Archives of Gerontology and Geriatrics 81 (2019) 1–7

Contents lists available at ScienceDirect

Archives of Gerontology and Geriatrics

journal homepage: www.elsevier.com/locate/archger

Inter-muscular adipose tissue is associated with adipose tissue inflammation and poorer functional performance in central adiposity

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ARTICLE INFO

Keywords:
Sarcopenic obesity
Intermuscular adipose tissue
Monocyte Chemoattractant Protein-1

ABSTRACT

Background: The presence of concomitant sarcopenia and obesity in sarcopenic obesity (SO) confers worse functional, morbidity and mortality outcomes compared to either alone. Excess adiposity and central redistribution of fats are associated with systemic inflammation and ectopic tissue fat infiltration in forms of Intermuscular adipose tissue (IMAT). Our study examines the profile of IMAT across a spectrum of body compositions and associations with physical performance and inflammatory biomarkers including Monocyte Chemoattractant Protein-1 (MCP-1), a novel biomarker of adipose tissue inflammation.

Methods: 187 community dwelling elderly participants were recruited and classified into 4 subgroups: normal, obese, sarcopenia and SO, using validated criteria for sarcopenia and waist circumference to define central obesity. We performed magnetic resonance imaging of mid-thigh sections to segment IMAT and muscle. Participants were assessed for muscle strength, physical performance and blood inflammatory biomarkers of interleukin-6, C-Reactive Protein and MCP-1. We examined correlation of IMAT(ratio) with muscle function measures and blood biomarkers. Multiple regression analyses were used to examine the association of body composition types and IMAT(ratio) with muscle function.

Results: IMAT(ratio) was highest in SO and obese groups. Overall, higher IMAT(ratio) is significantly associated with raised MCP-1, lower gait speed and muscle strength. SO had lowest scores in Short Physical Performance Battery (SPPB), gait speed, hand-grip and knee extension strength. IMAT(ratio) is independently associated with SPPB and handgrip strength, whilst SO is independently associated with muscle strength.

Conclusion: Our results suggest the possible role of IMAT as a candidate imaging biomarker for adipose tissue inflammation and associated poorer functional outcomes in SO.

1. Introduction

Aging is associated with loss of muscle and bone mass, and increase in fat mass and visceral fat (Sakuma & Yamaguchi, 2013; Riechman, Schoen, Weissfeld, Thaete, & Kriska, 2002). Different patterns of disordered body composition in the older adults are associated with different outcomes (Tyrovolas et al., 2016), with sarcopenia being one of the most extensively researched body composition type. The European Working Group on Sarcopenia in Older People (EWSOG) defines sarcopenia as a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength, leading to increased adverse outcomes such as falls, hospital admissions and mortality (Cruz-Jentoft et al., 2010). With an increasing proportion of the population becoming obese, the co-existence of obesity and sarcopenia is also increasingly observed in the elderly (Roubenoff, 2004). There is emerging evidence that concomitant sarcopenia and obesity confer worse functional, morbidity and mortality outcomes compared to either sarcopenia or obesity in isolation (Baumgartner et al., 2004; Huo et al., 2016; Rolland, Czerwinski, Van Kan, Morley, & Cesary, 2008). For instance, in the INCHANTI study, sarcopenic obese (SO) compared to sarcopenic

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https://doi.org/10.1016/j.archger.2018.11.006
Received 16 August 2018; Received in revised form 2 November 2018; Accepted 10 November 2018
Available online 13 November 2018
0167-4943/

non-obese participants exhibited a steeper decline in walking speed and a higher risk of developing new mobility disability over the 6-year follow-up (Stenholm, Alley, & Bandinelli, 2009).

The pathogenesis behind the poorer outcomes of SO individuals is hypothesized to be contributed by fat infiltration, mechanical stress, impairment of respiratory and cardiovascular function, metabolic and neuro-humoral signaling, and altered inflammatory responses (Roubenoff, 2000; Chung, Kang, Lee, Lee, & Lee, 2013). Adipose tissue inflammation has been shown to be the major contributor to disordered systemic inflammation that leads to metabolic syndrome and type 2 diabetes in obesity (Esser, Legrand-Poels, Piette, Scheen, & Paquot, 2014). Recent studies implicate the cross-talk between adipose tissue and skeletal muscle in the development of SO, with obesity-associated adipose tissue inflammation believed to play a dominant role in creating a vicious cycle which supports a state of chronic low grade systemic inflammation (Kalinkovich & Livshits, 2017). The pro-inflammatory milieu associated with excess adiposity, fat re-distribution and infiltration enhances catabolism of skeletal muscle through paracrine, autocrine and endocrine interactions between adipokines, myokines and chemokines, which in turn exacerbates the loss of muscle mass and muscle function (Kalinkovich & Livshits, 2017).

Obesity is also characterized by excessive production and diminished capacity to store lipids, leading to ectopic fat accumulation in skeletal muscle in the form of intramuscular and intermuscular adipose tissue (IMAT) (Aguiari et al., 2008). IMAT is defined as ectopic adipose tissue located between muscle groups beneath the muscle fascia and intramuscular adipose tissue which is visible on Magnetic Resonance Imaging (MRI) images (Vettor et al., 2009). The recent interest in IMAT is fueled by emerging evidence that myosteatosis is associated with visceral fat accumulation, elevated leptin levels, insulin resistance and adipose tissue inflammation (Zoico et al., 2013). Recent reports of significantly elevated levels of adipocyte chemokines such as MCP-1 in conjunction with impaired physical performance in SO suggest that IMAT may be a candidate imaging biomarker for adipose tissue inflammation and associated functional outcomes (Lim et al., 2015).

The influence of IMAT on functional performance and its association with inflammatory biomarkers in different body composition types amongst older adults is however less established. Studies on IMAT are usually performed on healthy older adult participants (Vettor et al., 2009) and few studies examine IMAT in relation to clinical phenotypes of disordered body composition and obesity (Addison, Marcus, LaStayo, & Ryan, 2014). Another issue is the lack of consensus in the definition of SO (Prado, Wells, Smith, Stephan, & Siervo, 2012), leading to different observations of clinical outcomes of SO. General measures of obesity such as body mass index, fat mass, fat-free mass index and muscle-to-fat ratio do not account for the central role played by fat redistribution in the pathogenesis of SO. In contrast, definitions for obesity such as waist circumference, waist-to-hip ratio and visceral fat area that focus on central obesity have been shown to be associated with increased systemic inflammation, metabolic syndromes and adverse cardiovascular outcomes (dos Santos et al., 2014; Spoto et al., 2014).

Our study therefore sets out to examine the profile of IMAT across a clinically defined spectrum of body composition types comprising: i) non-obese and non-sarcopenic (“normal”), ii) obese, iii) sarcopenic and iv) sarcopenic obese groups. Consistent with the study focus on adipose tissue inflammation, we defined obesity using waist circumference. We seek to examine the association of IMAT with physical performance and inflammatory biomarkers in the four body composition types. We hypothesize that SO participants will have worse physical performance compared to other body composition types, and this is mediated by increased IMAT and a pro-inflammatory state.

2. Methods

2.1. Study population and groups

We recruited 200 cognitively intact and community-dwelling older adults aged 50 years and older from August 2013 to November 2014, as part of a larger longitudinal study (GERILABS: Longitudinal Assessment of Biomarkers for characterisation of early Sarcopenia and predicting Frailty and Functional Decline in community-dwelling Asian older adults Study). Details of the study have been earlier described (Tay et al., 2015). In brief, inclusion criteria include community dwelling, cognitively intact, elderly participants between the ages of 50 to 99 years, and are independent in both basic activities of daily living (ADL) and instrumental ADL. For this study, we also excluded participants with preexisting inflammatory conditions (such as Rheumatoid Arthritis, Inflammatory Bowel Disease) and current use of immunosuppressants.

We classified each subject into 4 subgroups based on clinical criteria: normal, obese, sarcopenic, or sarcopenic obese. Obesity was defined with the revised National Cholesterol Education Program (NCEP)-obesity definition of waist circumference ($\geq$90 cm for Asian man and $\geq$80 cm for Asian women) (NCEP, 2001). Sarcopenia was defined by a presence of low gait speed and/or handgrip strength, and low muscle mass in accordance to the Asian Working Group for Sarcopenia criteria (Chen et al., 2014). The cutoff for usual gait speed is $<$ 0.8 m/s, and cutoffs for handgrip strength are $<$ 26 kg for men and $<$ 18 kg for women. Muscle mass measurements cutoffs are 7.0 kg/m^2 for men and 5.4 kg/m^2 for women by dual X-ray absorptiometry. SO individuals fulfilled criteria for both sarcopenia and obesity. Subjects were classified as normal if they did not fulfill criteria for the other 3 subgroups. Ethics approval was obtained from the Domain Specific Review Board of the National Healthcare Group, and written informed consent obtained from the study participant.

2.2. Data collection

2.2.1. Clinical measurements

Demographic and clinical data were obtained. Smokers were defined as respondents who answered positive to “currently smoking” and alcohol ingestion was defined as respondents who responded positive to “currently taking alcohol”. Standing height, body weight and waist circumference were measured, and body mass index (BMI) was calculated. Percentage body fat and lean mass measures were obtained via dual- energy X-ray absorptiometry (DXA: Discovery™ APEX 13.3; Hologic, Bedford, MA, USA). Appendicular skeletal mass (ASM) was derived from the summation of muscle mass measurements in the four limbs. Skeletal mass index (SMI) was defined as ASM/Height^2.

Grip strength was measured using a hydraulic hand dynamometer (North Coast Medical, Inc, Gilroy, CA, USA). Two trials of grip strength were obtained for each hand, with all four trials averaged to yield strength. Knee extensor muscle strength was measured using an electronic dynamometer (BASELINE PUSHPULL Dynamometer), with the participant seated at the edge of a chair and maintaining the trunk in the upright position. Each participant had 2 measures performed for each lower limb and the average of the 4 readings provided a measure of knee extension strength. Gait speed was based on the time to walk 3 m. Participants were instructed to walk at their usual pace and the best time for two trials was recorded. Physical performance was assessed on the three components of balance, gait speed and repeated chair stand of the Short Physical Performance Battery (SPPB) (Guralnik et al., 1994).

2.2.2. Laboratory assays

Venous blood samples obtained at baseline were centrifuged at 3000 rpm for 10 min, divided into aliquots, and frozen at −80 °C to ensure integrity of the specimens. Monocyte Chemoattractant Protein-1

(MCP-1) (DuoSet; R&D Systems, Minneapolis, MN, USA) and Interleukin 6 (IL-6) assays (eBioscience, San Diego, CA, USA) were performed according to manufacturer's instructions. C-Reactive Protein assays (CRP) were performed in a clinical laboratory of Tan Tock Seng Hospital. All serum samples were measured in duplicate, the lower detection limit for MCP-1 was set at 10 pg/mL and 0.04 pg/mL for IL-6. A cutoff of more than 5 mg/l was considered elevated for CRP.

2.2.3. Magnetic resonance imaging

We performed magnetic resonance imaging (MRI) of both thighs using two-dimensional modified Dixon T1- weighted gradient-echo pulse sequence with a 3 T system (Siemens Magnetom Trio, Germany). Muscle, subcutaneous adipose tissue and intermuscular adipose tissue were automatically segmented using an in-house machine learning based segmentation (Yang et al., 2016). Due to the anatomical variations among subjects, the middle third of both thighs was selected to avoid the complexity of the pelvic and knee joint regions. Volumes of muscle and fat were computed using the cylinder method for the middle third of the thigh (Yang et al., 2017). IMAT(ratio) was expressed as the ratio of IMAT volume to total volume of the imaged thigh (sum of muscle volumes and fat volumes). IMAT(ratio) was used to allow for comparable results by correcting for different body mass.

2.3. Statistical analysis

For comparison of baseline characteristics between the 4 subgroups, we used one-way analysis of variance with Bonferroni correction for post-hoc comparison of continuous variables, Chi-square test for categorical variables and Fischer Exact tests for categorial variables with small numbers. Cardiovascular risk factors of hypertension, hyperlipidemia, smoking, diabetes mellitus, atrial fibrillation and cardiovascular conditions of ischemic heart disease, peripheral vascular disease, stroke were pooled together to form a cardiovascular composite score for analysis across the subgroups (Tay, Lim, Chan, Chong, & Sitoh, 2011).

To study the relationship between IMAT(ratio) with physical performance and inflammatory biomarkers, we used Pearson correlation for parametric variables, and Spearman's rho for non-parametric variables. Finally, we performed multiple linear regression analyses to examine the association of functional performance (grip strength, knee extension strength, SPPB and gait speed) with body composition phenotype and IMAT(ratio), adjusted for age, gender, smoking status, alcohol ingestion, and SMI. The reference category for body composition phenotype was the non-obese non-sarcopenic group. Statistical Package for the Social Sciences (SPSS) version 22 was used for statistical analysis. The level of significance was set at 5%.

3. Results

Among 200 participants recruited, 10 participants were excluded as they did not complete MRI assessment and 3 further participants were excluded as they were taking immunosuppressants. The 13 participants who were excluded had a significantly higher proportion of males (χ^2 (1) = 5.81, p = 0.016), hypertension (χ^2 (1) = 4.66, p = 0.031) and stroke prevalence (χ^2 (1) = 9.47, p = 0.002) compared to included study group, however there were no significant differences in age (F (1,198) = 0.690, p = 0.407), body fat percentage (F(1.198) = 0.328, p = 0.568) nor SMI (F(1,198) = 3.329, p = 0.070). Out of the remaining 187 participants, 42 (22.5%) participants were in the normal group, 102 (54.6%) were obese, 42 (22.5%) were sarcopenic and 17 (9.1%) were SO. The mean age of the participants was 67.8 years old, with a female predominance of 70.1%. Table 1 shows the baseline characteristics of our study population. Of note, age and gender were significantly different between the groups. In particular the SO group tended to be older (M = 74.5, SD = 9.8) and had fewer males (11.8%). Besides stroke disease, there were no differences in cardiovascular risk factors, ischemic heart disease and other comorbid conditions between the groups.

3.1. Anthropometric data, physical performance and blood biomarkers

Significant differences in anthropometric measures of BMI, waist circumference, percentage body fat and SMI were noted between the groups, as expected per definition of the groups (Table 2). IMAT(ratio) was significantly different between the subgroups (F(3,183) = 6.619, p < 0.001), being highest in the SO (M = 0.0335, SD = 0.0178) followed by obese (M = 0.0274, SD = 0.0128) groups. Of note, post-hoc pairwise comparison with Bonferroni correction reveals statistically significant higher IMAT(ratio) for obese and sarcopenic obese groups compared to normal group, but no difference between sarcopenic with normal group. Compared with the sarcopenic group, SO subjects have significantly higher waist circumference, percentage body fat and IMAT(ratio) (p < 0.05, Bonferroni correction), and lower (albeit non-significant) SMI.

There was a statistically significant difference across all four groups in physical performance measures, with SO group having the lowest scores in SPPB, gait speed, grip strength and knee extension strength. Post-hoc comparisons with Bonferroni correction indicated that the mean SPPB scores were significantly lower in SO (M = 10.24, SD = 2.51) compared to sarcopenic (M = 11.62, SD 0.70, p = 0.007), obese (M = 11.26, SD = 1.41, p = 0.022), and normal (M = 11.71,SD = 0.55, p = 0.001) groups. Grip strength and knee extension strength were also lower in the SO compared to obese and normal groups (p < 0.05, Bonferroni correction).

For blood biomarkers, MCP-1 was significantly different across the subgroups [F(3,183) = 5.220, p = 0.002], being highest in the SO followed by obese groups. MCP-1 was also significantly higher in the SO group compared to the normal group. CRP was also shown to be statistically different across the subgroups [F(3,182) = 3.47, p = 0.017] being highest in the SO group, however post-hoc pairwise comparisons with Bonferroni correction of subgroup means did not demonstrate statistically significant differences. Another traditional inflammatory marker of IL-6 was also highest among SO participants, although no statistically significant difference was found between the subgroups.

3.2. Correlation between IMAT with physical performance and blood biomarkers

Grip strength, knee extension strength and gait speed were inversely correlated with IMAT(ratio) (Table 3: grip strength r = – 0.244, p = 0.001; knee extension strength r = -0.205, p = 0.005; gait speed r = -0.170, p = 0.020). Stratifying by subgroups, significant inverse correlations between IMAT(ratio) with gait speed (r= -0.240, p = 0.015), knee extension strength (r = -0.235, p = 0.017) and grip strength (r= -0.252, p = 0.011) were observed in the obese subgroup, with a similar trend observed in SO group although this did not reach statistical significance. In the sarcopenic subgroup, significant positive correlations were found between IMAT(ratio) with grip strength (r = 0.428, p = 0.029) and SPPB (r = 0.413, p = 0.036).

Among inflammatory biomarkers, only MCP-1 exhibits a significant correlation with IMAT(ratio) (Table 3: Pearson correlation coefficient = 0.286, p < 0.001). In subgroup analysis, this significant correlation is observed only in the SO group (r = 0.556, p < 0.05).

3.3. Multiple linear regression analysis

After adjusting for gender, age, SMI, smoking status and alcohol ingestion, body composition phenotypes of sarcopenia (β= -0.269, p < 0.001) and SO (β = -0.140, p < 0.05), and IMAT(ratio) (β= -0.101, p < 0.05) significantly predicted handgrip strength. This regression model with handgrip strength as the dependent variable has the highest R^2, accounting for 64.7% of the model variance (R^2 = 0.647, F(9,177) = 38.86, p < 0.001). Regression models

Table 1
Baseline Characteristics among Subgroups.

Variable	Overall (n = 187)	Non-obese, Non-sarcopenic (n = 42)	Obese (n = 102)	Sarcopenic (n = 26)	Sarcopenic Obese (n = 17)	p-value
Age (years)	67.8 ± 7.4	66.5 ± 7.4	66.7 ± 7.4	69.8 ± 7.1	74.5 ± 9.8	0.000
Male, n (%)	56 (29.9%)	19 (45.2%)	25 (24.5%)	10 (38.5%)	2 (11.8%)	0.021
Chinese, n (%)	171 (91.4%)	40 (95.2%)	88 (86.3%)	26 (100%)	17 (100%)	0.369
Cardiovascular composite score	1.51 ± 1.15	1.40 ± 1.23	1.59 ± 1.10	1.31 ± 1.12	1.65 ± 1.32	0.605
Hypertension, n (%)	86 (46.0%)	15 (35.7%)	51 (50.0%)	10 (38.5%)	10 (58.8%)	
Diabetes mellitus, n (%)	41 (21.9%)	9 (21.4%)	26 (25.5%)	2 (7.7%)	4 (23.5%)	
Hyperlipidemia, n (%)	121(64.7%)	26 (61.9%)	70 (68.6%)	14 (53.8%)	11 (64.7%)	
Ischemic Heart Disease, n(%)	4 (2.1%)	1 (2.4%)	1 (1.0%)	1 (3.8%)	1 (5.9%)	
Previous/Current Smoker, n(%)	20 (10.7%)	4 (9.5%)	10 (9.8%)	4 (15.4%)	2 (11.8%)	
Peripheral Vascular Disease	0	0	0	0	0	
Atrial fibrillation, n (%)	8 (4.3%)	3 (7.1%)	4 (3.9%)	1 (3.8%)	0 (0%)	
Stroke, n (%)	3 (1.6%)	1 (2.4%)	0 (0%)	2 (7.7%)	0 (0%)	
Alcohol ingestion, n (%)	4 (2.1%)	1 (2.4%)	3 (2.9%)	0 (0%)	0 (0%)	0.154
Advanced organ failure, n(%)	1 (0.5%)	0 (0%)	1 (1.0%)	0 (0%)	0 (0%)	–
Malignancy, n (%)	11 (5.9%)	3 (7.1%)	5 (4.9%)	2 (7.7%)	1 (5.9%)	0.858

Table 2
Comparison of Anthropometric Data, Physical Performance and Blood Biomarkers.

Variable	Overall (n = 187)	Non-obese, Non-sarcopenic (n = 42)	Obese (n = 102)	Sarcopenic (n = 26)	Sarcopenic Obese (n = 17)	p-value
Anthropometric Measurements						
Body Mass Index (kg/m^2)	23.8 ± 3.6	21.4 ± 2.5[a]	25.8 ± 3.5[ade]	20.8 ± 1.9[d]	22.8 ± 1.5[e]	0.00
Waist circumference (cm)	86.2 ± 9.0	78.5 ± 6.6[ac]	91.4 ± 7.0[ade]	77.6 ± 6.1[df]	87.7 ± 4.5[cf]	0.00
Body fat, %	36.4 ± 6.8	31.1 ± 5.9[ac]	38.9 ± 6.0[ad]	33.1 ± 5.7[df]	40.2 ± 4.9[cf]	0.00
Skeletal mass index (kg/m^2)	6.083 ± 1.03	5.98 ± 0.86	6.43 ± 1.06[de]	5.41 ± 0.75[d]	5.26 ± 0.59[e]	0.00
IMAT (ratio)	0.0254 ± 0.0134	0.0197 ± 0.0127[ac]	0.0274 ± 0.0128[a]	0.0214 ± 0.0087[f]	0.0335 ± 0.0178[cf]	0.00
Physical Performance /Muscle Strength						
SPPB total (0-12)	11.32 ± 1.38	11.71 ± 0.55[c]	11.26 ± 1.41[e]	11.62 ± 0.70[f]	10.24 ± 2.51[cef]	0.001
Gait speed (m/s)	1.15 ± 0.21	1.20 ± 0.23[c]	1.15 ± 0.20	1.10 ± 0.19	1.02 ± 0.24[c]	0.012
Handgrip strength (kg)	21.81 ± 6.59	24.65 ± 6.61[bc]	22.66 ± 6.49[de]	17.80 ± 3.82[bd]	15.87 ± 3.92[ce]	0.00
Knee extension strength (kg)	34.95 ± 7.76	36.23 ± 7.43[c]	36.19 ± 7.56[e]	32.38 ± 5.44	28.28 ± 8.93[ce]	0.00
Blood Biomarker						
MCP-1, pg/mL	126.10 ± 55.19	104.12 ± 33.36[ac]	133.16 ± 57.26[a]	114.11 ± 47.10	156.34 ± 74.86[c]	0.002
IL-6	1.60 ± 1.83	1.79 ± 2.10	1.58 ± 1.84	0.96 ± 0.77	2.21 ± 2.04	0.133
CRP	1.66 ± 2.40	1.11 ± 1.54	1.98 ± 2.75	0.75 ± 0.87	2.54 ± 2.94	0.017
Vitamin D	29.04 ± 9.54	30.29 ± 9.02	28.50 ± 9.63	29.81 ± 11.43	28.00 ± 7.22	0.705

IMAT = Inter-muscular adipose tissue, SPPB = Short Physical Performance Battery, MCP-1 = Monocyte Chemoattractant Protein-1; IL-6 = Interleukin-6, CRP = C-reactive Protein.
[a]post hoc results (with Bonferroni correction) between normal and obese ($p < 0.05$).
[b]post hoc results (with Bonferroni correction) between normal and sarcopenia ($p < 0.05$).
[c]post hoc results (with Bonferroni correction) between normal and SO ($p < 0.05$).
[d]post hoc results (with Bonferroni correction) between obese and sarcopenia ($p < 0.05$).
[e]post hoc results (with Bonferroni correction) between obese and SO ($p < 0.05$).
[f]post hoc results (with Bonferroni correction) between sarcopenia and SO ($p < 0.05$).

Table 3
Correlation between IMAT Ratio with Physical Performance and Blood Biomarker.

Correlation	Overall (n = 187)	Non-obese, Non-sarcopenic (n = 42)	Obese (n = 102)	Sarcopenic (n = 26)	Sarcopenic Obese (n = 17)
IMAT(ratio) and SPPB[†]	−0.127	−0.033	−0.189 (p = 0.057)	0.413*	−0.252
IMAT(ratio) and Gait Speed	−0.170*	−0.006	−0.240*	0.046	−0.065
IMAT(ratio) and Handgrip strength	−0.244*	−0.241	−0.252*	0.428*	−0.471 (p = 0.056)
IMAT(ratio) and Knee Extension strength	−0.205*	−0.187	−0.235*	0.118	−0.147
IMAT(ratio) and MCP-1	0.286*	0.013	0.166	0.296	0.556*
IMAT(ratio) and IL-6	−0.101	−0.096	−0.068	0.089	−0.016
IMAT(ratio) and CRP	0.084	0.044	−0.029	−0.207	0.235

IMAT = Inter-muscular adipose tissue, SPPB = Short Physical Performance Battery, MCP-1 = Monocyte Chemoattractant Protein-1; IL-6 = Interleukin-6, CRP = C-reactive Protein.
*Correlation is significant at the 0.05 level (2-tailed).
[†]Spearman correlation used for nonparametric variables.

Table 4a
Multiple Linear Regression Analysis for SPPB and Gait Speed.

Outcomes	SPPB^				Gait Speed^			
VARIABLES	R^2	Standardised Coefficient	B ± Std Error	P value	R^2	Standardised Coefficient	B ± Std Error	P value
Obese	0.145	−0.097	−0.269 ± 0.267	0.315	0.086	−0.108	−0.046 ± 0.042	0.279
Sarcopenic		0.034	0.136 ± 0.330	0.680		−0.108	−0.066 ± 0.052	0.208
Sarcopenic Obese		−0.158	−0.756 ± 0.405	0.064		−0.125	−0.092 ± 0.064	0.156
IMAT(ratio)		−0.171*	−17.603 ± 7.648	0.023		−0.093	−1.469 ± 1.215	0.228

IMAT = Inter-muscular adipose tissue, SPPB = Short Physical Performance Battery.
^Reference group: Non-obese, Non-sarcopenic; adjusted for age, gender, smoking status, alcohol ingestion and appendicular skeletal mass index.

Table 4b
Multiple Linear Regression Analysis for Muscle Strength.

Outcomes	Handgrip Strength^				Knee Extension Strength^			
VARIABLES	R^2	Standardised Coefficient	B ± Std Error	P value	R^2	Standardised Coefficient	B ± Std Error	P value
Obese	0.647	−0.021	−0.276 ± 0.819	0.736	0.285	0.076	1.176 ± 1.374	0.393
Sarcopenic		−0.268*	−5.099 ± 1.011	0.000		−0.123	−2.751 ± 1.695	0.106
Sarcopenic Obese		−0.140*	−3.205 ± 1.243	0.011		−0.161*	−4.325 ± 2.085	0.039
IMAT(ratio)		−0.101*	−49.493 ± 23.456	0.036		−0.134	−77.448 ± 39.331	0.050

IMAT = Inter-muscular adipose tissue; ^Reference group: Non-obese, Non-sarcopenic; adjusted for age, gender, smoking status, alcohol ingestion and appendicular skeletal mass index.

involving SPPB (R^2 = 0.145, F(9,177) = 4.50, $p < 0.001$), knee extension strength (R^2 = 0.285, F(9,177) = 9.22, $p < 0.001$) and gait speed (R^2 = 0.086, F(9,177) = 2.94, $p < 0.05$) demonstrated much lower R^2 (Tables 4a and 4b).

IMAT(ratio) independently predicted SPPB and handgrip strength (β = -0.171 and -0.101 respectively, $p < 0.05$), and showed a trend for knee extension strength (β= -0.134, p = 0.05). Comparing between body composition phenotypes, SO significantly predicted both handgrip strength and knee extension strength (β= -0.140 and -0.161 respectively, $p < 0.05$), while sarcopenia significantly predicted handgrip strength per se (β= -0.268, $p < 0.001$). Sarcopenic and obese subgroups were not significantly associated with knee extension strength, SPPB or gait speed.

4. Discussion

Our study highlights the differential profile of IMAT(ratio) across different body composition phenotypes, being highest in SO and obese groups and with no significant difference between sarcopenic and non-obese non-sarcopenic groups. In addition, our study contributes to the growing body of evidence about the synergistic impact of concomitant central obesity and sarcopenia by demonstrating that IMAT is positively correlated with MCP-1, a novel early biomarker of adipose tissue inflammation, and associated with poorer functional outcomes in SPPB and grip strength. These results corroborate the putative role of IMAT in the pathogenesis of SO via systemic inflammation and suggest that IMAT may be a candidate imaging biomarker for adipose tissue inflammation and associated functional outcomes in SO.

Interestingly, IMAT(ratio) is positively correlated with SPPB and handgrip strength amongst the sarcopenic group, such that higher IMAT(ratio) is paradoxically associated with better muscle function. This contrasts with the negative correlation between IMAT (ratio) and muscle function in the other body composition phenotypes. Notably, IMAT(ratio) in sarcopenic and normal groups were comparable. We therefore postulate that the contributing factors of poorer muscle function in sarcopenia are different from those in obese and sarcopenic obese groups. As IMAT(ratio) is a ratio of IMAT volume to total volume of the imaged thigh, the higher IMAT(ratio) values amongst sarcopenic subjects may instead be explained by concomitantly reduced thigh muscle volume, such that the major contribution of poorer muscle function is attributable to reduced skeletal muscle mass as opposed to IMAT volume.

The results in our study about the deleterious impact of SO highlight the importance of choosing a measure of central adiposity in the definition of SO. Waist circumference is a surrogate of central adiposity (Janssen, Katzmarzyk, & Ross, 2004) and may be more accurate in categorizing different systemic inflammation states, compared to general means of classifying obesity that do not take into account the effects of fat re-distribution. A study comparing the associations of SO with cardiovascular risks and inflammatory biomarker of CRP using different classifications of SO showed that waist circumference was superior to fat free mass, residual calculation and fat mass index in screening for metabolic and cardiovascular risk factors and had a stronger correlation with CRP (dos Santos et al., 2014; Oliveira et al., 2011; Dulloo, Jacquet, Solinas, Montani, & Schutz, 2010). Waist circumference was also more strongly correlated with MCP-1 compared with fat mass (Lim et al., 2015). Furthermore, it has the added advantage of ease of measurement which makes it a useful, relevant and low cost measure in clinical practice.

As opposed to earlier studies that utilized DXA or bioelectrical impedance analysis to study body composition in SO, the use of advanced MRI segmentation techniques enabled us to identify and quantify IMAT from muscle and subcutaneous adipose tissue. The novel use of IMAT(ratio) corrected for different body mass enhanced its discriminatory ability in differentiating the impact of IMAT on MCP-1 and physical performance among the body composition types. In addition, we examined MCP-1, a novel biomarker of inflammation, which may be more sensitive for adipose tissue inflammation in SO than general biomarkers of inflammation such as CRP or IL-6. This is supported by the observation that MCP-1 levels are more elevated in SO compared with sarcopenia or obesity in isolation (Lim et al., 2015). Besides being an early marker of systemic inflammation, MCP-1 may be also be a marker of muscle repair in tissue injury and impaired muscle strength (Deshmane, Kremlev, Amini, & Sawaya, 2009; Labbe et al., 2010; Mann et al., 2011). Taken together, this supports the utility of IMAT as a biomarker of inflammation and muscle performance in SO.

Two hypothetical scenarios of development of the SO phenotype have been proposed, derived from 2 different trajectories of weight gain and weight loss (Prado et al., 2012). The first scenario occurs in normal-weight individuals who gain weight, with gains from fat mass occurring

in excess of gains in fat free mass to result in a SO phenotype. The second scenario occurs in weight loss in morbidly obese individuals, who lose fat free mass in excess of fat mass. However, it is to be noted that these 2 scenarios characterized sarcopenia by muscle mass, without considering muscle strength and muscle quality. In contrast, Roubenoff (2000) noted that fat infiltration within the muscle in inflammatory conditions such as rheumatoid arthritis occur in an energy neutral state independent of weight changes, suggesting the primary role of inflammation above and beyond the effect of weight (Roubenoff, 2000). Building upon this observation, we propose a third mechanism of inflammation being the main driver in the development from obesity to SO, independent of weight changes. Our study result demonstrating the association of IMAT with MCP-1 in the SO group supports this hypothesis. In addition, IMAT is significantly associated with poorer physical performance independent of muscle mass, reflecting the importance of muscle quality in the pathogenesis of SO. In support of this, the Health Aging and Body Composition Study reported that muscle strength and muscle fat infiltration were independently predictive of incident mobility limitation (Visser et al., 2005).

Limitations of our study included inadequate representation of the male population resulting in the inability to conduct subgroup analysis by gender. Baseline characteristics of the excluded participants were also significantly different from the study participants in terms of gender proportion, prevalence of hypertension and stroke. However, there were no statistical differences in mean percentage body fat and ASMI between the excluded group and study group, suggesting that the 2 groups were not significantly different in body composition constitution. Larger studies which are adequately powered are required to ascertain if there is indeed a gender difference in the relationship between IMAT(ratio) and functional performance.

Data on menopausal status of female subjects were not specifically collected. This is an important consideration as men and postmenopausal women have more intra-abdominal fat than premenopausal women (Brown & Clegg, 2010). We believe that this is unlikely to have a material impact on the results, as the proportion of female subjects (N = 8, 6.10%) below the average age of menopause of 55yrs is relatively small, and sensitivity analysis performed excluding these 8 female subjects yielded the same results. Smoking and alcohol intake were not quantified, forming another limitation in this study due to self-reporting biases.

Another limitation is the cross-sectional design of our study, such that a causal relationship between IMAT, MCP-1 and SO cannot be concluded and needs to be confirmed in well-designed longitudinal studies. Lastly, we did not incorporate other MRI techniques such as magnetic resonance spectroscopy to study intramyocellular lipid, which has been implicated in the pathogenesis of insulin resistance (Nakagawa et al., 2007).

5. Conclusion

Using advanced MRI segmentation techniques, we demonstrated that IMAT(ratio) differs across the body composition types, being highest in the SO group. The significant association with MCP-1, a novel early biomarker of adipose tissue inflammation, and poorer physical performance support the etiologic role of IMAT in the pathogenesis of SO, and suggest the possible role of IMAT as a candidate imaging biomarker for adipose tissue inflammation and associated functional outcomes in SO. The results of our exploratory study should be confirmed in well-designed longitudinal studies to ascertain the utility of IMAT in predicting progression to SO.

Declarations of interest

None.

Acknowledgements

We would like to thank Prof Ding Yew Yoong and Dr Ismail Noor Hafizah for their invaluable guidance in the study design and implementation. This study was supported by Lee Foundation grant 2013.

References

Addison, O., Marcus, R. L., LaStayo, P. C., & Ryan, A. S. (2014). Intermuscular fat: A review of the consequences and causes. *International Journal of Endocrinology,* (2014), 309570. https://doi.org/10.1155/2014/309570.

Aguiari, P., Leo, S., Zavan, B., Vindigni, V., Rimessi, A., Bianchi, K., et al. (2008). High glucose induces adipogenic differentiation of muscle-derived stem cells. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 1226–1231.

Baumgartner, R. N., Wayne, S. J., Waters, D. L., Janssen, I., Gallagher, D., & Morley, J. E. (2004). Sarcopenic obesity predicts instrumental activities of daily living disability in the elderly. *Obesity Research, 12*, 1995–2004.

Brown, L. M., & Clegg, D. J. (2010). Central effects of estradiol in the regulation of food intake, body weight, and adiposity. *The Journal of Steroid Biochemistry and Molecular Biology, 122*(1-3), 65–73.

Chen, L. K., Liu, L. K., Woo, J., Assantachai, P., Auyeung, T. W., Bahyah, K. S., et al. (2014). Sarcopenia in Asia: Consensus report of the asian working group for Sarcopenia. *Journal of the American Medical Directors Association, 15*(2), 95–101.

Chung, J. Y., Kang, H. T., Lee, D. C., Lee, H. R., & Lee, Y. J. (2013). Body composition and its association with cardiometabolic risk factors in the elderly: A focus on sarcopenic obesity. *Archives of Gerontology and Geriatrics, 56*(1), 270–278.

Cruz-Jentoft, A. J., Baeyens, J. P., Bauer, J. M., Boirie, Y., Cederholm, T., Landi, F., et al. (2010). Sarcopenia: European consensus on definition and diagnosis. Report of the european working group on Sarcopenia in older People A. J. Cruz-Gentoft et al. *Age and Ageing, 39*(4), 412–423.

Deshmane, S. L., Kremlev, S., Amini, S., & Sawaya, B. E. (2009). Monocyte chemoattractant protein-1 (MCP-1): An overview. *Journal of Interferon & Cytokine Research, 29*(6), 313–326.

dos Santos, E. P., Gadelha, A. B., Safons, M. P., Nóbrega, O. T., Oliveira, R. J., & Lima, R. M. (2014). Sarcopenia and sarcopenic obesity classifications and cardiometabolic risks in older women. *Archives of Gerontology and Geriatrics, 59*(1), 56–61.

Dulloo, A. G., Jacquet, J., Solinas, G., Montani, J. P., & Schutz, Y. (2010). Body composition phenotypes in pathways to obesity and the metabolic syndrome. *International Journal of Obesity, 34*(S2), S4.

Esser, N., Legrand-Poels, S., Piette, J., Scheen, A. J., & Paquot, N. (2014). Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Research and Clinical Practice, 105*(2), 141–150.

Expert Panel on Detection, E (2001). Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *Jama, 285*(19), 2486.

Guralnik, J. M., Simonsick, E. M., Ferrucci, L., Glynn, R. J., Berkman, L. F., Blazer, D. G., et al. (1994). A short physical performance battery assessing lower extremity function: Association with self-reported disability and prediction of mortality and nursing home admission. *Journal of Gerontology, 49*(2), M85–M94.

Huo, Y. R., Suriyaarachchi, P., Gomez, F., Curcio, C. L., Boersma, D., Gunawardene, P., et al. (2016). Phenotype of sarcopenic obesity in older individuals with a history of falling. *Archives of Gerontology and Geriatrics, 65*, 255–259.

Janssen, I., Katzmarzyk, P. T., & Ross, R. (2004). Waist circumference and not body mass index explains obesity-related health risk. *The American Journal of Clinical Nutrition, 79*(3), 379–384.

Kalinkovich, A., & Livshits, G. (2017). Sarcopenic obesity or obese sarcopenia: A cross talk between age-associated adipose tissue and skeletal muscle inflammation as a main mechanism of the pathogenesis. *Ageing Research Reviews, 35*, 200–221.

Labbe, K., Danialou, G., Gvozdic, D., Demoule, A., Divangahi, M., Boyd, J. H., et al. (2010). Inhibition of monocyte chemoattractant protein-1 prevents diaphragmatic inflammation and maintains contractile function during endotoxemia. *Critical Care, 14*(5), R187.

Lim, J. P., Leung, B. P., Ding, Y. Y., Tay, L., Ismail, N. H., Yeo, A., et al. (2015). Monocyte chemoattractant protein-1: A proinflammatory cytokine elevated in sarcopenic obesity. *Clinical Interventions in Aging, 10*, 605.

Mann, C. J., Perdiguero, E., Kharraz, Y., Aguilar, S., Pessina, P., Serrano, A. L., et al. (2011). Aberrant repair and fibrosis development in skeletal muscle. *Skeletal Muscle, 1*, 21. https://doi.org/10.1186/2044-5040-1-21.

Nakagawa, Y., Hattori, M., Harada, K., Shirase, R., Bando, M., & Okano, G. (2007). Age-related changes in intramyocellular lipid in humans by in vivo 1H-MR spectroscopy. *Gerontology, 53*(4), 218–223.

Oliveira, R. J., Bottaro, M., Junior, J. T., Farinatti, P. T. V., Bezerra, L. A., & Lima, R. M. (2011). Identification of sarcopenic obesity in postmenopausal women: A cutoff proposal. *Brazilian Journal of Medical and Biological Research, 44*(11), 1171–1176.

Prado, C. M. M., Wells, J. C. K., Smith, S. R., Stephan, B. C. M., & Siervo, M. (2012). Sarcopenic obesity: A critical appraisal of the current evidence. *Clinical Nutrition, 31*(5), 583–601.

Riechman, S. E., Schoen, R. E., Weissfeld, J. L., Thaete, F. L., & Kriska, A. M. (2002). Association of physical activity and visceral adipose tissue in older women and men. *Obesity Research, 10*, 1065–1073.

Rolland, Y., Czerwinski, S., Van Kan, G. A., Morley, J. E., Cesary, M., Onder G., et al. (2008). Sarcopenia: Its assessment, etiology, pathogenesis, consequences and future

perspectives. *The Journal of Nutrition, Health & Aging, 12*(7), 433–450.
Roubenoff, R. (2000). Sarcopenic obesity: Does muscle loss cause fat gain?: Lessons from rheumatoid arthritis and osteoarthritis. *Annals of the New York Academy of Sciences, 904*(1), 553–557.
Roubenoff, R. (2004). Sarcopenic obesity: The confluence of two epidemics. *Obesity, 12*(6), 887–888.
Sakuma, K., & Yamaguchi, A. (2013). Sarcopenic obesity and endocrinal adaptation with age. *International Journal of Endocrinology,* (2013), 204164.
Spoto, B., Di Betta, E., Mattace-Raso, F., Sijbrands, E., Vilardi, A., Parlongo, R. M., et al. (2014). Pro-and anti-inflammatory cytokine gene expression in subcutaneous and visceral fat in severe obesity. *Nutrition Metabolism and Cardiovascular Diseases, 24*(10), 1137–1143.
Stenholm, S., Alley, S., Griswold, M. E., Koskinen, S., Rantanen, T., et al. (2009). The effect of obesity combined with low muscle strength on decline in mobility in older persons: Results from the InCHIANTI Study. *International Journal of Obesity, 2009*(33), 635–644.
Tay, L., Ding, Y. Y., Leung, B. P., Ismail, N. H., Yeo, A., Yew, S., et al. (2015). Sex-specific differences in risk factors for sarcopenia amongst community-dwelling older adults. *Age, 37*(6), 121.
Tay, L., Lim, W. S., Chan, P. C. M., Chong, M. S., & Sitoh, Y. Y. (2011). Influence of aggregation of vascular risk factors on neuropsychological performance and white matter lesions in early Alzheimer's disease. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association, 7*(4), S267.
Tyrovolas, S., Haro, J. M., Mariolis, A., Piscopo, S., Valacchi, G., Bountziouka, V., et al. (2016). Skeletal muscle mass and body fat in relation to successful ageing of older adults: The multi-national MEDIS study. *Archives of Gerontology and Geriatrics, 66*, 95–101.
Vettor, R., Milan, G., Franzin, C., Sanna, M., De Coppi, P., Rizzuto, R., et al. (2009). The origin of intermuscular adipose tissue and its pathophysiological implications. *American Journal of Physiology-Endocrinology and Metabolism, 297*(5), E987–E998.
Visser, M., Goodpaster, B. H., Kritchevsky, S. B., Newman, A. B., Nevitt, M., Rubin, S. M., et al. (2005). Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. *The Journals of Gerontology Series A, Biological Sciences and Medical Sciences, 60*(March (3)), 324–333.
Yang, Y. X., Chong, M. S., Tay, L., Yew, S., Yeo, A., & Tan, C. H. (2016). Automated assessment of thigh composition using machine learning for Dixon magnetic resonance images. *Magnetic Resonance Materials in Physics Biology and Medicine, 29*(5), 723–731.
Yang, Y. X., Chong, M. S., Lim, W. S., Tay, L., Yew, S., Yeo, A., et al. (2017). Validity of estimating muscle and fat volume from a single MRI section in older adults with sarcopenia and sarcopenic obesity. *Clinical Radiology, 72*(5), 427.e9–427.e14.
Zoico, E., Corzato, F., Bambace, C., Rossi, A. P., Micciolo, R., Cinti, S., et al. (2013). Myosteatosis and Myofibrosis: Relationship with aging, inflammation and insulin resistance. *Archives of Gerontology and Geriatrics, 57*(3), 411–416. https://doi.org/10.1016/j.archger.2013.06.001.