Marrow Adipose Tissue in Older Men:

Association with Visceral and Subcutaneous Fat, Bone Volume, Metabolism, and Inflammation

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Running title: Marrow fat in older men

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ABSTRACT

Marrow (MAT) and subcutaneous (SAT) adipose tissues display different metabolic profiles and varying associations with aging, bone density and fracture risk. Using a non-invasive imaging methodology, we aimed to investigate the associations between MAT, SAT and visceral fat (VAT) with bone volume, bone remodeling markers, insulin resistance and circulating inflammatory mediators in a population of older men. In this cross-sectional study, 96 healthy men (mean age 67 ± 5.5) were assessed for anthropometric parameters, body composition, serum biochemistry, and inflammatory panel. Using **single energy** computed tomography images, MAT (in L2 and L3 and both hips), VAT and SAT (at the level of L2-L3 and L4-L5) were measured employing Slice-O-Matic software (Tomovision), which enables specific tissue demarcation applying previously reported Hounsfield unit thresholds. MAT volume was similar in all anatomical sites and independent of BMI. In all femoral regions of interest (ROIs) there was a strong negative association between bone and MAT volumes ($r=-0.840$ to -0.972 , $p<0.001$), with location-dependent variations in the lumbar spine. Unlike VAT and SAT, no associations between MAT and serum glucose, inflammatory markers or insulin resistance indicators were found. In conclusion, strong inverse correlations between MAT and bone mass, which have been previously observed in women, were also confirmed in older men. However, MAT volume in all ROIs was interrelated and unlike women, mainly independent of VAT or SAT. The lack of strong association between MAT vs. VAT/SAT, and its discordant associations with metabolic and inflammatory mediators provide further evidence on MAT's distinct attributes in older men.

Keywords: Marrow fat, visceral fat, subcutaneous fat; osteoporosis; older men.

1. INTRODUCTION

Age-related bone loss and osteoporosis are linearly associated with increased marrow adiposity and declining bone mass and quality [1]. This increase in marrow adiposity has a deleterious impact on bone by releasing adipokines and inflammatory factors either locally or systemically [2, 3]. In healthy, obese and diabetic women of different ages marrow adipose volumes (MAT) are closely associated with subcutaneous (SAT) and visceral fat (VAT) volumes [4-8]. However, in older men where adipose tissues naturally undergo significant redistribution [9], the relationship between MAT, SAT, VAT and bone volumes, and the possible contribution of MAT to bone loss via induction of inflammation, metabolic alterations or systemic lipotoxicity has not been well characterized.

Therefore, using a non-invasive imaging methodology, we aimed to investigate the correlation between MAT, SAT, VAT and bone volumes in a population of older men. Also, regarding the known associations of fat depots with inflammation, diabetes and bone volume in other age and sex groups, we investigated their correlations with age, insulin resistance, circulating inflammatory mediators, calciotropic hormones (e.g. parathyroid hormone [PTH] and vitamin D), and bone remodeling markers in this population.

2. SUBJECTS AND METHODS

2.1. Subjects

Cross-sectional study of 96 apparently healthy men aged 60 and older recruited from the community. Ethical approval was obtained from the SingHealth Centralized Institutional Review Board before commencement of the study, and all study procedures were conducted in accordance with relevant guidelines and regulations. Written informed consent was obtained from all participants prior to their participation.

Participants were consecutively recruited from the attendees of community-based health fairs, based on eligibility and willingness to participate. A detailed medical history was obtained from all participants, which included history of chronic diseases (e.g. hypertension, ischemic heart disease, diabetes, etc.) and lifestyle such as smoking, alcohol use, physical activity and sun exposure. Subjects on antiviral/ anti-obesity/ corticosteroids/ anti-osteoporosis drugs, previous abdominal surgery, previous cancer, any investigational drugs for the past 3 months or excessive weight loss (>5% body weight) over the last 3 months were excluded from the study. Height (to the nearest millimeter) was recorded in all subjects without shoes, and weight (in kilograms) was measured with subjects in light clothing using electronic weighing scales (SECA model 220) to compute BMI (weight in kilograms divided by the square of the height in meters). Fracture history of the patients was not available.

2.2. Biochemical analysis

Fasting blood specimens were obtained from all respondents after an overnight fast of 10 hours for all analytes. Plasma glucose and lipid measurements (total cholesterol, triglyceride, HDL cholesterol), serum albumin, C-reactive protein and inorganic phosphate were analyzed on the same day as collection (Beckman Coulter Unicel DxC System, Beckman Coulter Inc., USA). LDL cholesterol was calculated by Fried Ewald's equation. For all other analytes, blood samples were stored as serum or plasma at -80 degrees C prior to assay. 25-hydroxy vitamin D was measured by radioimmunoassay (Diasorin, Italy). The adipokines leptin, resistin and adiponectin were measured using commercially available kits (Linco Research Inc., USA). Serum insulin and intact PTH were measured by immunoassay (Beckman Coulter Unicel Dxl System, Beckman Coulter Inc., USA). Plasma osteocalcin and serum procollagen type 1 n-terminal propeptide (P1NP) were measured by immunoassay (Roche Cobas 6000 analyzer, Roche Diagnostics, USA). These analytes were measured at the laboratory at Singapore General Hospital, which is Joint Commission International

(JCI) and College of American Pathologists Laboratory Accreditation Programme (CAP) accredited. Interferon gamma, interleukin-1 alpha (IL1 α), interleukin-1 beta, interleukin-6, macrophage inflammatory protein 1 alpha, receptor activator of nuclear factor kappa-B ligand, tumor necrosis factor alpha, insulin like growth factor-1, carboxy-terminal collagen crosslinks, osteopontin and osteoprotegerin were measured using flow cytometry (Luminex 100/200 System for multiplex assays, tests run by i-DNA Biotechnology, Singapore). The intra-assay and inter-assay coefficients of variation for the analytes measured by Luminex ranged from 6.2 to 21.8, and 6.9 to 19.8, respectively.

2.3. Image Acquisition and Analysis

Abdominopelvic imaging was conducted using a 64-slice multi-detector single-energy CT scanner (Somatom Definition, Siemens AG, Erlangen, Germany). Axial CT scan was performed with the subjects in supine, from the dome of the diaphragm down to the bottom of the pelvis, using a 35x35-cm field of view. Non-contrast enhanced scans using routine scan parameters of kVp (120); effective mAs (210); slice collimation 0.6 mm; slice width 5.0 mm; pitch factor 1.4 and increment 5.0 mm were acquired. The thin-slice raw data was reconstructed into 1mm sections at zero gap intervals. All image analyses were performed by a single research assistant, under the guidance of the radiologist. The 2D CT image data sets were saved in DICOM format and onto compact discs. These images were analyzed with an image analysis software [Tomovision's Slice-O-Matic v.4.3 Rev-6i (Tomovision, Montreal, QC, Canada)], previously used to assess VAT and SAT in humans [10, 11], and validated for MAT measurements using a similar single-energy CT system (MicroCT) in lab animals[12]. Only images of high quality and without artefacts were included in the study.

2.4. Anatomical Regions of interest (ROIs)

The lumbar ROIs (Fig. 1 A) were located by first identifying the most distinct landmark i.e. first sacral (S1) and then fifth lumbar (L5) vertebrae; followed cranially by L4, L3 then L2. Similar procedure was followed caudally to identify the proximal femur and the regions of interests within were determined from anatomical landmarks (Fig. 1A). Only images of high quality and without artefacts were included in the study.

2.5. Image analyses

For quantification of the abdominal VAT and SAT, axial CT slices at the level of the second and third lumbar vertebrae (L2 and L3 ROIs) were used. Pelvic VAT and SAT was quantified using CT slices at the level of the fourth and fifth lumbar vertebrae (L4&5 ROI) (Fig. 1D). Bone volume fraction (BV/TV) and MAT (defined as fat volume/total volume [FV/TV]) were quantified within lumbar vertebrae and proximal femur ROI: three axial slices at the level of second and third lumbar vertebra (L2, L3) and five axial slices of the left and right femoral necks and trochanters were selected for analysis (Fig. 1B, C and E). The cortical and trabecular sub-regions of all vertebral and femoral ROIs were delineated visually, and BV/TV and MAT were quantified in each sub-region and in total body.

CT Hounsfield values for bone, marrow fat and hematopoietic tissues were calculated. We used protocols previously validated in animals to quantify yellow/red marrow and bone using a single energy CT (MicroCT) and image analysis using Slice-O-Matic software [12]. To ensure artefacts resulted from larger tissue volumes of human (e.g. beam hardening) affect the results minimally, thresholds were adjusted to uniformly segment various fatty tissues (including spinal cord, VAT, SAT and MAT) and tissues with composition similar to red marrow (aorta and spleen). Aorta and spinal cord were intentionally chosen as they are partly/completely encased by vertebrae and

segmenting them accurately with thresholds that also detect VAT and SAT can ensure that MAT volume is not overestimated due to beam hardening. CT numbers for adipose tissue fell within negative values and soft tissues were between -100 and +100 Hounsfield units; whereas, the CT numbers of skeletal tissues took values from 100 up to 1524 [12-15]. However, final threshold ranges used for quantification of these parameters were further refined visually, using an image histogram [16]. Thus, the following resultant CT number thresholds were applied: fat ≤ 20 ; blood 21-129; trabecular bone 130-600 and cortical bone > 601 .

A pilot study was designed to check repeatability of measurements and make fine adjustments. Averages of 20 quantifications on images from 10 random subjects were used for this purpose. This procedure was repeated for 3 axial slices at L3 from 10 different subjects. Results were plotted on a histogram and the range of CT number values which best approximates the 95% confidence interval was chosen for the final quantification process. The volume function of Slice-O-Matic was used to compute the volume of the segmented ROIs. Bone, red marrow and yellow marrow segmentations were tagged as blue, red and yellow, respectively (Fig.1)

2.6. Densitometry (DXA Scans)

Using dual-energy X-ray absorptiometry (DXA; Hologic DPX-IQ Discovery, Hologic Inc., Bedford, MA, USA), bone mineral density (BMD), T- and Z-scores of femoral neck and lumbar spine were estimated in array mode, according to the manufacturer's protocols and software. At all stages of image processing, including the pilot study, the operator was blinded to the patient information.

2.7. Statistical analyses

Variables' scatter plots were used to visually confirm linearity of associations. Pearson's and Spearman's correlations (for normally and non-normally distributed or ordinal variables,

respectively) were used to investigate the association between regional fat volumes and fat volume/bone ratios with age, BMI, diabetes status, PTH, vitamin D, markers of bone resorption and formation and inflammatory mediators. Partial correlation was used to assess the independent associations between variables after adjusting for confounders. Where the results remained unchanged, only unadjusted correlations are presented to aid simplicity. The linearity of the correlations was checked visually using scatter plots. The IBM SPSS statistics for Windows, Version 23 (Armonk, NY; IBM Corp.) was used for the analyses. Variables were expressed as mean $\pm$ SD or median (interquartile range). *P*-values ≤ 0.05 were considered as significant.

3. RESULTS

3.1. Sample characteristics

Ninety six men (mean age $\pm$ SD = 67.5 $\pm$ 5.5; range: 60-87) fulfilled the inclusion criteria. Thirty five subjects had DXA reports available. The anthropometric, body composition and blood biochemistry profiles of the patients are presented in Table 1.

3.2. Distribution of MAT, VAT and SAT in older males

VAT and SAT volumes and MAT volume fractions in different ROIs have been presented in Table 2. VAT and SAT were significantly correlated within and between all abdominal and pelvic ROIs ($r=0.379$ to 0.864 ; $p<0.001$) and with BMI ($r=0.580$ to 0.674 ; $p<0.001$). Only vertebral MAT volume was associated with VAT and SAT volumes ($r=0.270$ to 0.327 , $p<0.042$). MAT volumes correlated across all marrow ROIs and in all tested anatomical sites ($r=0.307$ to 0.983 , $p<0.022$). This association was very strong ($r>0.9$) not only between adjacent ROIs (e.g. left neck vs left trochanter MAT; $r=0.916$, $p<0.001$), but also separate ROIs [e.g. between L2 vs L3 MAT ($r=0.955$,

$p<0.001$) or right vs. left femoral MAT ($r=0.930$, $p<0.001$]. MAT volumes of the vertebral bodies ROIs also correlated with proximal femoral ROIs ($r=0.288$, $p<0.032$).

3.3. Associations of fat depots with bone volume and mass

All proximal femoral ROIs showed a strong negative correlation between MAT and bone volume (BV/TV) (Fig. 2A). This negative correlation was less strong for the L2 and L3 vertebral ROIs (Table 3). There was also a consistent negative association between MAT vs DXA-derived BMD of femoral neck and total hip (Table 3).

In contrast, no correlations were found between VAT, SAT or BMI with BV/TV of any femoral ROI (Fig. 2B and C and Table 3). Nevertheless, with increasing BMI, abdominal and pelvic VAT, and SAT spinal BV/TV in both L2 and L3 showed some decline; but the correlations reached significance only for VAT vs BV/TV ($r=-0.360$ to -0.511 , $p<0.006$; Figure 3 and Table 3).

In proximal femoral ROIs, MAT volume fraction increased with age ($r=0.231$ to 0.315 , $p<0.025$). Concordantly, bone volume fraction decreased the same ROIs ($r=-0.226$ to -0.293 , $p<0.031$). We did not detect associations between age, bone and MAT volume fractions in L2 and L3 or VAT and SAT volumes.

3.4 Associations of red marrow with MAT and bone volume:

Unlike MAT, red marrow volume did not show any association with age ($R=-0.135$ to 0.050 , $p=0.194$ to 0.780) or BV/TV ($R=-0.13$ to 0.004 , $p=0.087$ to 0.966). With increasing MAT, red marrow volume slightly decreased in all ROIs, but the associations reached significance only in right trochanter ROI ($r=-0.221$, $p=0.033$). **MAT as a fraction of total marrow [i.e. $\text{MAT}/(\text{MAT}+\text{red marrow})$] was also negatively associated with bone marrow in all ROIs ($r=-0.811$ to -0.874 ; $p<0.001$).**

3.5. Associations of fat volume with inflammatory cytokines, insulin resistance indicators and bone biomarkers

Unlike VAT and SAT, there was no association between MAT and insulin resistance indicators or hormones involved in the metabolism of glucose and fat in any ROI (Table 4). No consistent associations were detected for any VAT, SAT or MAT ROIs with any of the tested inflammatory mediators, except a weak negative association between L2 and L3 MAT with IL-6 (Table 4).

No associations between bone and fat volumes vs. hormones and bone biomarkers ($R < 0.147$, $p > 0.238$) were found.

4. DISCUSSION

In this population of older men, we report that MAT in general is independent of VAT and SAT volumes and BMI. Also, compared to VAT and SAT, in older males MAT displays a distinctively different association with inflammatory mediators, insulin resistance markers and bone volume. Our results also confirm the highly negative association between MAT and bone volume [17], whereas VAT and SAT did not display associations with bone mass or volume.

The independence of MAT from VAT and SAT observed in this population of older men is contrary to the existing reports on the close association between MAT, VAT and SAT in normal, obese and diabetic females of different ages [4, 5, 8]. Our results support the association of VAT and SAT with insulin resistance [18-20]; however, the lack of association between VAT and SAT volumes with inflammatory mediators in older men is contrary to current understanding of the role of the adipose tissue in inflammation [18-20].

Recently, Malkov et al. [21] reported that older men with a high appendicular lean mass and low SAT had over 8 times the risk for hip fracture compared to those with lower appendicular lean

mass, but high SAT. Fat redistribution in older persons (decline in SAT and increased ectopic fat in other tissues such as muscle, bone marrow and viscera) [9, 22-24] leads to a pathological state in which adipocytes lose the ability to metabolize triglycerides, respond to hormones (e.g. insulin); and produce abnormal endo-, para- and auto-crine factors (e.g. leptin, adiponectin, resistin and inflammatory cytokines) [22-24].

In the case of MAT, there is a growing body of evidence suggesting that high-volume fat relocation into bone marrow can create a lipotoxic environment [2, 3, 25-27]. For example, the concentration of several pro-inflammatory cytokines in the MAT of old male mice increase by few folds to several thousand times compared to younger animals leading to lipotoxic and anti-osteoblastogenic effects [2]. In addition, palmitic acid (a by-product of fatty acid biosynthesis, produced in abundance by MAT in human cells in vitro [25] and in vivo [28]) is toxic to human osteoblasts [26]. In agreement with this, bone loss due to ovariectomy in dogs leads to MAT expansion which is inversely related to bone apposition rate [29]. In this study, bone exposed to MAT showed significantly less formation surface, compared to the bone that faces red marrow [29]. In agreement with our results, it has been established that fat infiltration into bone marrow is associated with lower bone mineral density in trabecular bone (where bone is more metabolically active), and prevalent vertebral fracture [30].

In addition, further support for probable causative roles of MAT in bone mass decline comes from the observation that bone decline occurred without red marrow expansion; thus lost bone was mainly (if not exclusively) replaced by MAT. This may be in line with the decreased red marrow due to MAT expansion in osteoporotic patients previously reported by Griffith et al. [31]. Overall, the potential negative consequences of fat redistribution in aging men could be associated with

the development of a highly lipotoxic bone microenvironment [30] that affects bone and the hematopoietic system, however, further studies are still required.

Regarding the relationship between MAT and inflammation, it has been shown that MAT-released palmitate induces a pro-inflammatory response [32]. Interestingly, in our subjects the associations between IL-6 and MAT in femoral and vertebral ROIs had opposite trends (positive and negative associations, respectively) and other inflammatory factors in the bloodstream did not show associations with MAT volumes thus suggesting that the pro-inflammatory role of MAT is site-dependent; a hypothesis that should be tested in future human studies.

Compared with other imaging methods, our quantifications of MAT vary from the reported values by magnetic resonance spectroscopy, which could be due to artefacts such as beam hardening. However, the single-energy CT and Slice-O-Matic measurements have been previously validated in the bone of a rodent model [12] and widely in clinical studies looking at VAT and SAT in humans [10, 11]. Considering the larger size of humans and thicker bones that make beam hardening artefacts more likely, we set the thresholds in a way that both non-bone-encased (subcutaneous and visceral) and bone-encased (spinal cord) fat tissues could be reliably segmented. Plus, for every image we visually checked for non-accounted voxels (e.g. a vessel within SAT) and ensured that we do not include artefacts as a particular tissue. Nevertheless, further validation studies in humans are still required.

Major strengths of this study are the use of a recently validated non-invasive method to quantify MAT. To our knowledge, this is the first attempt to quantify MAT and red marrow in humans using single-energy CT. In addition, to increase the validity of our data, only associations that were repeatable in similar ROI (e.g., all femoral ROI or in both L2 and L3 vertebrae) were reported here.

As limitations, the cross-sectional nature and relatively small sample size of this study limits our ability to determine causality relations for the associations found here., In addition, the resolution of CT scan is not ideal for differentiation of tissue volumes in sub-millimeter scale; the MAT variations may affect the measurements of bone density in single energy CT [33] and we could not explore MAT saturation degree, which may have effects on inflammatory and diabetic markers. In addition, only few but representative ROI (6 MAT, 2 SAT and 2 VAT ROI) were analyzed in this population, which may limit the generalizability of these results.

In summary, MAT volumes are independent of BMI and VAT/SAT volumes in older men. Without requiring an invasive technique [17], our results confirmed the negative association between MAT and bone volumes in older subjects. In addition, our results suggest that MAT displays different associations with bone volume and metabolic/inflammatory profiles compared to VAT and SAT. Longitudinal studies in both genders are warranted to confirm whether these are age-, gender-or ROIs-specific findings. In conclusion, unravelling the molecular mechanism of crosstalk between bone and fat would lead to a better understanding of their relationship and its role in the pathophysiology of osteoporosis in older persons.

Funding: This project was partially funded the National Medical Research Council of Singapore [Singapore] and Australian Institute for Musculoskeletal Science [AIMSS; St Albans, Australia]

Human and Animal Rights and Informed Consent: Ethical approval was obtained from the Singapore General Hospital Institutional Review Board before commencement of the study, and written informed consent obtained from all participants prior to their participation.

Conflict of interest: The authors have no conflict of interest or any competing financial interests. We have full control of all primary data and that agree to allow the journal to review the data if required.

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TABLES

Table 1: Demographic, Clinical, Body Composition and Serological Characteristics of Participants (mean $\pm$ SD). BMI: Body mass index; IFN: Interferon; IL: Interleukin; MIP-1: macrophage inflammatory protein 1; OPG: Osteoprotegerin; RANKL: Receptor activator of nuclear factor kappa-B ligand; IGF: Insulin-like growth factor 1; CTX: Collagen type 1 cross-linked C-telopeptide; P1NP: Procollagen type 1 N-terminal propeptide; HDL: High density lipoprotein; LDL: Low density lipoprotein; HOMA-IR: Homeostatic model assessment-Insulin resistance; CRP: C-reactive protein.

Morphometric and bone density characteristics	
Variables	Mean (SD)
Age (years)	67.5 (5.5)
BMI (kg/m ²)	24.6 (4.2)
Height (cm)	164.7 (6.4)
Lumbar spine BMD (g/cm ²)	1.01 (0.136)
T-score	0.143 (1.179)
Z-Score	0.958 (1.225)
Fem Neck BMD (g/cm ²)	0.764 (0.092)
T-score	-1.180 (0.729)
Z-Score	0.306 (0.790)
Biochemistry results	
Analyte	Median (IQR)
Vitamin D (μ g/L)	22.4 (19.9, 27)
Parathyroid Hormone (pmol/L)	3.8 (3.4, 4.3)
Serum phosphate (mmol/L)	0.97 (0.88, 1.08)
Serum albumin (g/L)	39 (37, 40)
IFN gamma (IU/ml)	18.2 (10.2-28.6)
IL-1 alpha (pg/mL)	5.6 (4.4, 7.4)
IL-1 beta (pg/mL)	2.0 (1.2, 2.1)
IL-6 (IU/mL)	4.6 (2.6, 5.6)
MIP-1 alpha (μ g/mL)	5.75 (4.5, 7)
TNF alpha (ng/mL)	3.14 (2.7, 3.9)
OPG (ng/mL)	1.4 (0, 5.8)
RANKL (ng/mL)	58.4 (47.5, 83.5)
Osteopontin (μ g/L)	4.4 (2.8, 7.7)
Resistin (ng/ml)	7.4 (5.7, 10.4)
IGF1 (ng/mL)	116.6 (101.1, 145.7)
Leptin (μ g/L)	4 (3.3, 6.5)
Adiponectin (ng/mL)	6040 (4368, 7364)
Osteocalcin (ng/mL)	15 (12, 16)
CTX (mg/L)	0.26 (0.22, 0.36)
P1NP (μ g/L)	30.1 (24.5, 34.5)
Glucose (mmol/L)	5.2 (4.9, 5.4)
Cholesterol (mmol/L)	5.28 (4.9, 5.8)
Triglyceride (mmol/L)	1.09 (0.77, 1.6)
HDL (mmol/L)	1.22 (1.1, 1.4)
LDL (mmol/L)	3.56 (2.9, 3.8)
Cholesterol: HDL ratio	4.33 (3.5, 4.7)
Insulin (μ unit/mL)	5.3 (4.1, 8.5)
HOMA-IR	1.23 (0.92, 2.47)
CRP (mg/L)	0.9 (0.5, 2.4)

Table 2: MAT (FV/TV) and VAT/SAT volumes ($\times 10^3$ voxels) in different regions of interest.

	Region of interest									
	MAT (%)						VAT and SAT (voxels)			
	Lt fem. neck	Lt fem. troch.	Rt fem. neck	Rt fem. troch.	L2 Vert.	L3 Vert.	L2-3 VAT	L2-3 SAT	L4-5 VAT	L4-5 SAT
Mean (SD)	8.8 (9.0)	9.1 (10.3)	9.2 (8.8)	9.2 (9.9)	10.5 (13.5)	12.1 (13.5)	77.3 (34.6)	56.8 (26.5)	64.9 (25.9)	89.8 (37.9)
CV (%)	101.7	113.4	95.7	107.8	129	112	44.8	46.7	0.4	40

Rt: right, Lt: left; L2 and L3: second and third lumbar vertebra; BV/TV: bone volume fraction; marrow fat volume fraction: MAT; visceral and subcutaneous adipose tissue: VAT and SAT; fem: femoral; troch: trochanteric; CV: coefficient of variation

Table 3: Correlations between MAT and BV/TV at several ROIs. Significant associations have been highlighted in bold and p -values ≤ 0.001 have not been displayed.

	Lt neck MAT%	Rt neck MAT%	Lt troch. MAT%	Rt troch. MAT%	Lt Ttl hip MAT%	Rt Ttl hip MAT%	L2 MAT%	L3 MAT%
Lt neck BV/TV% (n=96)	-0.964	-0.892	-0.896	-0.850	-0.942	-0.893	-0.333 (0.012)	-0.330 (0.013)
Rt neck BV/TV% (n=96)	-0.884	-0.963	-0.840	-0.883	-0.870	-0.946	-0.376 (0.004)	-0.346 (0.009)
Lt troch. BV/TV% (n=96)	-0.880	-0.836	-0.966	-0.906	-0.953	-0.892	-0.372 (0.005)	-0.347 (0.009)
Rt troch. BV/TV% (n=96)	-0.841	-0.886	-0.906	-0.967	-0.893	-0.950	-0.424	-0.386 (0.003)
Lt Ttl hip BV/TV% (n=96)	-0.925	-0.865	-0.953	-0.894	-0.969	-0.900	-0.362 (0.006)	-0.347 (0.009)
Rt Ttl hip BV/TV% (n=96)	-0.884	-0.947	-0.893	-0.947	-0.903	-0.972	-0.409	-0.374 (0.009)
L2 BV/TV (n=58)	-0.398 (0.002)	-0.336 (0.010)	-0.348 (0.008)	-0.317 (0.015)	-0.375 (0.004)	-0.3433 0.011	-0.240 (0.090)	-0.213 (0.138)
L3 BV/TV% (n=58)	-0.471	-0.409	-0.440	-0.397 (0.002)	-0.460	-0.410	-0.274 (0.052)	-0.264 (0.06)
Lt neck BMD (g/cm², n=35)	-0.370 (0.031)	-0.276 (0.115)	-0.340 (0.049)	-0.284 (0.103)	-0.364 (0.035)	-0.295 (0.090)	-0.282 (0.23)	-0.262 (0.27)
Lt Ttl hip BMD (g/cm², n=35)	-0.368 (0.032)	-0.274 (0.117)	-0.322 (0.063)	-0.302 (0.082)	-0.355 (0.040)	-0.305 (0.079)	-0.105 (0.57)	-0.031 (0.87)

Rt: right, Lt: left; Ttl: total; L2 and L3: second and third lumbar vertebra; BV/TV: bone volume fraction; MAT: BV/TV; troch: trochanteric

Table 4: Associations between metabolic and inflammatory markers and fat volumes at different ROIs. Significant associations are in bold and p -values ≤ 0.001 have not been displayed.

	Adiponectin (ng/mL)	Leptin (μ g/L)	Glucose (mmol/L)	Insulin (μ U/mL)	HOMA-IR	CRP (mg/L)	IL-1 α (pg/mL)	IL-6 (IU/mL)	TNF- α (ng/mL)
L2-3 VAT (voxel, n=96)	-0.350	0.310 (0.002)	0.207 (0.044)	0.463	0.460	0.302 (0.003)	-0.051 (0.623)	-0.034 (0.744)	0.025 (0.806)
L2-3 SAT (voxel, n=96)	-0.115 (0.265)	0.496	0.127 (0.216)	0.334	0.330	0.460	0.067 (0.516)	-0.109 (0.291)	0.100 (0.334)
L4-5 VAT (voxel, n=96)	-0.267 (0.009)	0.344	0.161 (0.118)	0.400	0.396	0.254 (0.013)	-0.007 (0.947)	-0.099 (0.338)	0.022 (0.829)
L4-5 SAT (voxel, n=96)	-0.157 (0.128)	0.513	0.153 (0.136)	0.332	0.328	0.453	0.058 (0.577)	-0.112 (0.278)	0.102 (0.324)
Lt neck MAT% (n=96)	-0.08 (0.442)	-0.126 (0.228)	0.029 (0.779)	-0.018 (0.86)	-0.023 (0.828)	-0.158 (0.128)	0.042 (0.69)	0.183 (0.078)	0.047 (0.651)
Rt neck MAT% (n=96)	-0.154 (0.139)	-0.125 (0.229)	0.004 (0.967)	-0.02 (0.981)	-0.012 (0.91)	-0.152 (0.145)	0.007 (0.946)	0.114 (0.276)	0.004 (0.973)
Lt troch. MAT% (n=96)	-0.051 (0.627)	-.12 (0.248)	.037 (0.724)	0.034 (0.748)	0.025 (0.807)	-0.128 (0.22)	-0.014 (0.894)	0.242 (0.019)	-0.014 (0.892)
Rt troch. MAT% (n=96)	-0.002 (0.985)	0.151 (0.145)	-0.012 (0.911)	-0.131 (0.21)	-0.083 (0.428)	0.027 (0.796)	0.069 (0.511)	0.054 (0.603)	-0.105 (0.316)
Lt Ttl hip MAT% (n=96)	-0.065 (0.532)	-0.12 (0.249)	0.033 (0.749)	0.014 (0.891)	0.007 (0.943)	-0.143 (0.17)	0.019 (0.856)	0.221 (0.032)	0.021 (0.839)
Rt Ttl hip MAT% (n=96)	0.003 (0.975)	0.135 (0.193)	-0.002 (0.985)	-0.146 (0.159)	-0.106 (0.308)	0.016 (0.875)	0.036 (0.731)	0.024 (0.819)	-0.132 (0.204)
L2 MAT% (n=57)	-0.169 (0.208)	0.252 (0.059)	0.215 (0.108)	0.116 (0.39)	0.11 (0.414)	.075 (0.578)	-0.185 (0.167)	-0.286 (0.031)	-0.217 (0.105)
L3 MAT% (n=57)	-0.123 (0.363)	0.204 (0.127)	0.125 (0.353)	0.088 (0.517)	0.076 (0.575)	0.147 (0.276)	-0.194 (0.148)	-0.318 (0.016)	-0.204 (0.128)

Rt: right, Lt: left; Ttl: total; L2, L3, L4 and L5: second, third fourth and fifth lumbar vertebra, respectively; marrow fat volume fraction=MAT; VAT and SAT: visceral and subcutaneous adipose tissue; troch: trochanteric

FIGURE LEGENDS

Fig. 1: **A:** Definition of the regions of interest on a scout scan; L2 and L3: second and third vertebra where bone volume and MAT were quantified; L2&3 and L4&5 abdominal and pelvic regions of interest (at the level of 2nd+3rd and 4th+5th lumbar vertebrae, respectively) where VAT and SAT were quantified; N and T imply femoral neck and trochanteric regions of interest where bone volume and MAT were quantified; **B:** A screenshot of Slice-O-Matic detailing the segmentation and tagging process on a lumbar vertebral body; **C:** A magnification of the segmented lumbar vertebral body displayed in B; Bone, red marrow and yellow marrow segmentations have been tagged as blue, red and yellow, respectively. **D:** A screenshot of Slice-O-Matic detailing the segmentation and tagging process of a CT slice of pelvic region VAT, SAT and muscles have been tagged in khaki, yellow and red, respectively; **E:** A magnification of segmented slice of femoral head displaying definition of the femoral neck and trochanteric ROIs. N and T imply femoral neck and trochanteric regions of interest respectively. Bone, red marrow and yellow marrow segmentations have been tagged as blue, red and yellow, respectively.

Fig. 2 **A)** All proximal femoral ROIs showed a strong negative correlation between MAT and bone (BV/TV). **B-C)** The negative correlations were moderate for the L2 and L3 vertebral ROIs and non-existent for VAT and SAT.

Fig. 3 A-C) With increasing BMI, abdominal and pelvic VAT and SAT, spinal BV/TV in both L2 and L3 vertebral bodies declined; but, the associations were significant only for abdominal and pelvic VAT (B). **D-I)** Only vertebral MAT (D-F), but not femoral MAT (G-I) volume was associated with VAT and SAT volumes.