Marrow adipose tissue spectrum in obesity and type 2 diabetes mellitus

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Abstract

Objective: To assess the association of bone mass and marrow adipose tissue (MAT) with other fat depots, insulin resistance, bone remodeling markers, adipokines and glucose control in type 2 diabetes and obesity.

Design and methods: The study groups comprised 24 controls (C), 26 obese (O) and 28 type 2 diabetes. Dual-energy X-ray absorptiometry was used to determine bone mineral density (BMD). Blood samples were collected for biochemical measurements. ¹H Magnetic resonance spectroscopy was used to assess MAT in the L3 vertebra, and abdominal magnetic resonance imaging was used to assess intrahepatic lipids in visceral (VAT) and subcutaneous adipose tissue. Regression analysis models were used to test the association between parameters.

Results: At all sites tested, BMD was higher in type 2 diabetes than in O and C subjects. The C group showed lower VAT values than the type 2 diabetes group and lower IHL than the O and type 2 diabetes groups. However, MAT was similar in the 3 groups. Osteocalcin and C-terminal telopeptide of type 1 collagen were lower in type 2 diabetes than those in C and O subjects. Moreover, at all sites, BMD was negatively associated with osteocalcin. No association was observed between MAT and VAT. No relationship was observed among MAT and HOMA-IR, leptin, adiponectin or Pref-1, but MAT was positively associated with glycated hemoglobin.

Conclusions: MAT is not a niche for fat accumulation under conditions of energy surplus and type 2 diabetes, also is not associated with VAT or insulin resistance. MAT is associated with glycated hemoglobin.

Introduction

Obesity and type 2 diabetes mellitus are part of a spectrum respectively linked as cause and consequence, embedded in a metabolic environment of insulin resistance generated by adipose tissue and muscle. The performance of pancreatic β cells in insulin secretion determines the transition between normal glucose tolerance, pre-diabetes and type 2 diabetes, a typical pathway followed by obese individuals.

Obesity and type 2 diabetes are also independent risk factors for several disorders, including arterial hypertension, dyslipidemia, macroangiopathy and nonalcoholic steatohepatitis. These disorders have mechanisms closely related to insulin resistance, such as visceral obesity, ectopic lipid deposition and an altered profile in circulating levels of adipokines (1). On the other hand, obesity and type 2 diabetes are usually associated with normal bone mass, albeit this feature does not necessarily mean that both have a protective effect against fractures (2, 3, 4). Several studies have shown that fracture risk is higher in obese postmenopausal women (5) and obese men (6) and that fracture risk is significantly higher in type 2 diabetes (7, 8). However, there are also data showing that, among obese individuals, fracture incidence is increased in those with low bone mineral density (BMD) (9).
The pathophysiology of bone disorder in obesity and especially type 2 diabetes most likely includes specific factors that do not pertain to the universe of other chronic complications usually associated with these metabolic diseases. Osteoblasts and adipocytes within the bone marrow originate from the same mesenchymal stem cell. Several lines of evidence indicate that nutritional deprivation influences the commitment of progenitor cells toward differentiation into osteoblasts or adipocytes, whereas far less is known about the effect of energy surplus.

The mechanisms related to higher fracture risk in obesity and type 2 diabetes have not been clearly delineated. A previous study reported derangement of collagen crosslinking and cortical porosity as possible mechanisms of decreased bone strength in type 2 diabetes (10). However, there is no consensus in the literature about the impact of insulin resistance, adipose tissue distribution and fat overflow to marrow adipose tissue (MAT) on BMD. Russel et al. reported that BMD has an inverse relationship with the rate of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) in adolescent girls (11). There are also results suggesting that bone volume is negatively associated with MAT and VAT in obese individuals. On the other hand, improvement in insulin sensitivity does not necessarily lead to a beneficial effect on bone mass. For instance, both calorie restriction and treatment with thiazolidinediones have a positive effect on insulin action and glucose tolerance, but concomitantly provoke bone loss and MAT enhancement. The relationship between serum insulin levels and insulin resistance and different niches of fat depot, including MAT, has been recently evaluated in non-diabetic women (12). The study showed an inverse relationship between VAT and intrahepatic lipid (IHL) and serum levels of insulin, but no relationship was observed between MAT and circulating insulin levels. Despite these lines of evidence, the effect of insulin resistance and type 2 diabetes on bone quantity and quality has not been assessed.

The focus of this study was to assess BMD and diverse fat depots in obese and type 2 diabetes individuals and to investigate the association of bone mass and MAT with VAT, IHL and insulin resistance. The second objective was to analyze the association of MAT with factors originating in adipocyte cell lines and osteoblasts (leptin, adiponectin, Pref-1 and osteocalcin) as well as with metabolic control.

Subjects and methods

Subjects

Our study group comprised 24 controls (C: 14 females and 10 males), 26 obese subjects (O: 16 females and 10 males) and 28 patients with type 2 diabetes (15 females and 13 males). The clinical characteristics of these groups are shown in Table 1. The study was approved by the Institutional Review Board of the Ribeirao Preto Medical School, USP (#1149/2012), and all subjects gave written informed consent to participate.

Exclusion criteria were pregnancy, presence of a chronic disease known to affect bone metabolism, abnormal thyroid functioning, hypothalamic or pituitary disorders, use of estrogen/oral contraceptive, hormone replacement therapy, glucocorticoid or osteoporosis therapy (bisphosphonates, denosumab, teriparatide, strontium ranelate and calcitonin).

Due to claustrophobia, 1 control, 1 obese and 5 type 2 diabetes subjects did not undergo any MRI examination. In addition, 3 individuals in the obese group and 4 in the type 2 diabetes group refused to complete the abdominal examination. Serum insulin levels were measured in 17 C and 17 O subjects, but not in type 2 diabetes individuals due to antidiabetic therapy.

Methods

Biochemical assessment

Blood samples were collected between 0800 and 0900h after a 12-h overnight fast. Biochemical measurements (calcium, glucose, glycated hemoglobin, phosphorous, albumin, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine) were performed on the day of blood collection. Calcium, phosphorus, alkaline phosphatase, fasting glucose, albumin, AST, ALT and creatinine were determined using an automatic biochemistry analyzer (Wiener lab, CT 600 i, Thermo Fisher Scientific). The levels of glycated hemoglobin (HbA1c) were measured by high-performance liquid chromatography (D10 – Hemoglobin A1C Testing System, Bio Rad). The serum aliquots for the other parameters were stored at −70°C until the day of the assay.

25-Hydroxyvitamin D (25 (OH) D) (Liaison, DiaSorin, Saluggia VC, Italy), intact PTH (Immulate I, Siemens) and IGF-I (Immulate 2000 Siemens) were determined...
by chemiluminescence. The serum levels of osteocalcin (host-Easia Diasource, Louvain-la-Neuve, Belgium), Pref-1 (Quantikine Human Pref-1 R&D Systems), leptin (Quidel, TECOMedical Group, Gewerbestrasse, Sissach, Switzerland) and adiponectin (Millipore) were determined by enzyme immunoassay. The C-terminal telopeptide of type I collagen (CTX) was measured by electrochemiluminescence (Cobas E 411, Roche Diagnostics). All intra- and inter-assay coefficients of variation were lower than 10% and 20% respectively.

\[ ^1 \text{H-MR spectroscopy of bone marrow in L3} \]

The volunteers underwent spine MRI in a 1.5T system (Philips ACHIEVA, Philips Medical Systems), as described previously (12).

Briefly, the subjects were positioned head first in the magnet bore in the prone position. A phased-array coil was positioned over the lumbar region. Sagittal T2-weighted fast spin echo acquisition was used as a reference for the spectroscopy voxel placement. A single voxel of 3.37 mL was positioned in the center of the third lumbar (L3) vertebral body. The point resolved spectroscopy (PRESS) technique was applied using the following parameters: repetition time (TR)=2000ms, three echo times (TE)=40/60/80ms, 8 averages, without fat or water suppression.

MRS data were processed with LCModel software (Version 6.1, http://www.s-provencher.com/pages/lcmodel.shtml). The area values of the CH2 lipid peak at 1.3 ppm and the water peak at 4.7 ppm were T2-corrected using a fitting to a mono-exponential decay curve. Finally, the MAT content of L3 was expressed as lipid/(water+lipid) estimated from the corrected water and fat concentrations.

\[ \text{Abdominal magnetic resonance imaging} \]

Abdominal images were acquired with a phased-array torso coil. A coronal turbo-spin-echo (TSE) T2-weighted sequence with breath-holding was applied to localize the following scan volumes. Consecutively, a breath-holding
axial gradient double-echo T1-weighted sequence, in phase (echo time=4.2 ms) and out of phase (echo time=2.1 ms, slice thickness=6.0 mm), was acquired including the whole abdomen, centered on the umbilical region.

The formula: fat = (SI in phase – SI out of phase)/(2SI – in phase) was used to calculate IHL, VAT and SAT from the averaged signal intensity (SI) in each region of interest (ROI) or labels defined by image segmentation. The SI values in the previously mentioned formula refer to the pair in/out phase images. In the liver, a manual segmentation was performed to select four ROIs as representative segments of the liver at the level of the main portal vein. The label of the visceral and subcutaneous fat areas was defined using the Display software (http://www.bic.mni.mcgill.ca/software/Display/Display.html) and a semiautomatic segmentation of an axial slice at the level of the umbilicus. This methodology has been described previously in detail elsewhere (12).

**Dual-energy X-ray absorptiometry**

Bone mineral density in the lumbar spine (L1–L4), total hip and femoral neck was determined by dual-energy X-ray absorptiometry (Hologic Discovery Wi, QDR series, Waltham, MA, USA). The precision error was 1.2% for L1–L4, 2.3% for the femoral neck and 2.7% for total hip. BMD values are expressed as g/cm².

**Statistical analysis**

The data for the three groups were analyzed by one-way ANOVA followed by the Duncan post-test. The confidence interval was 95%. ANOVA was calculated with the aid of the SAS 9.4 software (SAS Institute Inc., SAS/STAT User’s Guide, Version 9.4, Cary, NC, USA: SAS Institute INC., 2013).

The linear regression model was applied, with age and BMI considered as covariates. To determine the association of the variables of interest, simple linear regression models (model 1) were adjusted by age and BMI (model 2), yielding the regression coefficients and R-square. All analyses were carried out using the SAS 9.4 software. The level of significance was set at 0.05.

**Results**

The study included 24 healthy controls, 26 obese subjects and 28 type 2 diabetes patients. Table 1 shows the anthropometric characteristics and biochemical evaluation of the subjects enrolled in this study. The O and type 2 diabetes groups were appropriately matched for age, sex, height, weight and BMI, but both showed higher weight and BMI than the C group. The circulating levels of glucose and glycated hemoglobin (HbA1c) were higher in the type 2 diabetes group. The three groups had similar serum levels of calcium, phosphorus and alkaline phosphatase.

No significant differences in the serum levels of ALT, AST, creatinine, PTH, 25(OH)D and IGF-I were observed between groups (Supplements). The C and O groups had similar serum levels of osteocalcin and CTX and their values were higher than those observed in type 2 diabetes patients.

BMD values for the lumbar spine (Fig. 1A), total hip (Fig. 1B) and femoral neck (Fig. 1C) were higher in type 2 diabetes than those in O and C subjects (P<0.05), but did not differ between the O and C groups (Table 1). The number of individuals with osteopenia (C=11, O=12, type 2 diabetes=9) and osteoporosis (C=3, O=3, type 2...
diabetes=0) indicated that the rate of low bone mass was 58%, 57.6% and 32% in the C, O and type 2 diabetes groups respectively.

VAT was higher in type 2 diabetes than that in C subjects (Fig. 1E). Also, the type 2 diabetes and O groups showed significantly higher IHL than the C group (P<0.05; Fig. 1F). In contrast, no difference in MAT values was found between the 3 groups (Table 1; Fig. 1F).

Table 2 shows the results of linear regression analysis. There was no association among lumbar spine BMD and IHL, VAT or VAT/SAT rate. Also, no relationship was observed between BMD and serum insulin levels or HOMA-IR. A tendency to a negative association between BMD and MAT was observed in the lumbar spine (P=0.061), but this weak relationship was not maintained after adjustment for age and BMI.

As expected, VAT and IHL exhibited a positive association (Fig. 2A), which persisted after adjustment for age and BMI. However, no association was observed between MAT and VAT (Fig. 2B) and VAT/SAT. Moreover, in contrast to VAT, MAT exhibited no relationship with HOMA-IR (Fig. 2C) or serum insulin levels.

**Table 2**  Linear regression results.

<table>
<thead>
<tr>
<th>Associations</th>
<th>Estimate</th>
<th>P value</th>
<th>IC (95%)</th>
<th>R²</th>
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<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>L1–L4 BMD × IHL</td>
<td>0.001</td>
<td>0.55</td>
<td>-0.003</td>
<td>0.006</td>
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<tr>
<td>Total hip BMD × IHL</td>
<td>0.003</td>
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<td>0.008</td>
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<td>Femoral neck BMD × IHL</td>
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<td>0.15</td>
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<td>1E-05</td>
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<td>Total hip BMD × VAT</td>
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<td>Femoral neck BMD × VAT</td>
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<td>-3E-06</td>
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<td>-0.068</td>
<td>0.203</td>
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<td>Femoral neck BMD × VAT/SAT</td>
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<td>L1–L4 BMD × insulin</td>
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<td>-5E-04</td>
<td>0.001</td>
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<td>Total hip BMD × insulin</td>
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<td>0.36</td>
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<td>0.001</td>
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<tr>
<td>Femoral neck BMD × insulin</td>
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<td>0.34</td>
<td>-5E-04</td>
<td>0.001</td>
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<td>L1–L4 BMD × HOMA-IR</td>
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<td>0.08</td>
<td>-0.002</td>
<td>0.047</td>
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<td>Total hip BMD × HOMA-IR</td>
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<td>0.08</td>
<td>-0.002</td>
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<td>Femoral neck BMD × HOMA-IR</td>
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<td>0.14</td>
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<td>0.043</td>
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<td>L1–L4 BMD × MAT</td>
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<td>-0.008</td>
<td>0.0</td>
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<td>Adiponectin × MAT</td>
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<td>0.87</td>
<td>-0.34</td>
<td>0.29</td>
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<td>Adiponectin × VAT</td>
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<td>0.01</td>
<td>-0.001</td>
<td>-2E-04</td>
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<td>Adiponectin × SAT</td>
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<td>0.83</td>
<td>-2E-04</td>
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<td>Adiponectin × VAT/SAT</td>
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<td>&lt;0.01</td>
<td>-22.48</td>
<td>-3.64</td>
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<td>Leptin × MAT</td>
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<td>Leptin × VAT</td>
<td>-7E-04</td>
<td>0.39</td>
<td>-0.002</td>
<td>0.001</td>
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<tr>
<td>Leptin × SAT</td>
<td>0.0015</td>
<td>&lt;0.01</td>
<td>0.001</td>
<td>0.002</td>
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<td>Leptin × VAT/SAT</td>
<td>-48.08</td>
<td>&lt;0.01</td>
<td>-71.67</td>
<td>-24.49</td>
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<td>Pref-1 × MAT</td>
<td>-0.003</td>
<td>0.18</td>
<td>-0.008</td>
<td>0.001</td>
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<td>Pref-1 × IHL</td>
<td>0.001</td>
<td>0.81</td>
<td>-0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Pref-1 × VAT</td>
<td>1E-05</td>
<td>0.11</td>
<td>-1E-06</td>
<td>1E-05</td>
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<tr>
<td>Pref-1 × VAT/SAT</td>
<td>0.03</td>
<td>0.64</td>
<td>-0.11</td>
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<tr>
<td>IHL × VAT</td>
<td>0.001</td>
<td>&lt;0.01</td>
<td>0.0003</td>
<td>0.001</td>
</tr>
<tr>
<td>MAT × VAT</td>
<td>1E-05</td>
<td>0.955</td>
<td>-4E-04</td>
<td>0.0004</td>
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<tr>
<td>MAT × VAT/SAT</td>
<td>2.737</td>
<td>0.49</td>
<td>-4.999</td>
<td>10.473</td>
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<td>MAT × HOMA-IR</td>
<td>-0.055</td>
<td>0.16</td>
<td>-0.131</td>
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<td>MAT × insulin</td>
<td>-0.857</td>
<td>0.34</td>
<td>-2.616</td>
<td>0.903</td>
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<td>L1–L4 BMD × osteocalcin</td>
<td>-0.012</td>
<td>&lt;0.01</td>
<td>-0.019</td>
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<tr>
<td>Total hip BMD × osteocalcin</td>
<td>-0.012</td>
<td>&lt;0.01</td>
<td>-0.018</td>
<td>-0.005</td>
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<td>Femoral neck BMD × osteocalcin</td>
<td>-0.013</td>
<td>&lt;0.01</td>
<td>-0.02</td>
<td>-0.006</td>
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<tr>
<td>Insulin × osteocalcin</td>
<td>0.751</td>
<td>0.58</td>
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<td>Osteocalcin × glucose</td>
<td>-0.28</td>
<td>0.09</td>
<td>-0.6</td>
<td>0.05</td>
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<tr>
<td>MAT × glucose</td>
<td>0.033</td>
<td>0.5</td>
<td>-0.063</td>
<td>0.129</td>
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<tr>
<td>MAT × HbA1c</td>
<td>0.321</td>
<td>0.4</td>
<td>-0.436</td>
<td>1.078</td>
</tr>
</tbody>
</table>

**Table 2** Linear regression results.

BMD, bone mineral density; HbA1c, glycated hemoglobin; IHL, intrahepatic lipids; L1–L4, lumbar spine; MAT, marrow adipose tissue; Pref-1, preadipocyte factor 1; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue. Model 1 is a non-adjusted model and model 2 is a model with age and BMI considered as covariates.
There were significant differences in the behavior of circulating levels of adipocyte-originated factors among the 3 groups. Although adiponectin was higher in C than that in O and type 2 diabetes subjects, the serum levels of leptin tended to be lower in the C group \((P=0.064)\). The 3 groups showed similar values of Pref-1. These 3 peptides produced by adipocyte cell lines showed no association with MAT (Table 2).

A negative association was detected among osteocalcin and lumbar spine (Fig. 2D), total hip (Fig. 2E) and femoral neck (Fig. 2F) BMD. However, no association was observed between the serum levels of osteocalcin and insulin or glucose.

MAT showed no association with glucose, but was positively associated with HbA1c.

**Discussion**

Obesity reflects the high capacity of white adipose tissue (WAT) to store lipids. However, the overflow of lipids from WAT to other tissues reveals that a certain limit exists concerning the ability of WAT to retain lipids in its domain. Excessive accumulation of fat within and subsequently outside adipose tissue leads to functional (insulin resistance) and structural disorders not only in adipose tissue itself but also in muscle, liver and pancreas (13). Type 2 diabetes and nonalcoholic steatohepatitis (NASH) are illustrative consequences of lipotoxicity triggered by the overflow of lipids (14, 15). Bone disease in type 2 diabetes remains as a conundrum in which, unexpectedly, the role of MAT and insulin resistance has scarcely been investigated. This study suggests that MAT represents a diverse and sole type of adipose tissue: (a) it is not a site for fat storage during energy surplus, (b) it seems to have no relationship with VAT, serum levels of insulin or insulin resistance and (c) is positively associated with HbA1c.

Several studies have observed that bone mass seems not to be negatively affected by type 2 diabetes and hyperglycemia (16, 17, 18). This study reaffirms these data and provides some additional interesting information. Bone mass was higher in type 2 diabetes not only compared with the control group but also compared with the obese group. These results indicate that BMD in type 2 diabetes individuals results from a more complex configuration than simply the mechanical and endocrine/paracrine influence of the adipose tissue existing in obese non-diabetic individuals. For instance, non-severely obese individuals do not share a low bone turnover profile with type 2 diabetes patients (19, 20, 21). Rochefort et al. (22) evaluated the serum concentrations of osteocalcin and insulin in a population of prepubertal obese and control children and observed that the circulating levels of osteocalcin were similar in the two groups. Similarly, another study conducted on non-diabetic overweight and obese adults reported that BMI and body weight did not differ across tertiles of total or undercarboxylated osteocalcin (23). On the other hand, the present results seem to conflict with those obtained in a previous study performed by Pittas et al. in a cohort study of individuals aged 65 years and older, showing that osteocalcin is inversely associated with BMI and fat mass (24). There are several differences between this study and the one by Pittas et al. which render them incompatible for comparison: the age difference between the two groups studied and, more importantly, the inclusion of some obese subjects who also had diabetes in the Pittas study. The present results agree with those reported in most studies of bone remodeling in type 2 diabetes, which identified low bone formation and resorption

**Figure 2**

Regression analysis among (A) lumbar spine, (B) total hip and (C) femoral neck bone mineral density (BMD) and osteocalcin, (D) HOMA-IR and marrow adipose tissue (MAT), (E) visceral adipose tissue (VAT) and MAT, (F) intrahepatic lipids (IHL) and MAT.
activity based on the determination of biochemical markers (25, 26). Obese patients exhibited osteocalcin and CTX values similar to control. Moreover, there was a negative association between serum levels of osteocalcin and lumbar spine BMD, suggesting that the high BMD in type 2 diabetes is closely related to low bone turnover.

Although obesity is a major determinant of type 2 diabetes mellitus, the site of fat allocation is more important than the amount of fat (27). Several steps are involved in the onset of insulin resistance, especially the accumulation of fat in VAT and subsequently its redistribution to pancreas, muscle and liver (28). Next, β cell dysfunction becomes the last step in the onset of type 2 diabetes (29). Our data confirm previous studies showing that VAT and IHL are significantly increased in obese individuals (30) and even more so in type 2 diabetes patients (15). This study contributes information showing that MAT did not differ significantly among control, obese and type 2 diabetes subjects. It should be highlighted that the difference in IHL was significantly higher between the control and obese groups (253%) than that between the obese and type 2 diabetes groups (50%). The same pattern was observed when we calculated the variation of VAT between control and obese subjects (36%) and between obese and type 2 diabetes subjects (5.3%). Conversely, MAT showed a different and unique behavior, i.e., it was slightly lower in the obese group compared with control (11%) and was higher in type 2 diabetes subjects than that in the obese subjects (14.8%). These results clearly show that marrow adipocytes do not accumulate fat under conditions of energy surplus and adapt differently to nutritional variation. Indirectly, these results support previous clinical and experimental investigations showing that MAT increases under conditions of calorie restriction, whereas the other fat depots shrink (31). MAT in humans and mice exhibits the same pattern of adaptation under conditions of caloric restriction, with MAT expansion occurring in both species (31, 32, 33). Accumulated experimental evidence indicates that the incremental amount of MAT under caloric restriction results from progenitor recruitment and adipocyte differentiation rather than from adipocyte hypertrophy, arguing against a target for energy support in this process (34, 35). Intriguingly, mice and humans respond differently when exposed to a high-fat diet. The former exhibit a concomitant enhancement of WAT and MAT under a high-fat diet regimen (36), whereas the total amount of MAT remains unchanged in obese humans (37). Conceivably, the relatively smaller quantity of MAT in smaller animals such as mice accounts for trace level changes in several physiological conditions. Further, human studies are necessary to investigate MAT quality and the profile of changes in bone adipocyte secretion during fat surplus.

A recent study on a group of non-diabetic participants predominantly consisting of normal and overweight women demonstrated a positive relationship between MAT and circulating glucose levels and showed no relationship between serum insulin levels or HOMA-IR and MAT (12). This study, which included normal weight, obese and type 2 diabetes individuals of both sexes, also supports these findings, demonstrating that MAT has a positive relationship with HbA1c values and no association with serum insulin levels or HOMA-IR. Moreover, an association was observed between VAT and IHL and the association was maintained after correction for age and BMI. In contraposition, no association was verified between MAT and VAT. The present results are not in line with those obtained by Bredella et al. in a previous study evaluating obese women (38). The authors described a weak positive association between MAT and VAT in 47 healthy premenopausal women, whose BMI ranged from 18.1 to 41.4 kg/m² with a mean age of 32.8±7.1 years. Also, they found no association between age and MAT. The two studies are not directly comparable; this study also included diabetic patients, not only women but also men, and the mean age of the volunteers differed (38). Indirectly, the present results are supported by a recent study showing that higher HOMA-IR is associated with greater volumetric BMD and generally favorable bone microarchitecture, independent of body weight (39). Furthermore, the associations between HOMA-IR and bone microarchitecture persisted after adjusting for multiple potential covariates including time since menopause. Based on these results, the authors hypothesized that insulin resistance may protect, at least in part, against bone loss due to estrogen deficiency and/or aging in postmenopausal women (39).

Peptide secretion by VAT is largely influenced by the amount of lipids stored in adipocytes. Although leptin and proinflammatory adipokines increase with progressive triglyceride accumulation, adiponectin secretion is reduced during cell enlargement. As expected, leptin was slightly higher in the obese group, whereas adiponectin was higher in the control group, but there was no relationship between BMAT or BMD and leptin or adiponectin levels. A previous study reported a positive correlation between BMD and leptin, but the relationship disappeared after adjustment for fat mass (40). Experimental approaches indicate that
leptin input through the hypothalamic ventromedial nucleus stimulates the sympathetic nervous system, hampering osteoblast activity (41). Conversely, there is also some evidence that leptin also exerts a direct stimulatory action on osteoblasts. In the clinical setting, another relevant factor accounting for difficulties in understanding the action of leptin on bone is the evolving process of leptin resistance with obesity. Pref-1, a peptide produced by adipocyte precursor cells, inhibits adipocyte and osteoblast differentiation. A previous study showed that patients with anorexia nervosa have higher or similar serum Pref-1 levels compared with age-matched healthy women (42). In this study, the circulating levels of Pref-1 were similar in control, obese and type 2 diabetic subjects. To our knowledge, only one study determined the serum levels of Pref-1 in these 3 groups (43). No significant difference was detected between control and obese subjects, but type 2 diabetes individuals had higher Pref-1 levels than control. It should be emphasized that the cited study was conducted solely on women with a mean BMI value compatible with severe obesity.

In anorexia nervosa, serum Pref-1 levels are increased and are positively correlated with MAT, whereas they are negatively correlated with BMD (42). More recently, it was shown that in women with anorexia nervosa, Pref-1 is responsive to either clinical recovery or transdermal estrogen therapy, which has an inhibitory effect on Pref-1 production (44, 45). Enhanced BMD was detected in both studies, and the first investigation showed a reduction in MAT (44). These relationships were not observed in the present normal weight, obese or type 2 diabetes subjects. Previous studies also indicate that estrogen deficiency is associated with MAT expansion (46); although estrogen levels were not measured, it can be predicted that estrogen did not account for the differences between the groups in relation to adipose tissue parameters. The women included were appropriately matched in relation to premenopausal and postmenopausal status: 36% and 64% in C group, 31% and 69% in O group and 27% and 73% in type 2 diabetes group respectively.

This study has some limitations. The sample size was small, and the study had a cross-sectional design. On the other hand, the groups of patients were well defined, and all diabetic patients had been diagnosed with the disease at least five years before the study. Also, no diabetic patient had nephropathy, clinical neuropathy or proliferative retinopathy. In addition, the methods used for fat assessment are accurate, allowing precise measurements of fat within different fat pads.

Conclusion
This study shows that marrow is neither a niche for fat accumulation in energy surplus nor has any association with insulin resistance. Our findings also suggest that glucose excess may be of pathophysiologic significance for the development of marrow adipose tissue.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
I M A and F J A P conceived and designed the experiments; I M A, C E G S, A K N, M H N B, J E Jr and F J A P performed the experiments; I M A, C E G S, M H N B, J E Jr and F J A P analyzed the data; A K N did the statistical analysis; I M A and F J A P wrote the manuscript; C E G S, A K N, M H N B and J E Jr reviewed/edited the manuscript. Guarantor's name: I M A and F J A P.

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