

# Cold-activated brown adipose tissue is an independent predictor of higher bone mineral density in women

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## Abstract

**Summary** In animals, defective brown adipogenesis leads to bone loss. Whether brown adipose tissue (BAT) mass relates to bone mineral density (BMD) in humans is unclear. We determined the relationship between BAT mass and BMD by cold-stimulated positron-emission tomography (PET) and dual-energy X-ray absorptiometry (DXA) in healthy volunteers. Higher BAT mass was associated with higher BMD in healthy women, but not in men, independent of age and body composition.

**Introduction** Contrary to the traditional belief that BAT is present only in infants, recent studies revealed significant depots of BAT present in adult humans. In animals,

defective brown adipogenesis leads to bone loss. While white adipose tissue mass is a known determinant of BMD in humans, the relationship between BAT and BMD in humans is unclear. We thus examined the relationship between BAT and BMD in healthy adults.

**Methods** BAT volume (ml) and activity (standard uptake value) were determined by  $^{18}\text{F}$ -fluorodeoxyglucose PET after overnight mild cold exposure at 19 °C, and BMD was determined by DXA.

**Results** Among 24 healthy adults (age  $28 \pm 1$  years,  $F=10$ ), BAT volumes were  $82.4 \pm 99.5$  ml in women and  $49.7 \pm 54.5$  ml in men. Women manifested significantly higher BAT activity, by  $9.4 \pm 8.1$  % ( $p=0.03$ ), than men. BAT volume correlated positively with total and spine BMD ( $r^2=0.40$  and  $0.49$ , respectively,  $p<0.02$ ) in women and remained a significant predictor after adjustment for age, fat, and lean body mass ( $p<0.05$ ). Total and spine BMD were higher in women who harbored visually detectable BAT on PET images than those without by  $11 \pm 2$  % ( $p=0.02$ ) and  $22 \pm 2$  % ( $p<0.01$ ), respectively. No associations were observed between BAT parameters and BMD in men. **Conclusions** This study demonstrated higher BMD among healthy women with more abundant BAT, independent of age and other body compositional parameters. This was not observed in men. The data suggest that brown adipogenesis may be physiologically related to modulation of bone density.

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## Introduction

There are two kinds of adipose tissues. White adipose tissue specializes in energy storage and is in excess in obesity. In

contrast, brown adipose tissue (BAT) regulates adaptive thermogenesis and energy homeostasis [1]. Through the action of uncoupling protein-1 (UCP1), BAT dissipates the electrochemical energy in the mitochondrial respiratory chain as heat. Animals with abundant BAT are protected from diet-induced obesity, while UCP1 knockout animals become obese on a normal diet at thermoneutrality [2]. Although BAT was traditionally believed to disappear after infancy in humans, recent evidence has revealed significant depots of BAT in the cervico-supraclavicular region of adults [3–6]. Consistent with its energy-dissipating function, BAT abundance is associated with reduced fat mass (FM) in humans [3, 6].

Body composition is an important predictor of bone mineral density (BMD). Greater lean body mass (LBM) predicts higher BMD [7] and has been attributed to the stimulatory effects of mechanical stress on osteoblastogenesis. Conversely, some [8, 9], but not all [10, 11], studies showed positive correlations between higher FM and BMD, with variations apparently arising from differences in age, gender, health status, and/or adipose distribution [12]. For example, excess visceral adipose tissue correlates negatively with BMD, attributable to actions of inflammatory cytokines on bone modeling [12].

These apparently conflicting data beg the question as to whether adiposity exerts differential impact on the bone in an adipose composition-specific manner and whether a causal relationship between adiposity and BMD may reside within BAT, at least in some humans. This may be supported by recent studies that revealed CCAAT/enhancer-binding protein  $\beta$  (C/EBP $\beta$ ) to be a regulator of bone remodeling and adipogenesis. Animals with C/EBP $\beta$  deficiency demonstrate both bone loss and impaired BAT formation [13, 14], reminiscent of age-related decline in bone mass and BAT activity observed in humans. Whether BAT impacts BMD in humans is unclear. Given these findings, we hypothesize that greater BAT abundance may be associated with higher BMD in humans. To investigate this, we capitalized our recently developed technique for quantifying BAT volume and correlate it with BMD in healthy adults.

## Subjects and methods

### Subjects

Study participants consisted of 24 volunteers (ten women) recruited through local advertisement to participate in an energy metabolism-related protocol at the National Institutes of Health (ClinicalTrials.gov identifier number NCT00521729). All subjects were healthy and on no medications. The women were studied during the early follicular phase of the menstrual cycle (days 2–5). Volunteers were

admitted to the Metabolic Clinical Research Unit of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in Bethesda, MD, USA, for assessment of body composition, BMD, and cold-stimulated BAT. The protocol was approved by the NIDDK-NIAMS institutional review board, and informed consent was obtained from all subjects.

### BMD and body composition

BMD and body composition were measured by dual-energy X-ray absorptiometry (Lunar iDXA; GE Healthcare, Madison, WI, USA). Scan analysis was performed using GE Encore 11.10 software. The NHANES database was used as reference for body Z-scores. The following parameters were measured in each volunteer: total body, total arm, total leg, spine, and pelvis BMD; FM; trunk fat; and LBM. The coefficients of variation of BMD, FM, and LBM were 0.5, 1.0, and 0.5 %, respectively.

### Cold-stimulated BAT

As BAT functions as a thermogenic organ, cold exposure increases its activity while thermoneutrality mutes it [3, 4]. The current standard for BAT detection is by positron-emission tomography (PET) scanning following cold exposure. This method utilizes  $^{18}\text{F}$ -fluorodeoxyglucose (FDG) as a tracer to quantify overall BAT metabolic activity. Previous studies showed reliable detection and estimation of BAT volume in humans by FDG-PET following cold exposure [3, 4]. We therefore employed cold-stimulated PET scanning, an established and validated technique, to measure BAT volume and activity for correlation with BMD.

Volunteers, wearing standardized hospital gowns, slept for 12 h in a room where the temperature was set at 19 °C. A 15-mCi injection of FDG was given 1 h prior to the end of the cold exposure, which continued during the hour of FDG equilibration. PET scanning was performed immediately thereafter and completed within 30 min.

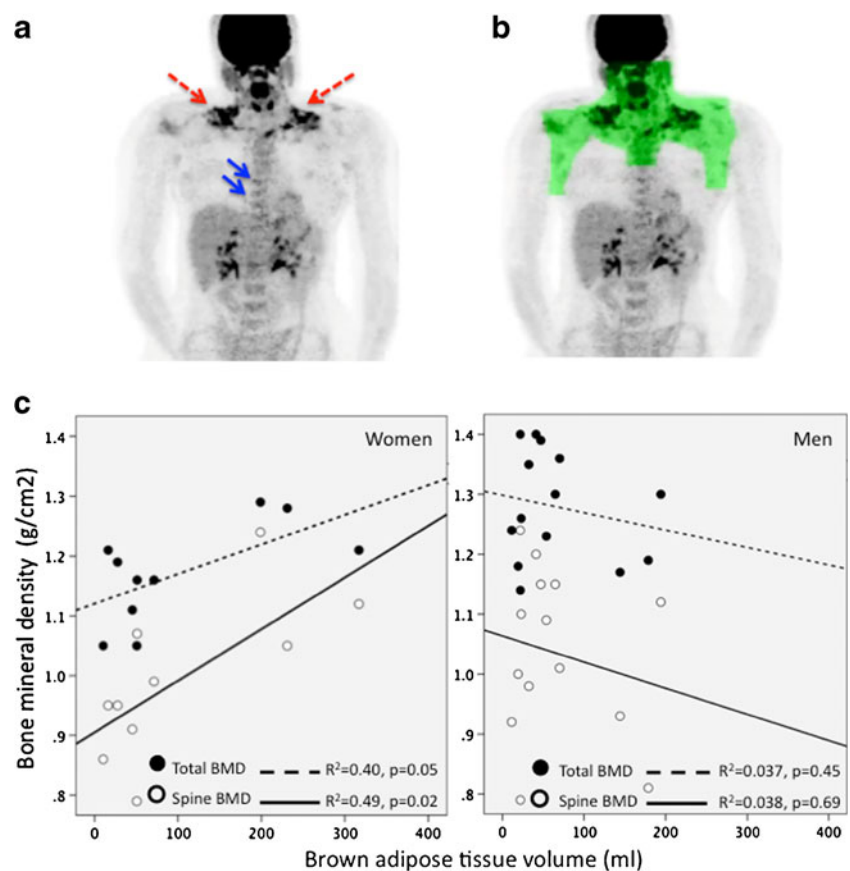
Each scan was first examined for visually detectable BAT in the ventral neck, superficial and lateral to the sternocleidomastoid muscles, the established common reservoir of BAT in humans [3, 6]. However, BAT activity varies between individuals, and previous histology-imaging correlative studies showed that FDG uptake can be below the visually detectable threshold on PET scans obtained from individuals who harbor lower abundance of BAT [5]. In other words, low-grade BAT activity is visually undetectable but histologically present in some humans [5]. We therefore constructed a torso-mantle to define a region of interest (ROI) to capture the cervical–supraclavicular–thoracic BAT depot using a customized program (IDL software, Exelis Visual Information Solutions, Inc., Boulder, CO,

USA). Mean standardized uptake values (SUVs), which represent the activity per milliliter within the ROI divided by the injected dose in megabecquerels per gram of body weight, were determined within the torso-mantle. The volume of BAT, defined as the sum of the volume of all voxels with SUV values greater or equal to 2.0 g/ml, was quantified within the torso-mantle. Quantification of mean SUV within the BAT-specific torso-mantle allows a systematic and objective estimation of BAT volume and activity, which is more sensitive than visual examination alone. A representative image of BAT uptake and the three-dimensional reconstruction of the ROI are shown in Fig. 1.

### Statistical analysis

Statistical analysis was performed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). Data are expressed as mean  $\pm$  standard deviation for normally distributed variables. Comparisons between results were performed using the unpaired *t* test. Multivariate linear regression models using analysis of variance were constructed to determine the predictors of BMD, including age, gender, FM, and LBM. An  $\alpha$  error of 0.05 was considered the threshold for statistical significance.

**Fig. 1** **a** PET image of a 20-year-old woman demonstrating FDG uptake in cervical–supraclavicular BAT depots (*red arrows*). Uptake was also evident within the vertebra (*blue arrows*) that harbored the bone marrow with BAT characteristics [18]. **b** A representative image of BAT uptake and the three-dimensional reconstruction of the torso-mantle (*green*) that was constructed to allow quantification of FDG uptake within BAT-specific region. **c** Positive correlations between BAT volume with total and spine BMD in women (*left*), but not in men (*right*)



## Results

### Clinical characteristics

Table 1 summarizes clinical characteristics of participating subjects. All subjects had normal BMD. BAT was visually detectable in six out of 24 (three females and three males) individuals following cold exposure. Using the BAT-specific torso-mantle quantification method, the amount of BAT ranged from 10.4 to 316.9 ml in the entire cohort, with mean torso-mantle SUV between 0.74 and 1.10.

As expected, men had significantly higher LBM as well as total and regional BMD, but lower FM, than women (Table 1). Z-scores were within the normal range for all subjects. There was a trend for higher BAT volume in women. BAT activity, as quantified by SUV, was  $9.4 \pm 8.1$  % higher in women than in men ( $p=0.03$ ).

### BMD and body composition

Total BMD correlated positively with LBM ( $r=0.75$ ,  $p<0.001$ ), but not with FM ( $p=0.8$ ). Positive correlation between BMD and LBM was seen in men ( $r=0.62$ ,  $p=0.02$ ), and similar positive correlation approached significance in women ( $r=0.59$ ,  $p=0.06$ ).

**Table 1** Clinical characteristics

|                                      | All       | Men       | Women      |
|--------------------------------------|-----------|-----------|------------|
| N                                    | 24        | 14        | 10         |
| Age (years)                          | 28±7      | 28±7      | 28±8       |
| Weight (kg)                          | 70.2±11.1 | 74.8±2.8  | 64.4±2.8   |
| Body mass index (kg/m <sup>2</sup> ) | 22.5±2.1  | 22.7±2.2  | 22.3±2.1   |
| Fat mass (kg)                        | 16.0±6.1  | 13.5±5.4  | 19.2±5.6*  |
| Trunk fat mass (kg)                  | 67.7±27.3 | 63.9±29.2 | 74.5±23.2  |
| Fat-free mass (kg)                   | 54.2±10.6 | 61.4±7.8  | 45.2±5.6*  |
| Lean body mass (kg)                  | 51.0±10.2 | 57.9±73.4 | 42.2±53.3* |
| Total BMD (g/cm <sup>2</sup> )       | 1.23±1.10 | 1.28±0.09 | 1.17±0.08* |
| Total Z-score                        | 0.77±0.64 | 0.59±0.55 | 1.04±0.64  |
| Spine BMD (g/cm <sup>2</sup> )       | 1.01±0.13 | 1.04±0.14 | 0.99±0.13* |
| Pelvis BMD (g/cm <sup>2</sup> )      | 1.10±0.15 | 1.15±0.17 | 1.04±0.11* |
| Leg BMD (g/cm <sup>2</sup> )         | 1.34±0.16 | 1.44±0.13 | 1.22±0.10* |
| Arm BMD (g/cm <sup>2</sup> )         | 0.98±0.13 | 1.07±0.10 | 0.86±0.08* |
| BAT volume (ml)                      | 81.0±83.1 | 49.7±54.5 | 82.4±99.5  |
| Mean torso-mantle SUV                | 0.85±0.1  | 0.82±0.06 | 0.90±0.10* |

BMD bone mineral density, BAT brown adipose tissue, SUV standard uptake values

\* $p < 0.05$  compared with men

### BAT and body composition

As BMD, LBM, and FM were different between men and women, we next investigated the relationship between BAT and body composition in each gender. In men, there was no association between BAT parameters, LBM, or FM. In contrast, BAT volume correlated positively with FM ( $r=0.74$ ,  $p=0.02$ ) in women, and a trend for a positive correlation between mean BAT SUV and FM ( $r=0.58$ ,  $p=0.08$ ) was also observed. There was no association between BAT parameters and LBM.

### BAT and BMD

Figure 1 depicts the relationship between BAT and BMD. There were no associations between BAT parameters with total or regional BMD in men. However, in women, BAT volume correlated positively with total and spine BMD. In a forward stepwise multiple regression analysis controlling for age, FM, and LBM, BAT volume was the independent predictor of total ( $\beta=0.63$ ,  $p=0.049$ ) and spine BMD ( $\beta=0.70$ ,  $p=0.02$ ) (Tables 1 and 2 of the “[Electronic supplementary material](#)”). Total and spine BMD were higher in women with visually detectable BAT activity than those without by  $11 \pm 2$  % ( $p=0.02$ ) and  $22 \pm 2$  % ( $p < 0.01$ ), respectively.

### Discussion

This is the first study investigating the relationship between cold-activated BAT and BMD in healthy men and women. Our findings revealed a positive correlation between BAT volume with total and spine BMD in women, independent of FM and LBM. These findings suggest a possible regulatory link between brown adipogenesis and bone density in humans.

While numerous studies have demonstrated association of higher body mass on BMD, little is known about whether adipose subtypes relate to bone mass. This is due to the absence of a sensitive BAT detection method, rendering research into BAT and bone mass difficult. The recent re-discovery of BAT in adulthood by PET scanning has made such research pursuit feasible for the first time. However, given the protective role of BAT in diet-induced obesity, all studies to date have focused on its potential anti-obesity property. Animals with defective BAT activity have been evaluated for their metabolic vulnerability to obesity. Interestingly, animal models with defective brown adipogenesis also manifest reduced bone mass [13, 15], suggesting mechanistic links between BAT function and bone mass regulation [16]. This is concordant with findings in a recent study revealing lower BMD and BAT mass in women with anorexia nervosa [17]. Our study further uncovers a positive correlation between cold-activated BAT mass and BMD in healthy women, but not in men. Collectively, these findings suggest a previously unrecognized regulatory role of BAT on bone mass in humans, which appears to be gender-specific.

The mechanisms underlying the positive association between BAT activity and bone mass are unknown. Recent studies have identified a brown adipocyte-like phenotype in marrow adipocytes [18]. The bone marrow is a unique microenvironment, where cellular and stromal-vascular components of adipose and bone juxtapose. As brown adipogenesis is capable of generating a hypoxic gradient, which fuels chondrocytogenesis and bone formation [19], we speculate that individuals with higher BAT mass harbor a more osteoblastogenic bone marrow microenvironment, leading to greater BMD. Consistent with this hypothesis, brown adipocyte-specific genes in marrow fat are reduced in ageing and diabetes, conditions which also exhibit bone loss [16]. As shown in Fig. 1, FDG uptake was evident within the vertebra following cold stimulation, suggestive of the presence of a BAT-enriched bone marrow. Given that such uptake was not observed in other bones, it is our speculation that it may explain our data which show that BAT volume most strongly correlates with spine BMD. It is also possible that BAT regulates bone mass through the local milieu of adipokines and bone growth factors, given recent discoveries of the role of bone morphogenic proteins on bone remodeling and brown adipogenesis [20].

It is uncertain why correlations between BMD and BAT were only seen in women, but not in men. While this could be a type II error, sexual dimorphism in brown adipogenesis cannot be excluded, given that women harbor more BAT than men [3]. Indeed women tended to have higher total Z-scores than men, supportive of a positive effect of BAT on age-adjusted BMD in women, who also tended to have more abundant BAT than men in our study. It is possible that the impact of BAT on BMD is magnified in women due to their lower overall LBM than in men. Collectively, these findings suggest complex interplay between BMD, BAT, LBM, age, and gender. Future studies should include subjects with wider range of body composition and BMD to decipher these relationships.

The cross-sectional design and small sample size are limitations of the current study. Despite careful adjustment for potential confounders, a causal relationship between brown adipogenesis and higher BMD cannot be established. We cannot exclude the possibility that the observed relationship between BAT and BMD represents a type I error, and larger confirmatory studies are required. However, validity of our data is supported by their demonstration of the expected relationship between LBM and BMD. We did not perform regional BMD measurement, which precludes the assessment of association between BAT parameters and clinically relevant regions such as the femoral neck. The positive relationship observed between BAT volume and FM contrasts with previous studies [3, 6], which may be explained by our inclusion of only lean individuals. Whether the interaction between BAT and FM is dependent on absolute adiposity merits further studies in the future.

Our study provides exploratory, hypothesis-generating insights into a novel interactive domain of adipose–bone biology. The reason for bone loss beginning in the second decade in women, long before the onset of estrogen deficiency, is unclear. We speculate that BAT decline-related bone loss may be a contributing mechanism as BAT abundance falls rapidly after early adulthood [3, 6]. Given that bone loss results in osteoporosis, a highly prevalent condition with significant morbidity and mortality, identification of a novel positive regulator of bone formation may shed therapeutic insight into treatment of osteoporosis in the future.

In summary, the current study reveals healthy women with more abundant BAT to have higher BMD, independent of age and other body compositional parameters. These positive correlations are absent in men. While we emphasize that causality cannot be proven in our associative study, we hypothesize that BAT may be a novel regulator of bone mass in humans. Whether age-related BAT atrophy contributes to bone loss merits future longitudinal studies.

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**Conflicts of interest** None.

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