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RESEARCH ARTICLE

Bone marrow adipose tissue mass and dipeptidyl peptidase-4 link aging and metabolic health to biomarkers of bone turnover

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Abstract

Bone marrow adipose tissue (BMAT) has been linked to negative bone health outcomes, and a high level of bone marrow adipocyte accumulation is observed during aging and in individuals with diabetes and obesity. This study explores the relationships between BMAT, age, metabolic health, and the impact of their interactions on bone turnover in a cross-sectional cohort of healthy women and men. Levels of bone turnover biomarkers, procollagen type 1 N-terminal propeptide (P1NP) and β-CrossLaps, were determined alongside dipeptidyl peptidase-4 (DPP4) concentration and activity as biomarkers of metabolic health. We used magnetic resonance imaging to assess proton density fat fraction to quantify BMAT mass in healthy individuals and correlated results to sex, age, body mass index (BMI), and glycated hemoglobin A1c (HbA1c), which represents long-term glycemic control. Age was the strongest determinant of increased BMAT mass, explaining more than a third of its overall variation, as well as a robust determinant of bone turnover. A sex-specific correlation pattern was observed between BMAT and bone turnover: women displayed a trend for a positive correlation of BMAT, which depended on age. In men, BMAT mass correlated significantly, but inversely, with both biomarkers, which was also age-dependent. DPP4 concentration and activity were positively associated with P1NP in both sexes, and these relationships were independent of age, BMI, or HbA1c. These findings indicate that the impact of BMAT on bone turnover may be age-dependent, whereas metabolic regulator DPP4 is linked to bone turnover independently of metabolic health or aging.

NEW & NOTEWORTHY Our findings highlight the central role of bone marrow adipose tissue in the relationship between bone health, age, and metabolism. Increased marrow adipocytes produce endocrine signals, such as dipeptidyl peptidase-4, which modulate these associations. We show that women and men have distinct associations between bone turnover, age, and bone marrow adipocytes.

aging; bone marrow adipose tissue (BMAT); bone turnover; dipeptidyl peptidase-4; metabolic health

INTRODUCTION

Bone health is influenced by a range of systemic pathologies, such as aging and metabolic diseases. It stands to reason that these disorders are interlinked and that diabetes and obesity exacerbate the functional decline of bone health during aging. Bone marrow adipose tissue (BMAT) is increasingly recognized as a potential connecting element between these factors, linking it to clinically relevant conditions, such as osteoporosis and hematological diseases (1). Its expansion occurs progressively with age, and it is found to be elevated in individuals with obesity and patients with either type 1 or type 2 diabetes (T1D and T2D) (2). Bone marrow adipocytes (BMAds), however, also exist in healthy bones, and some reports challenge the view of an unambiguously inverse relationship between bone marrow adipogenesis and bone health, suggesting that BMAds can constitute a critical component of the healthy bone niche (2, 3). Thus, BMAT's precise role in bone pathophysiology remains unclear, and recent findings further illustrate its complex relationship with anatomical location, age, and gender (2). For instance, levels of BMAT were found to be increased during aging in proximal-to-distal distribution in both axial and appendicular skeletons, an effect that was more pronounced in women,

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especially postmenopause (4). These results indicate that BMAT is regulated differently in men and women, likely due to changes in hormonal cues. Moreover, the study highlights that the degree of fatty acid unsaturation in BMAds varies across the skeleton, with higher unsaturated fatty acid levels found in the axial skeleton. Although somewhat contradicting the data from animal models, these findings further support the concept of distinct roles for BMAT in different skeletal areas (4).

A growing body of research highlights the potential use of biomarkers based on chemical-shift-encoded magnetic resonance imaging (CSE-MRI) in assessing bone health, mainly expressed as proton density fat fraction (PDFF) and its use for quantifying BMAT mass (5, 6). For instance, such analyses have demonstrated that fat depositions in bone and skeletal muscle are positively correlated and are also linked to degenerative disorders, such as intervertebral disk degeneration, altogether linking degenerative processes within the musculoskeletal system to ectopic fat accumulation (7). These findings align with the observation that PDFF measurements could predict vertebral compression fractures (VCFs) (8). Such results suggest that increases in BMAT mass may serve as an early marker of elevated fracture risk, offering a noninvasive tool for assessing bone composition changes linked to VCFs in aging populations.

Parallel to the impact of BMAT on bone, metabolic regulators like dipeptidyl peptidase-4 (DPP4) are increasingly recognized as influential in bone health. Our previous studies in preclinical animal models have linked expansion of BMAT in aged mice to increased DPP4 release from cells of the bone-resident adipocyte lineage, which resulted in impaired bone healing (9). Detrimental effects of DPP4 have also been reported for hematopoiesis in the bone marrow (10). DPP4, a membrane-shed protease, is responsible for the inactivation of various secreted proteins, including the incretin hormones, glucagon-like peptide 1 (GLP1), and glucose-dependent insulinotropic polypeptide (GIP), which are vital for stimulating postprandial insulin secretion. DPP4 inhibitors, known as gliptins, are a class of oral drugs commonly used for the management of T2D (11). Individuals with metabolic diseases, such as diabetes, frequently show inverse relationships between DPP4, its molecular targets, and bone health (12). High DPP4 activity correlates with low bone mineral density (BMD) and increased fracture risk, which may depend on two signaling pathways: the entero-endocrine-osseous axis involving gastrointestinal substrates of DPP4, that is, GIP and GLP-1, and a pancreatic-endocrine-osseous axis, linking DPP4 to bone and energy metabolism through expression of RANKL and blood glucose regulation (13). Circulating DPP4 is also elevated in patients with osteoporosis and in otherwise metabolically healthy individuals, as well as in newly diagnosed patients with T2D (14, 15). These findings are supported by the observation that gliptins may exert beneficial effects on bone by enhancing the function of osteogenic progenitor cells (9). It should be noted, however, that many drugs that are used to treat T2D are not clearly linked to a reduction in fracture risk. In fact, although some gliptins may produce protective effects on bone health, at least one member of this class, trelagliptin, correlated with increased fracture risk, altogether suggesting that the impact of DPP4 inhibition on bone requires further study (16). In contrast, the use of GLP-1 receptor agonists in patients with T2D may stabilize or even improve BMD and bone turnover marker profiles, an effect that is also supported by corresponding observations in preclinical animal models (17, 18). However, the use of GLP-1 receptor agonists showed no clear risk reduction for fractures per se (17). Interestingly, direct infusions of GLP-1 or GIP suppressed bone resorption, and simultaneous administration of both hormones resulted in a synergistic effect (19). In summary, the interplay between DPP4 activity, BMAT accumulation, and bone pathology in older individuals with or without metabolic disease presents a compelling area of investigation. To further investigate these relationships, we here examined the links between markers of bone turnover and MRI-based measurements of BMAT mass and body fat distribution, in combination with analyses of DPP4 concentrations and activities in men and women across different ages and levels of overweight and obesity.

METHODS

Study Cohort and Assessment of Clinical and Anthropometric Data

The study cohort consisted of 76 healthy volunteers (40 women and 36 men) without bone pathologies (e.g., osteoporosis) or overt T2D in their medical history. Specifically, exclusion criteria were as follows: acute illness or infection within the last 4 wk; conditions, as assessed by a study physician, that call into question the success of the study or indicate a risk to the participant; existing type 1 diabetes; patients with type 2 diabetes and pharmacological diabetes therapy other than Metformin and DPP4 inhibitors; HbA1c $>$10.0%; diabetes duration $\geq$2 yr. All enrolled participants completed the study, that is, there was no attrition. Group assignments in this study were generated retroactively, as determined by sex, age, and/or body mass index (BMI). Therefore, no randomization or blinding during recruitment and data collection was performed. Plasma analyses were performed in a blinded manner. Upon inclusion, individuals' information on age, gender, and body mass index (BMI) was recorded. Glycosylated hemoglobin A (HbA1c) was determined in the majority of individuals to monitor metabolic health status. Although some cases of prediabetes, based on HbA1c levels, were included in the study (9 women and 6 men), no individuals surpassed the diabetes-defining threshold of 48 mmol/mol (Table 1). The study and its protocol were reviewed and approved by the Ethics Committee of the University of Tübingen, and written informed consent was obtained from all subjects before participation.

MR Imaging Protocol and Image Analysis

Magnetic resonance (MR) measurements were performed in the early morning after overnight fasting on a 3 T whole body imager (Magnetom Vida, Siemens Healthineers, Erlangen, Germany). For the first part of the examination, volunteers were placed head first in a supine position, and the spine-array coil in combination with two body-array coils placed on the trunk was applied as receiver coils. For assessment of adipose tissue distribution in the trunk, a three-dimensional (3-D) two-point volumetric interpolated breath-hold examination (VIBE)

Table 1. *Overview of subgroups after stratification by age and BMI*

Parameter/Group	Group: Age ≤ 46/BMI < 25		Group: Age ≤ 46/BMI > 25		Group: Age > 46/BMI < 25		Group: Age > 46/BMI > 25	
Sex	F	M	F	M	F	M	F	M
Participants	10	9	6	8	11	7	13	12
Age, yr	27.4 ± 3.1	29.8 ± 6.7	40.0 ± 5.1[a]	31.9 ± 5.4	56.1 ± 6.6[a,b]	56.6 ± 6.8[a,b]	59.2 ± 7.0[a,b]	59.0 ± 7.5[a,b]
BMI, kg/m^2	20.8 ± 1.9	22.3 ± 1.8	35.4 ± 3.9[a],§	29.3 ± 5.5[a]	21.4 ± 2.4[b]	23.0 ± 1.5[b]	33.5 ± 3.0[a,c],§	29.5 ± 3.6[a,c]
HbA1c, mmol/mol	33.0 ± 1.9 (9)	35.4 ± 4.1 (8)	37.8 ± 3.1 (5)	33.7 ± 2.7 (7)	37.7 ± 4.2[a] (11)	35.4 ± 3.6 (7)	38.9 ± 4.4[a] (10)	37.8 ± 3.3 (10)

Women (F) and men (M) were separated into subgroups of individuals either aged 46 yr or below or older than 46 yr of age and, second, by BMI, indicating either normal body weight (BMI <25 kg/m^2) or overweight/obesity (BMI >25kg/m^2). Data are depicted as means ± standard error (SE) whenever applicable. Letters and symbol indicate statistical significance by one-way ANOVA ($P < 0.05$) as specified below. Values in parentheses depict instances when the dataset is incomplete, that is, deviating from the regular *n* with the actual number of individuals in the group indicated in the 'participants' row. BMI, body mass index; HbA1c, glycated hemoglobin A1c. [a]Compared with group: age ≤ 46/BMI < 25 (within same sex); [b]Compared with group: age ≤ 46/BMI > 25 (within same sex); [c]Compared with group: age > 46/BMI < 25 (within same sex); §Women compared with men within age- and BMI-matched group.

Dixon sequence was applied for quantification of visceral (VAT) and subcutaneous adipose tissue (SAT) of the abdominal (aSAT) and thoracic (tSAT) regions. Segmentation was performed as previously described (20). Bone marrow PDFF was quantified in vertebral bodies by applying a 3-D six-point VIBE CSE sequence covering the lumbar spine (6). In addition, intrahepatic lipids (IHLs) were determined by localized proton MR spectroscopy in the posterior part of segment 7 applying a single-voxel STEAM technique (21).

Plasma Analyses

β-CrossLaps (CTX) and procollagen type 1 N-terminal propeptide (P1NP) were assessed by Labor Berlin—Charité Vivantes GmbH (Berlin, Germany) using standardized procedures for clinical markers. DPP4 activity was measured by enzymatic conversion of the substrate Gly-Pro *p*-nitroanilide (Gly-Pro-pNA) into *p*-nitroaniline (pNA), as published before (22). Briefly, 40 μL of plasma was diluted 1:5 in assay buffer (50 mM glycine, 1 mM EDTA, and pH 8.7). A standard dilution series was prepared by serial dilution of 1 mM pNA (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany; RRID: SCR_008988). Ninety microliters of diluted samples, standards, and blanks were measured in duplicates at 405 nM as a kinetic over 30 min, starting directly after the addition of 10 μL of 5 mM Gly-Pro-pNA (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany; RRID: SCR_008988). The DPP4 activity was calculated by the slope of the standard series in nmol/min/mL. For DPP4 concentrations, we used the Human DPPIV/CD26 Quantikine ELISA Kit (Bio-Techne GmbH, Wiesbaden, Germany) according to the manufacturer's instructions.

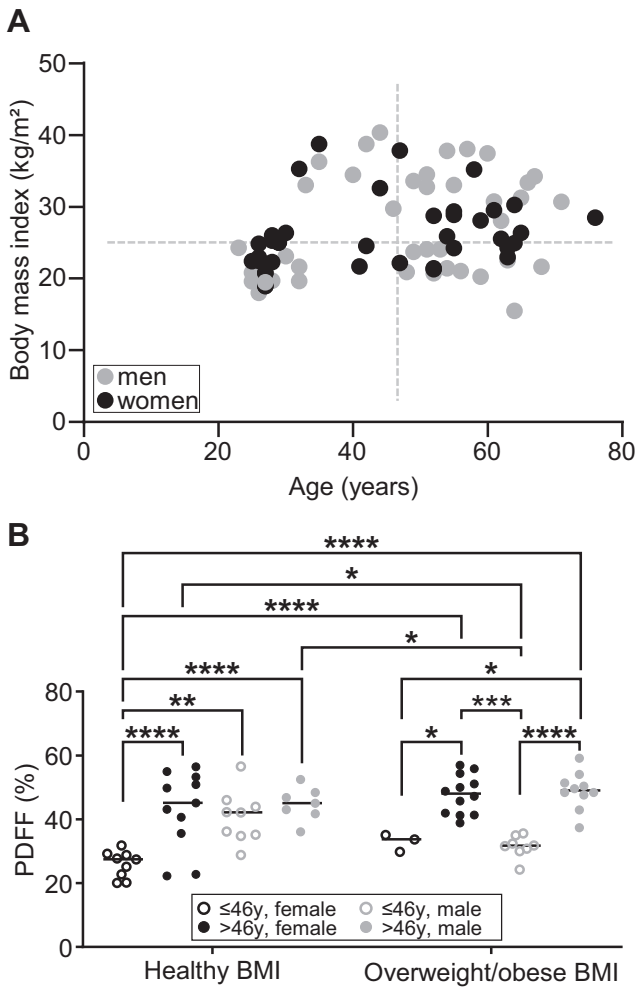
Statistical Analysis

For initial group-based analyses, women (W) and men (M) were considered separately. We next separated our individuals by the mean age of 46 yr into individuals belonging to a younger subgroup and an older subgroup. The cohort was additionally stratified by BMI into individuals with a healthy body weight, that is, with a BMI <25 kg/m^2, and individuals with overweight/obesity, including all individuals with a BMI ≥25 kg/m^2. Accordingly, the resulting eight groups consisted of *1*) women up to 46 yr of age with a BMI <25 kg/m^2, *2*) women up to 46 yr of age with a BMI ≥25 kg/m^2, *3*) women older than 46 yr of age with a BMI <25 kg/m^2, *4*) women older than 46 yr of age with a BMI ≥25 kg/m^2, *5*) men up to 46 yr of age with a BMI <25 kg/m^2, *6*) men up to 46 yr of age with a BMI ≥25 kg/m^2, *7*) men older than 46 yr of age with a BMI <25 kg/m^2, and *8*) men older than 46 yr of age with a BMI ≥25 kg/m^2 (Table 1 and Fig. 1*A*). The software GraphPad Prism (v. 10.4.0 and 10.5.0; RRID: SCR_002798) was used to conduct grouped analyses by two- and three-way analyses of variance (2-way ANOVA, factors: age and BMI; 3-way ANOVA, factors: age, BMI, and sex) with full model fitting of individual factor interactions and the Tukey test for multiple comparisons correction. To further investigate the relationship between age, BMI, and HbA1c with the biomarkers of interest, Spearman correlations were performed using software R (v. 4.4.1; RRID: SCR_001905). Standard pairwise Spearman correlations without controlling for covariates were computed using the cor() function among selected variables. Statistical significance was evaluated using permutation-based tests implemented via cor.test(). Semipartial Spearman correlations were calculated using the ppcor package's pcor.test() function, controlling for age, BMI, or HbA1c. All individuals lacking data for individual parameters were excluded from correlation analyses.

RESULTS

Cohort Characteristics and Stratification into Subgroups: Impact of Age and BMI on BMAT

A total of 76 individuals (40 women and 36 men) were included in the study. Age stratification by the cohort's mean age of 46 yr yielded 33 individuals (16 women and 17 men) up to 46 yr of age and 43 individuals (24 women and 19 men) that were older than 46 yr. BMI stratification resulted in 37 individuals with a healthy BMI, that is, BMI <25 kg/m^2 (21 women and 16 men) and 39 individuals with a BMI ≥25 kg/m^2, indicating overweight/obesity (19 women and 20 men; all data are summarized in Table 1 and Fig. 1*A*). HbA1c, as a standard diagnostic marker of long-term glycemic control, was mostly similar between groups, but we observed significantly higher levels in women of both older groups with either a healthy or overweight-indicating BMI compared with women in the younger group with a healthy BMI. Despite its common use as a diagnostic marker in diabetes care, we found no BMI-dependent differences for HbA1c in any of the groups. This may be due to the fact that none of the participants in our cohort met the T2D criterion for HbA1c, which is defined by a value above 48 mmol/mol (Table 1).



Source of Variation	% variation	Significance	
BMI	0.5194	ns	(0.3875)
Sex	3.151	*	(0.0360)
Age	35.68	****	(<0.0001)
BMI x Sex	3.430	*	(0.0290)
BMI x Age	1.483	ns	(0.1466)
Sex x Age	1.363	ns	(0.1636)
BMI x Sex x Age	3.042	*	(0.0393)

Figure 1. Bone marrow adipogenesis is influenced by age and sex. *A*: distribution of body mass index (BMI) and age in the study cohort (men: gray circles; women: black circles; summarized in Table 1). Broken lines indicate stratification into individuals with a healthy BMI (BMI <25 kg/m^2) or a BMI indicating overweight/obesity (BMI ≥ 25 kg/m^2), and individuals grouped into the younger (age ≤ 46 yr) and older (age >46 yr) subgroups. *B*: quantification of MR-based measurements of bone marrow proton density fat fraction (PDFF) as a marker of BMAT mass in the groups separated by BMI (healthy: BMI <25 kg/m^2) and (overweight/obese: BMI ≥ 25 kg/m^2), sex (men: gray open/filled circles; women: black open/filled circles), and age (age ≤ 46 yr: open circles; age >46 yr: filled circles). Data are depicted as grouped scatter plots with lines indicating medians for each group; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$ indicate significant differences by multiple comparisons testing after three-way ANOVA. Source of variation analysis is summarized in the box below the plot for significances for the independent variables' (body weight, sex, and age) contribution to variation and for the pairwise and triplewise interactions (i.e. BMI $\times$ sex $\times$ age) between factors (ns, not significant; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$). MR, magnetic resonance.

Using three-way analysis of variance (3-way ANOVA), we compared the eight subgroups to examine which of the three factors, age, BMI, and sex, contributed to variation in BMAT mass in our study participants. Although in this statistical model BMI did not associate with BMAT mass, age as an independent variable was the most significant determinant of BMAT mass, explaining 36% of its variation. Sex also contributed statistically significantly to variation but only explained 3.1% of the variation in BMAT mass. There were significant interactions between BMI and sex, as well as all three factors together regarding BMAT mass (Fig. 1*B*). These findings indicate that distinct, sex-specific, biological processes could be regulating marrow adipogenesis.

Analysis of Biomarkers of Bone Turnover and Metabolic Health

To examine the link between bone health, BMAT, and age-related metabolic dysfunction, we next assessed markers of bone turnover in women and men separately. Procollagen type 1 N-terminal propeptide (P1NP) and β-CrossLaps (CTX) are clinical markers of bone formation and resorption, respectively, and were analyzed alongside the concentration and activity of circulating DPP4. Among the various biomarkers of bone turnover, CTX and P1NP are widely used with a broad availability of reference datasets (23). Overall, no major sex-specific differences were found: when comparing all women to all men in our cohort, we observed no significant differences for all four markers (P1NP $P = 0.3136$; CTX $P = 0.7741$; DPP4 concentration $P = 0.9238$; DPP4 activity $P = 0.7537$ by Mann–Whitney test; data not shown). No significant differences were observed when comparing measurements of the four markers in women and men in the stratified subgroups (as listed in Table 2), with the exception of older women with BMI <25 kg/m^2 displaying a significantly higher value compared with men ($P = 0.0162$).

For P1NP, women in the older group with a normal BMI had significantly higher levels of this marker in comparison with women in the other three groups, whereas no significant differences between strata were found in men (Fig. 2*A*). Two-way ANOVA, however, showed that age was a significant factor in predicting P1NP, explaining 18% and 14% of its variance in women and men, respectively. BMI was only a significant variable in women, explaining 23% of variance (Fig. 2*A*). For bone resorption marker CTX, we observed similar results, with age and BMI explaining a significant proportion of its variance in women (12% and 16%, respectively), whereas only age was a significant predictor of CTX in men (19% explained variance; Fig. 2*B*). We next assessed whether age or overweight predicted the concentration or activity of circulating DPP4. However, no major statistically significant differences were observed between the subgroups. Analysis of variance showed that age explained a significant proportion of variance of DPP4 concentration and activity in women (10% and 23% explained variance, respectively), but no statistically significant differences were observed in men (Fig. 2, *C* and *D*).

Correlational Analysis of the Link between BMAT, Bone Turnover, and Metabolic Health

To further evaluate the relationship of DPP4 and bone health in the context of aging and metabolic disease, we

Table 2. *Summary of subgroup MRI quantifications and biomarker measurements*

Parameter	Group: Age ≤ 46/BMI < 25		Group: Age ≤ 46/BMI > 25		Group: Age > 46/BMI < 25		Group: Age > 46/BMI > 25	
Sex	M (n = 9)	F (n = 10)	M (n = 8)	F (n = 6)	M (n = 7)	F (n = 11)	M (n = 12)	F (n = 13)
CTX, ng/mL	0.57 ± 0.30	0.41 ± 0.14	0.53 ± 0.22	0.16 ± 0.10	0.37 ± 0.16	0.57 ± 0.32	0.32 ± 0.15	0.44 ± 0.27
P1NP, µg/L	59.9 ± 20.8	55.8 ± 13.2	65.3 ± 22.5	38.8 ± 15.2	48.0 ± 14.3	80.0 ± 22.5 (P = 0.0162)	46.5 ± 19.8	53.0 ± 17.1
DPP4, ng/mL	413.4 ± 111.0	360.5 ± 76.9	425.2 ± 130.5	383.3 ± 99.3	369.5 ± 86.1	467.5 ± 110.1	434.4 ± 113.4	401.8 ± 86.1
DPP4, nmol/min/mL	35.1 ± 4.7	31.1 ± 4.7 (9)	37.7 ± 10.1	28.4 ± 3.4	33.2 ± 5.7	36.8 ± 5.5	33.0 ± 9.3	35.1 ± 6.9
PDFF, %	40.7 ± 8.1	26.0 ± 4.1 (9)	31.4 ± 3.5	32.9 ± 2.7 (3)	44.8 ± 5.2	43.2 ± 12.0	49.0 ± 5.8 (10)	47.8 ± 6.1 (12)
Abdomen SAT, L	4.1 ± 1.5	4.3 ± 2.0	7.2 ± 4.9	16.6 ± 2.3	4.4 ± 0.7	4.5 ± 1.7	8.3 ± 5.0	15.3 ± 5.3
Thorax SAT, L	1.7 ± 0.6	1.7 ± 1.0	2.6 ± 1.5	5.8 ± 1.3	1.9 ± 0.4	2.1 ± 0.8	3.2 ± 1.3	5.5 ± 1.4
VAT, L	2.1 ± 1.0	0.9 ± 0.4	3.4 ± 2.1	3.8 ± 1.3	3.5 ± 1.1	1.4 ± 0.7	6.2 ± 2.7	5.0 ± 2.3
IHL, %	1.2 ± 0.8	0.7 ± 0.6	1.9 ± 2.6	4.1 ± 5.6	2.1 ± 1.5	1.0 ± 0.7	7.6 ± 8.8	6.7 ± 5.2

Analyses of biomarker levels CTX, P1NP, DPP4 concentration, and activity, as well as quantifications from the MRI-based analyses in the stratified subgroups. Study participants were divided into individuals separated by BMI (healthy: BMI <25 kg/m^2) and overweight/obese (BMI >25 kg/m^2) or age, that is, individuals up to 46 yr of age or individuals older than 46 yr. Data are depicted as means ± standard error (SE) whenever applicable. Values in parentheses depict instances when the dataset was incomplete, that is, deviating from regular n with the actual number of individuals in the group indicated in the row 'Sex'. BMI, body mass index; CTX, β-CrossLaps; DPP4, dipeptidyl peptidase-4; IHL, intrahepatic lipids; P1NP, procollagen type 1 N-terminal propeptide; PDFF, proton density fat fraction; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

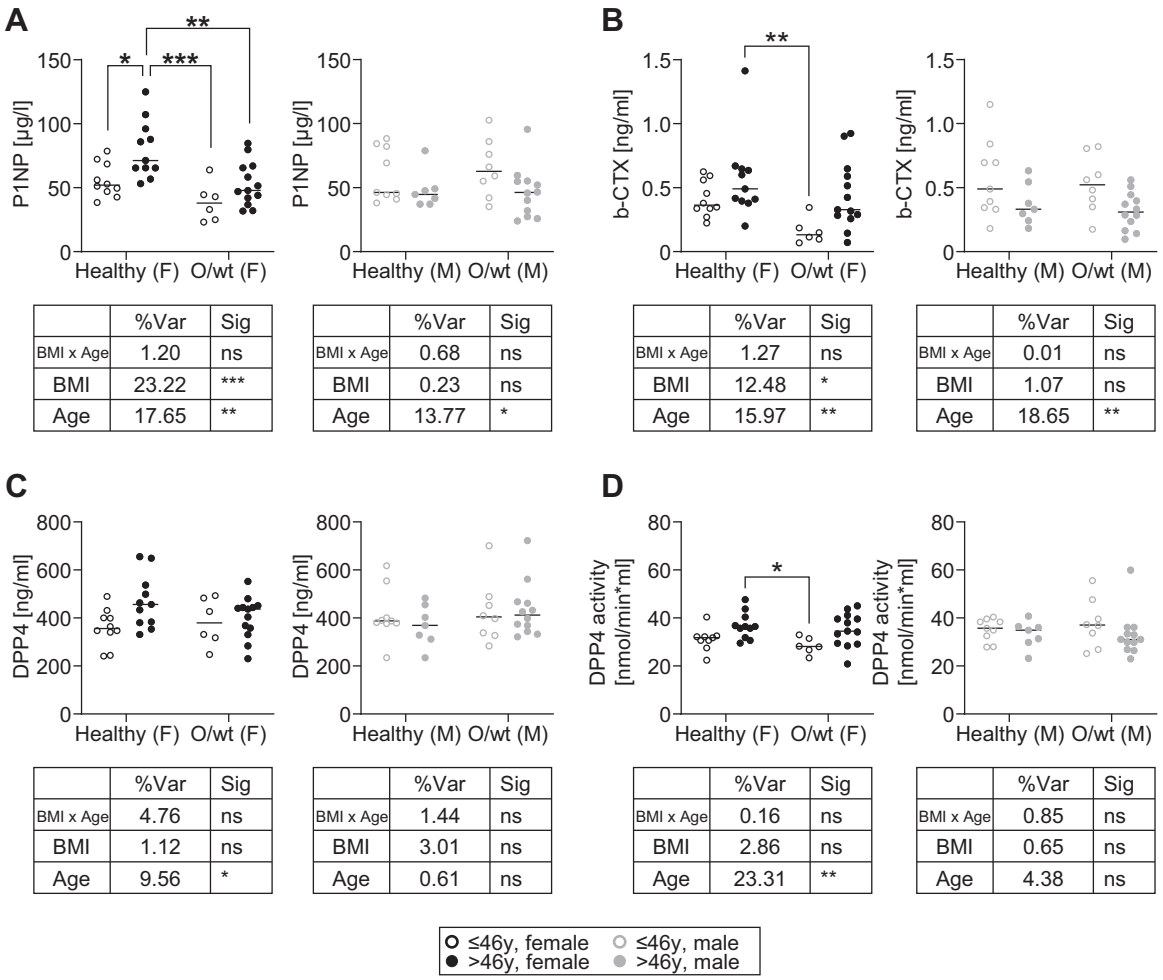
used Spearman correlations to examine the relationship of age, BMI, and HbA1c with the vertebral PDFF as a marker of BMAT mass. To evaluate bone health, bone turnover markers P1NP and CTX were included, as well as DPP4 concentration and activity, to further evaluate the impact of metabolic health status. DPP4 concentration and activity correlated significantly with each other in women and men in all analyses, indicating that both biomarkers are useful as robust representations of circulating DPP4. To better estimate the impact of each variable on these correlations, we subsequently adjusted all correlations for either of the individual variables: age, BMI, or HbA1c (Fig. 3).

In women, we observed a positive correlation of age with BMAT mass, which is consistent with the literature on aging and BMAd accrual (Fig. 3*A*). Moreover, we observed a trend for a positive correlation between both bone turnover markers and BMAT mass ($P = 0.067$ and $P = 0.099$ for P1NP and CTX, respectively). Regarding the relationship to DPP4, we observed significant positive correlations between BMAT mass and DPP4 concentration and activity. Notably, DPP4 was also positively correlated with bone turnover markers, suggesting that these three biomarker categories, that is, of BMAT, of bone turnover, and of metabolic health, are linked with each other. BMI correlated inversely with bone turnover and showed no association with DPP4 or BMAT mass (Fig. 3*A*). HbA1c was significantly associated with age and BMAT mass and showed a less strong, but significant association with both DPP4 markers (Fig. 3*A*). When adjusting these correlations for age, the positive correlation between BMAT mass and DPP4 markers became less evident suggesting a high degree of age-dependency for this relationship (Fig. 3*B*). The significant positive correlations between P1NP and both DPP4 markers were retained upon adjustment for age, and CTX was now also significantly correlated to DPP4 concentration (Fig. 3*B*). Age is therefore not a strong determinant of the correlation between bone turnover markers and DPP4 markers. Adjustment for BMI retained or increased most positive correlations and significance levels. For instance, the correlation between BMAT mass and both bone turnover markers became significant, suggesting that BMI is a mild confounder of the impact of age on the correlations (Fig. 3*C*). Correction for HbA1c had a small effect on the correlations, especially between DPP4 and BMAT mass or bone turnover markers (Fig. 3*D*).

A different pattern emerged when performing the same set of correlations in men. Age was also significantly and positively correlated to BMAT mass (Fig. 3*E*). Contrasting the observations in women, in men a significant inverse relationship was observed between age and bone turnover markers, P1NP and CTX, and this inverse relationship was also evident between both turnover markers and BMAT mass (Fig. 3*E*). Although DPP4 markers did not correlate with age or BMAT mass, a positive association, similar to women, was found between DPP4 markers and bone turnover biomarker P1NP (Fig. 3*E*). Further paralleling the observation in women, age-adjustment mitigated most correlations, with the exception of the positive correlations between bone turnover markers and DPP4, which were partially retained (Fig. 3*F*). Adjustments for BMI or HbA1c only had limited impact (Fig. 3, *G* and *H*). In summary, the positive correlation between DPP4 concentration and activity on one side and bone turnover marker P1NP on the other side remained robust in men, regardless of adjustment, thus displaying a similar pattern as in women. A similar effect, albeit somewhat less pronounced, was evident for CTX.

Correlation Analyses of BMAT, Bone Turnover, and Markers of Adiposity

BMAds represent a type of fat cell that is distinct from other adipocytes, displaying different responses to some typical physiological stimuli (2, 24). We therefore asked whether the relationship between bone turnover and other adipose tissue depots would be comparable with those observed for BMAT. For this purpose, we examined the correlations between BMAT mass and other adipose tissue depots throughout the body, that is, aSAT, tSAT, VAT, and IHL, to bone turnover markers, P1NP and CTX. The adipose tissue compartments displayed highly significant correlations among each other, to IHL, and to BMI in men and women (Fig. 4). Conversely, although significant for tSAT, VAT, and IHL, there were only moderate positive correlations with BMAT mass in women (Fig. 4*A*) and none in men (Fig. 4*B*). Mirroring the findings for BMI in the previous group-based



	%Var	Sig
BMI x Age	1.20	ns
BMI	23.22	***
Age	17.65	**

	%Var	Sig
BMI x Age	0.68	ns
BMI	0.23	ns
Age	13.77	*

	%Var	Sig
BMI x Age	1.27	ns
BMI	12.48	*
Age	15.97	**

	%Var	Sig
BMI x Age	0.01	ns
BMI	1.07	ns
Age	18.65	**

	%Var	Sig
BMI x Age	4.76	ns
BMI	1.12	ns
Age	9.56	*

	%Var	Sig
BMI x Age	1.44	ns
BMI	3.01	ns
Age	0.61	ns

	%Var	Sig
BMI x Age	0.16	ns
BMI	2.86	ns
Age	23.31	**

	%Var	Sig
BMI x Age	0.85	ns
BMI	0.65	ns
Age	4.38	ns

Figure 2. Analysis of biomarkers of bone turnover, P1NP and CTX, and DPP4 concentration and activity in stratified subgroups. *A–D*: analysis of the two bone turnover biomarkers, P1NP (*A*) and CTX (*B*), as well as DPP4 concentration (*C*) and activity (*D*) in plasma samples. Stratified groups are separated into women (F, black open/filled circles, *left*) and men (M, gray open/filled circles, *right*) and subdivided by BMI [healthy: BMI <25 kg/m^2 and overweight/obese (O/wt): BMI ≥25 kg/m^2] and age (age ≤46 yr: open circles; age >46 yr: filled circles). Data are depicted as grouped scatter plots with lines indicating medians in each group; *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate significant differences by multiple comparisons testing after two-way ANOVA. Source of variation analysis is summarized in the box below the plot for significances (Sig) for the two independent variables' (BMI and age) contribution to variation (%Var) and for the interaction between both factors (BMI × age; ns, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). BMI, body mass index; CTX, β-CrossLaps; DPP4, dipeptidyl peptidase-4; P1NP, procollagen type 1 N-terminal propeptide.

analysis, bone turnover markers were negatively correlated with most adipose tissue depots but showed some sex-specific differences: although P1NP reached statistical significance for aSAT, tSAT, and VAT in women, only CTX displayed significant correlations for all four depots and BMI in men (Fig. 4, *A* and *B*). Upon correction for either age or HbA1c as independent variables, similar correlation patterns between adipose tissue compartments among each other and the inverse correlations to bone turnover markers were largely retained, while significances of correlations to BMAT mass were lost, altogether indicating that only limited connection exists between vertebral marrow adipogenesis and fat accumulation in other depots (Fig. 4, *C–F*).

DISCUSSION

In the present study, we investigated the relationship between aging and biomarkers of bone turnover and the modulating effects of bone marrow adipogenesis, as a marker of bone disorder, and circulating DPP4, which is frequently considered a marker of metabolic health and an established target of diabetes therapies. Age correlated positively with vertebral BMAT mass in women and men, whereas markers of metabolic health, BMI, and HbA1c did not, suggesting that they may only have a more limited impact on marrow adipogenesis. It is noteworthy, however, that other studies report significant associations between various markers of obesity, such as BMI or visceral fat, and BMAT, suggesting that the small group size of our subgroups may somewhat limit data interpretation (25, 26). However, one study exclusively assessed postmenopausal women, limiting full comparability to our dataset (25). Another study showed significant positive associations between spinal PDFF and certain adiposity traits, that is, visceral fat mass in particular, whereas the positive association with BMI became fully evident only after correction for BMD (26). Interestingly, other BMAT depots, in the femoral head, diaphysis, and total hip, showed significant inverse correlations

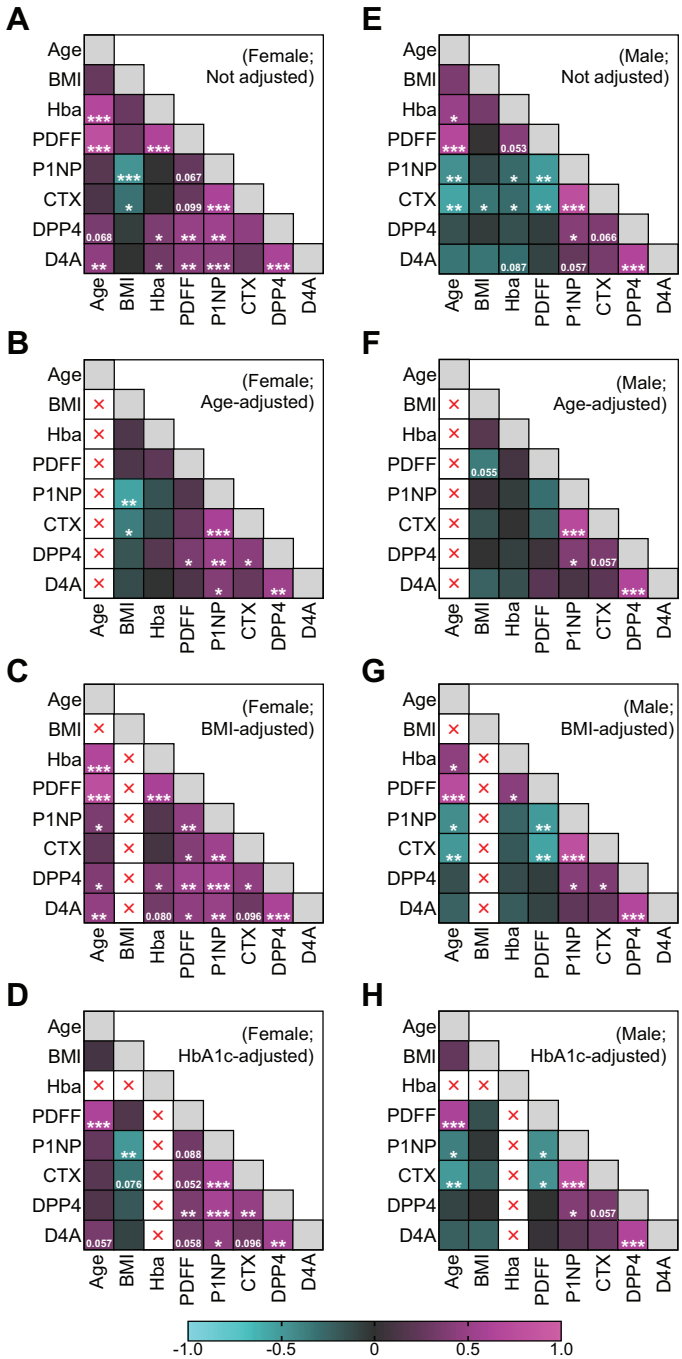


Figure 3. Interactions between bone marrow adipogenesis, bone turnover, and DPP4 occur and are mainly affected by age. *A–D*: Spearman correlation between age, BMI, HbA1c (Hba), and multiple biomarkers representing bone marrow adipogenesis (PDFF), bone turnover (P1NP and CTX), and DPP4 concentration (DPP4) and DPP4 activity (D4A), as summarized in Table 2, in women without (*A*) and after adjustment for the factors age (*B*), BMI (*C*), or HbA1c (*D*). *E–H*: corresponding correlation analyses in men without (*E*) adjustment and after adjustment for the factors age (*F*), BMI (*G*), or HbA1c (*H*). Purple color indicates positive correlations with a correlation coefficient >0, and turquoise color indicates negative correlations with a correlation coefficient <0. Statistically significant correlations are indicated as $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$; strong trends are indicated as numbers ($P < 0.1$ and ns, not significant). BMI, body mass index; CTX, β-CrossLaps; DPP4, dipeptidyl peptidase-4; HbA1c, glycated hemoglobin A1c; P1NP, procollagen type 1 N-terminal propeptide; PDFF, proton density fat fraction.

between PDFF and BMI, altogether presenting a highly complex relationship between obesity, regional fat distribution, and BMAT accumulation throughout the body, whereas age was consistently positively associated with PDFF measurements in all bone regions. Age also emerged as the main parameter that determined the correlations between BMAT mass and bone turnover markers. These associations displayed a sex dimorphism and were positively correlated in women and inversely correlated in men. Conversely, significant positive correlations between DPP4 and bone turnover markers, mainly P1NP, were observed in women and men, and these associations were independent of age, BMI, or HbA1c, suggesting a direct connection between bone health and DPP4.

Our findings on the age-related increase of marrow adipogenesis are consistent with previous studies, recapitulating the established consensus of increased bone marrow adipocyte accumulation in individuals with increased age, despite differences having been reported in men and women and in different anatomical locations (2, 4). Conversely, the link of obesity parameters, such as BMI and other fat depots, to marrow adipocyte accumulation is comparably weak in women and essentially absent in men in our dataset, further supporting the notion that BMAds represent a distinct type of fat cells with unique metabolic properties and distinct regulatory mechanisms (24). Although the impact of age on BMAT mass is well documented in men, menopause has been identified as an independent factor that increases marrow adipogenesis. In women, we can, therefore, not fully separate the impacts of these two processes, aging and menopause, on marrow adipogenesis and, therefore, also not on other biomarkers assessed in our study. The literature consensus for women is that the two markers of bone turnover assessed in our study, P1NP and CTX, increase markedly in women at the onset of menopause. Similar dynamics are also described for other markers, such as osteocalcin and bone alkaline phosphatase (23). A decline of these markers is mainly observed in early life, plateauing for some markers in adult women before menopause. We observed mild age-dependent increases for P1NP and CTX in the age-stratified groups, that is, individuals aged either above or below 46 yr, which likely split the female sub-cohort into women before and after menopause, and this was true for both BMI-separated subgroups, that is, healthy BMI versus overweight-indicating BMI. Thus, stratification of our cohort using age as a categorical variable was able to explain a significant proportion of bone turnover variance in women. Age as a continuous variable, as used in our Spearman correlations, also robustly correlated with bone turnover markers, which was statistically significant upon BMI adjustment, altogether suggesting that age might be partially responsible for increased levels of the bone turnover markers in women, but that menopause status is a major main driver of this effect. This assumption is supported by a study that linked aging to elevated levels of bone turnover biomarker bone alkaline phosphatase in postmenopausal women, that is, after the age of 50 yr. The increase could be explained by the absence of estrogen, as estrogen treatments resulted in reduced levels in postmenopausal individuals (27).

In men, the correlation of age with bone turnover biomarkers seems to be inverse for most individual markers,

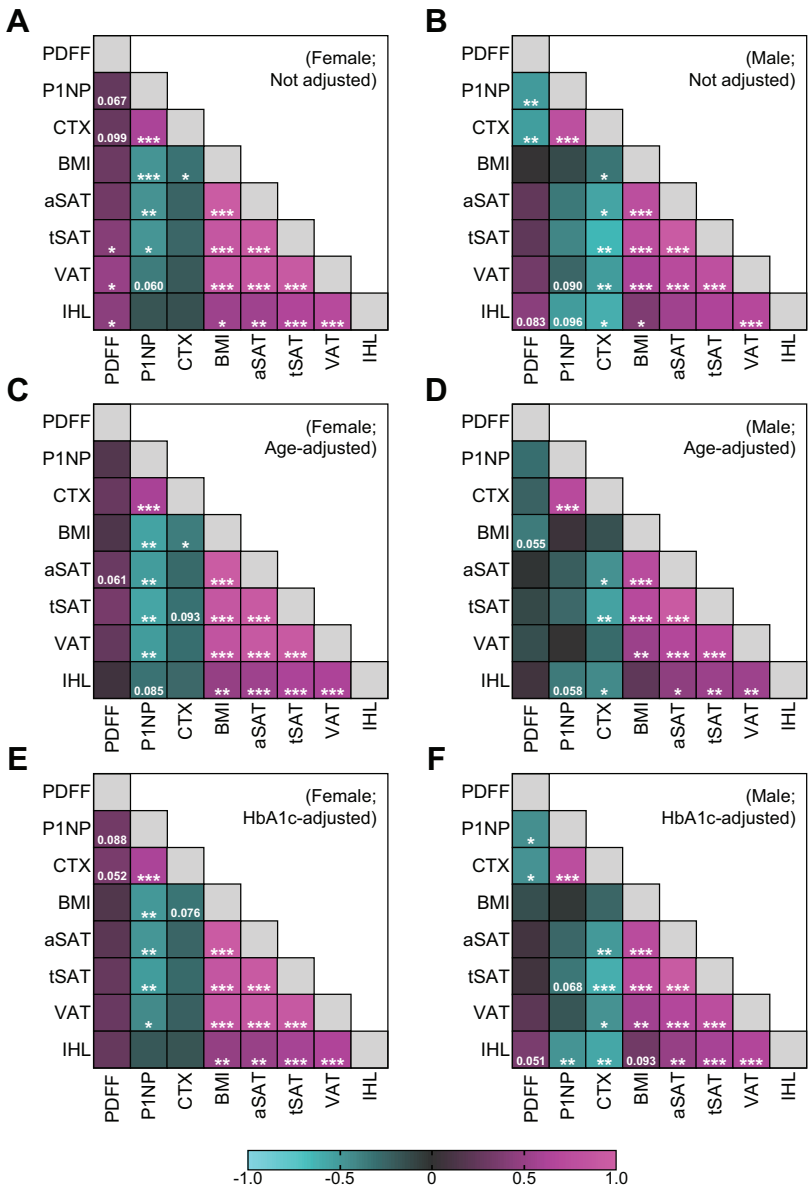


Figure 4. Sex-specific correlations of adiposity and bone turnover markers CTX and P1NP. *A–F*: Spearman correlation between BMAT mass (PDFF), bone turnover markers (P1NP and CTX), and obesity markers (BMI, aSAT, tSAT, and VAT) and IHL in women (*A*, *C*, and *E*) and men (*B*, *D*, and *F*) without adjustment (*A* and *B*) and after adjustment either for age (*C* and *D*) or for HbA1c (*E* and *F*). Purple color indicates positive correlations with a correlation coefficient >0, and turquoise color indicates negative correlations with a correlation coefficient <0. Statistically significant correlations are indicated as $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$; strong trends are indicated as numbers ($P < 0.1$ and ns, not significant). aSAT, abdominal subcutaneous adipose tissue; BMAT, bone marrow adipose tissue; BMI, body mass index; CTX, β-CrossLaps; HbA1c, glycated hemoglobin A1c; IHL, intrahepatic lipids; P1NP, procollagen type 1 N-terminal propeptide; PDFF, proton density fat fraction; tSAT, thoracic subcutaneous adipose tissue; VAT, visceral adipose tissue.

although some are reported to be unchanged, and there is some contradictory literature suggesting that mild increases in elderly men may occur, depending on estrogen availability (23, 28). For instance, P1NP was found to be decreased, but only until ∼50 yr of age, with little further change after this (29). This observation is in principle consistent with our own data, as only age explained a significant proportion of variation for P1NP and CTX in the age- and BMI-stratified subgroups in men, whereas BMI had no significant effects. Although no data on CTX were reported in the article, markers like osteocalcin and urinary N-telopeptides of type I collagen displayed similar patterns. Other markers, like bone alkaline phosphatase, showed no age-related changes in men (29). These observations are consistent with other datasets (27).

Unlike women, DPP4 was not affected by age in men, and no link to BMAT mass was evident in men in our dataset, regardless of whether stratified group analyses or correlation analyses were performed. These data taken together show a sex dimorphism for the age dependency of bone turnover biomarkers, although it stands to reason that some of this is determined by menopause in women, rather than a generalized aging process. In opposition to this, the impact of obesity and the associated measurements in our study, that is, the adipose tissue volumes and liver fat content, were more consistent between sexes, showing a significant negative correlation between bone turnover biomarkers, P1NP in women and CTX in men, and obesity, with little impact of age- or HbA1c adjustments.

In our study, we observed significant positive correlations between the two DPP4 markers and the bone turnover markers. This was particularly pronounced for bone formation marker P1NP in women but also evident in men, showing a significant correlation with DPP4 concentration and a trend with DPP4 activity ($P = 0.057$). Similar correlations were also observed for CTX, although not always statistically significant. These effects were retained after adjustments for age, BMI, or HbA1c, altogether suggesting that DPP4 may

affect bone turnover markers and that its effect is independent of metabolic health and age-related pathological factors. The impact of DPP4-associated molecular signaling pathways, such as incretin hormones, on bone turnover is well documented but mainly exists in the context of diabetes. Our preclinical studies in mice showed that DPP4, secreted from adipogenic cells of the bone marrow, could contribute to impaired bone healing, whereas administration of sitagliptin, which inhibits DPP4, resulted in activation of osteogenic progenitor cells and improved bone healing (9). This is consistent with a meta-analysis showing that diabetes drugs targeting GLP1 signaling promote osteogenic differentiation while inhibiting adipogenesis of mesenchymal stromal cells (30). Corresponding results have also been reported in clinical settings, although these data for the most part focus on patients with diabetes, where ongoing treatments may confound the relationship between DPP4, bone health/turnover, and BMAT. In patients with newly diagnosed T2D, DPP4 activity correlated with severity of osteoporosis and fracture risk, suggesting that DPP4 may impact bone health in the context of metabolic disease (15). Although DPP4 has a wide range of substrates for proteolytic cleavage, the available literature primarily addresses incretin hormones, as GLP1 and GIP are also key targets for diabetes treatment. Incretin hormone receptors are widely expressed in the skeletal system and promote bone formation, suggesting that an elevated DPP4 activity might negatively impact bone health (31). Although mouse models with deletion of the individual incretin receptors display signs of compensation, deletion of the GIP receptor in mice resulted in lower bone size and mass and altered bone metabolism, whereas deletion of the GLP1 receptor impaired bone mechanical quality (18, 32). Mice with simultaneous deletion of both receptors also showed impaired bone health (33). Finally, DPP4 may target certain immune cells and impact bone metabolism through immunomodulatory mechanisms (31). Altogether, these data support the conclusion that changes in DPP4 may directly impact bone health by altering incretin hormone activity. Further studies are needed to evaluate the impact of this endocrine axis on marrow adipogenesis. Moreover, the understanding of the impact of more recently approved diabetes drugs, like liraglutide and semaglutide, on bone remains scarce and is limited by several factors, including lack of bone turnover marker analyses as primary endpoints and short observation periods. Moreover, the analysis of the impact of such drugs has frequently been conducted in preclinical models using much higher concentrations than those used in clinical practice (reviewed in Ref. 17). Both incretin hormones and their signaling cascades have also been implicated in bone health and maintenance (18, 34). It is therefore conceivable that correlations between bone turnover and DPP4 levels, as observed in our study, may be linked to the impact of DPP4 on incretins and their ability to regulate bone formation and resorption. For instance, direct infusion of both hormones in nondiabetic men showed a significant acute impact on bone resorption (19).

Our study has limitations. The cohort size is relatively limited, which could impact broad applicability, for instance, to various ethnically diverse populations. The small group size also resulted in our single BMI threshold for the stratified group analyses by age, sex, and BMI. Since none of the participants in our study had manifest diabetes, the findings on the relationships between BMAT, DPP4, and bone turnover may not extend to patients with T2D. Moreover, our study does not consider individual participants' medication, lifestyle interventions, and bone mineral density as a marker of bone health, which should be assessed more precisely in future studies. The cross-sectional design of our study also limits interpretation of longitudinal effects of age and diabetes on bone turnover, and follow-up studies are needed to further establish the role of such changes over time. DPP4's connection with bone turnover markers is presently correlative, and our study does not explore mechanisms or provide insight into how DPP4 could influence bone or marrow health. Additional mechanistic studies are necessary to clarify the physiological role of DPP4 in this context. The study applied complex statistical adjustments to isolate the effects of variables like age, BMI, and HbA1c, which could introduce interpretation challenges. Although these adjustments provide insights, they also increase the likelihood of statistical noise, making it harder to draw definitive conclusions about the relationships observed. Related to this, a limitation of this study is the relatively small number of participants in each of the eight subgroups, potentially limiting the statistical power of subgroup analyses and increasing the likelihood of failing to detect true differences (type II errors). The study's findings suggest that age, rather than BMI, plays a prominent role in determining marrow adipogenesis and bone health in women and men. Sex-specific differences indicate that aging processes affecting bone health biomarkers, such as P1NP and CTX, are complex and vary significantly between sexes. Notably, DPP4 correlated with bone turnover markers in both sexes, and this relationship appears unrelated to age or metabolic health. Overall, the study emphasizes the need to consider sex and age independently when examining the link between bone health and marrow adipogenesis. Future research may explore the molecular mechanisms driving these sex-specific differences and the potential utility of biomarkers like DPP4 in predicting bone health outcomes across the lifespan.

DATA AVAILABILITY

Data will be made available upon reasonable request.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.G., J.M., and T.J.S. conceived and designed research; S.H., S.G., and T.H. performed experiments; S.H., S.G., O.K., T.H., J.M., and T.J.S. analyzed data; S.H., S.G., N.S., A.L.B., J.M., and T.J.S. interpreted results of experiments; S.H., S.G., and T.J.S. prepared figures; J.M. and T.J.S. drafted manuscript; S.H., J.M., and T.J.S. edited and revised manuscript; S.H., S.G., O.K., T.H., N.S., A.L.B., J.M., and T.J.S. approved final version of manuscript.

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