356)**	**-2.14 (-4.200, -0.084)**

Abbreviations: HDL; high-density lipoprotein, LDL; low-density lipoprotein, CRP; C-reactive protein, IMAT; inter- and intra-muscular adipose tissue. Bold text indicates P<0.05.
**Adjusted for age, sex and ALM/BMI*

Table 2. Associations of calf IMAT and muscle density with biochemical parameters.

multivariable regression analyses (Table 2), after adjustment for age, sex and ALM/BMI, higher IMAT CSA remained significantly associated with lower HDL and higher CRP, but not with higher glucose. Higher muscle density was also associated with lower CRP in these adjusted analyses.

Table 3 presents associations between each of calf IMAT CSA and muscle density with each of physical function, balance and gait parameters, after adjustment for age, sex and ALM/BMI. Participants with higher muscle density and lower IMAT CSA took significantly less time to complete the sit-to-stand test, and, amongst 41 participants who completed computerised posturography, also demonstrated significantly lower values for path length, and anteroposterior and mediolateral amplitude, indicating less postural sway. However, muscle density was more

	IMAT	Muscle density
	B-coefficient (95% CI)*	
Hand grip strength (kg)	0.003 (-0.122, 0.127)	**0.507 (0.055, 0.960)**
Knee extension strength (kg)	0.020 (-0.082, 0.123)	0.254 (-0.146, 0.655)
Sit-to-stand (s)	**0.232 (0.122, 0.341)**	**-0.744 (-1.259, -0.228)**
BOOMER tests		
Step test (number of steps)	-0.040 (-0.120, 0.039)	**0.395 (0.089, 0.701)**
TUG (s)	**0.063 (0.014, 0.112)**	**-0.244 (-0.439, -0.049)**
FRT (cm)	-0.104 (-1.609, 1.401)	4.726 (-0.930, 10.382)
Total BOOMER score	-0.13 (-0.030, 0.004)	**0.090 (0.021, 0.159)**
Standing balance test (N = 41)		
Path length (cm)	**0.927 (0.558, 1.295)**	**-3.119 (-4.740, -1.497)**
Anteroposterior amplitude (cm)	**0.032 (0.016, 0.048)**	**-0.116 (-0.183, -0.050)**
Mediolateral amplitude (cm)	**0.010 (0.001, 0.019)**	**-0.070 (-0.111, -0.029)**
Gait parameters (N = 40)		
Gait speed (cm/s)†	**-0.007 (-0.013, -0.001)**	0.016 (-0.009, 0.040)
Cadence (steps/min)	**-0.303 (-0.546, -0.060)**	0.754 (-0.237, 1.745)
Step time (s)	**0.002 (0.001, 0.003)**	-0.003 (-0.007, 0.001)
Step length (cm)	-0.140 (-0.306, 0.025)	0.191 (-0.474, 0.856)
Step width (cm)	**0.075 (0.011, 0.139)**	**-0.347 (-0.587, -0.107)**
Swing time (%)	-0.037 (-0.078, 0.004)	0.067 (-0.097, 0.231)
Double support time (%)	0.076 (-0.005, 0.156)	-0.145 (-0.470, 0.181)

Abbreviations: IMAT; inter- and intra-muscular adipose tissue, TUG; timed up and go, FRT; functional reach test. Bold text indicates P<0.05.
**Adjusted for age, sex and ALM/BMI.*
†Normalised to leg length

Table 3. Associations of calf IMAT and muscle density with physical performance and gait parameters.

strongly associated with BOOMER assessment performance than IMAT CSA, with participants with higher muscle density demonstrating significantly shorter time to perform the TUG and better scores on the step test and total BOOMER score. Amongst BOOMER assessments, participants with high IMAT CSA demonstrated significantly worse performance for the TUG only. The static standing with eyes closed test was not included in these analyses as all participants except one achieved the highest attainable score of 90.

Conversely, IMAT CSA appeared to be more strongly associated with gait performance than muscle density in 40 (83%) participants who completed GAITRite analysis. Those with higher IMAT CSA demonstrated significantly slower gait peed, cadence and step time, and greater step width. A trend approaching significance was also observed for higher IMAT CSA with double support time (P=0.066). Higher muscle density was significantly associated only with smaller step width.

Discussion

The results from this cross-sectional study demonstrate that higher pQCT-determined calf IMAT CSA and lower muscle density are significantly associated with higher CRP levels, and poorer balance and mobility, in community-dwelling older adults, independent of muscle mass. These findings suggest that IMAT is an important contributor to the deleterious metabolic and functional consequences of sarcopenia.

A novel finding of the present study is that higher calf IMAT CSA and lower calf muscle density were associated with greater postural sway (i.e. more instability). A previous study has also reported that high levels of gluteal IMAT are associated with poorer static and dynamic balance in older adults (mean age 74 years; 58% female)[8], however ours is the first study we are aware of to demonstrate this association for calf IMAT via a validated computerised posturography method. The association was stronger in the anterior-posterior compared to medio-lateral axis. The ankle plantar and dorsiflexors act alone to maintain balance in this plane via the inverted pendulum strategy during quiet stance and so, at least for the bipedal balance task performed in this study, it is possible calf IMAT may have a greater impact on balance than IMAT levels for other lower-limb muscle groups[23]. However, studies examining effects of IMAT in different lower-limb muscle groups on balance performance are required to confirm this.

Lower calf IMAT CSA and higher muscle density were associated with improved performance in the sit-to-stand and TUG tests, even after adjustment for lean mass in the present study. Higher muscle density was also associated with higher step test and total BOOMER scores. A previous study has similarly reported that thigh IMAT, but not lean mass, assessed by MRI was

significantly associated with worse performance in stair ascent and descent tasks, and the TUG, in 77 men and 32 women with a mean age of 74 years[5]. In the Health Aging and Body Composition study including 3,075 men and women aged 70 to 79 years, lower thigh muscle attenuation (indicating higher fat infiltration) measured by CT was associated with worse performance in a lower-extremity performance assessment including the sit-to-stand test[24].

Our study is also the first to report associations of IMAT with several different gait parameters in older adults. Higher calf IMAT CSA was associated with slower gait speed and cadence, and greater step time and width, while higher muscle density was associated with smaller step width only. In the Health ABC study, a 1SD higher baseline thigh IMAT CSA was associated with 25 and 50% increased odds in 1,552 women and 1459 men respectively, for poor performance (defined as gait speed <100 cm/s) nine years later[25]. Conversely, a recent analysis of 2,725 participants (57% women) with a mean age of 74 years in the AGES-Reykjavik study reported that mid-thigh IMAT and muscle attenuation did not consistently influence incident mobility disability over five years[26]. An intervention study has also demonstrated that reductions in calf, but not thigh, IMAT were associated with improved walking speed in 27 obese women with a mean age of 64 years[9]. Furthermore, the only other study we are aware of to examine associations of IMAT with gait parameters assessed by the GAITRite® system was conducted by Addison and colleagues and reported that older adults with high step-to-step variability in stride width, swing and double support time, have higher IMAT of the gluteal muscles, but not thigh muscles[8]. It is possible that IMAT accumulation in the calf and gluteal muscles is more consistently associated with poorer gait performance in older adults than IMAT of the thigh muscles, although prospective studies are required to confirm this.

It is interesting to note that high IMAT CSA appeared to be consistently associated with poorer gait performance but not BOOMER tasks, while low muscle density was generally associated with poorer balance but not gait performance. A recent study demonstrated that MRI-derived muscle adiposity represents only 50% of CT-derived muscle density[27], and we similarly observed a moderate correlation between IMAT and muscle density ($r=-0.62$; $P<0.01$) in this study. It has been suggested that pQCT-assessed IMAT represents intermuscular adipose tissue (visible fat beneath the fascia lata) while muscle density is indicative of greater intramuscular fat content (fat between muscle fibres and fat within muscle cell)[28]. Thus, high IMAT and low muscle density may be separate but related components of muscle quality that have differing effects on aspects of physical function during ageing.

High calf IMAT CSA and low muscle density were positively and independently associated with serum CRP, a biomarker for systemic inflammation. High CRP levels have previously been associated with lower calf muscle density and higher thigh IMAT, as well as hyperinsulinaemia and insulin resistance[29,30]. Addison and colleagues have demonstrated a significant positive correlation between IMAT and each of interleukin-6 mRNA and interleukin-6 protein in 26 frail and non-frail older adults with a mean age of 81 years[31]. It has been suggested that IMAT may contribute to poor muscle function through increasing systemic inflammation[5], and indeed, a study of 542 older adults (mean age 80 years, 49% female) demonstrated that high levels of inflammation were associated with poor physical performance[32]. However, in our analyses, adjustment for CRP did not attenuate associations of IMAT CSA or muscle density with physical function outcomes (data not shown). It is possible that inflammatory markers (such as IL-6) not measured in our study influence this relationship, although prospective studies are required to clarify whether higher IMAT contributes to increased levels of localised and/or systemic inflammation, or whether increased inflammation is explained by effects of ageing and general obesity, rather than IMAT *per se*.

It is also possible that insulin resistance and diabetes, independent of or related to high IMAT levels[33], contributed to the poorer physical function observed in participants with higher IMAT. NHANES III data from 3,475 men and 3,113 women aged ≥60 years has demonstrated that older adults with diabetes have two- to three-fold increased odds of poor physical performance[7]. In the present study, IMAT CSA was positively correlated with fasting blood glucose and negatively correlated with HDL, but only the association with HDL remained significant after adjustment for confounders, suggesting that IMAT does not independently cause insulin resistance. Furthermore, adjustment for diabetes status (according to fasting glucose levels) in physical function analyses did not attenuate observed associations (data not shown), suggesting that insulin resistance does not explain the effects of IMAT on physical function. The improved HDL levels of participants with low IMAT is consistent with a previous cross-sectional study of 82 premenopausal women (mean age 39 years) which reported higher calf muscle density is associated with higher HDL levels[10], and an aerobic exercise intervention in 33 women and 40 men aged 40 to 65 years demonstrated that reductions in thigh IMAT were associated with improvements in lipid profile, albeit in men only[34]. Thus, while further research is required to determine its associations with long-term cardiometabolic outcomes, IMAT may be an important target for improving cardiometabolic health and physical function in older adults, particularly given it can be substantially reduced with exercise[4].

The primary limitations of our study include its cross-sectional design, which restricts comments on causation, and the potential bias attributable to our recruitment methods. Indeed, the results indicated that our cohort generally had good physical function and cardiometabolic health, and few co-morbidities, despite a relatively high prevalence of obesity. It is likely that our findings may not be generalisable to less healthy older adult populations. The sample size was relatively small which restricted our ability to adjust for multiple confounders in multivariable models, and it is possible that residual confounding remains for lifestyle factors including physical activity and diet. We did not adjust for total body or visceral fat in our models as the primary aim was to investigate IMAT effects independent of sarcopenia, but the ALM/BMI measure is indicative of muscle mass relative to body fat. Finally, whilst pQCT has several advantages over other IMAT measurement techniques, it is limited in that it is not capable of distinguishing intra- and inter-muscular adipose tissue[10] and it is not possible to isolate individual muscle groups, although a previ-

ous MRI study indicates that calf IMAT is highest in the gastrocnemius[35]. There is also no standardised thresholds for separating skeletal muscle from other tissues using linear attenuation coefficients within CT images[36], and differences in observed associations of IMAT with cardiometabolic health and physical function between previous studies and our own may be influenced by differences in IMAT measurement sites, techniques and thresholds.

In conclusion, community-dwelling older adults with higher calf IMAT CSA and lower muscle density determined by pQCT have poorer balance and inflammatory status. As these effects are independent of muscle mass, IMAT is likely to represent a novel and important target for interventions aimed at improving physical function in older adults.

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