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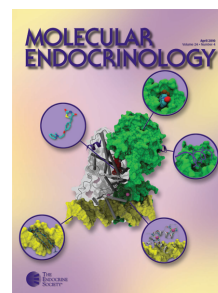
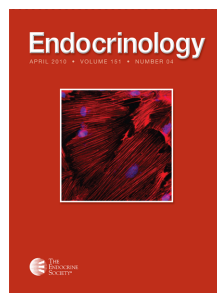
THE JOURNAL
OF CLINICAL
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J. Clin. Endocrinol. Metab. 2011 96:782-786 originally published online Dec 22, 2010; , doi: 10.1210/jc.2010-1922

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Objective: Recent studies suggest a close local link between bone marrow adiposity and endosteal bone formation. Using magnetic resonance imaging, we examined whether the relation between the amount of marrow fat and cortical bone is present at multiple sites along the diaphyses of the long bones of young and old males and females.

Design: The relations between values for cortical bone area and percent marrow fat in each 5-mm section along the midthird of both femoral shafts were determined using magnetic resonance imaging in eight healthy young (aged <25 yr), and nine healthy old (aged >55 yr) men and women.

Results: Strong inverse correlations were observed between values for cortical bone area and percent marrow fat along the shafts of all 34 femurs; r values between -0.54 to -0.97 ; all P values = 0.01 – 0.0001 . The strength of this local association was comparable in the young and the elderly and in males and females.

Conclusion: Our results underscore the strength of the local connections between bone and marrow adiposity. Increasing our understanding of the mechanism for this association could lead to better diagnosis and treatment approaches for osteoporosis. (*J Clin Endocrinol Metab* 96: 782–786, 2011)

Fat is a key determinant of bone acquisition during growth and bone loss with aging, a link that persists at both a systemic and local level throughout life. Knowledge that bone and fat cells arise from the same mesenchymal precursor cells within the bone marrow has generated considerable interest on the relation between marrow adiposity and osteoblast proliferation. A large body of *in vitro* and animal literature indicates that, depending on the interplay of molecular and biochemical stimuli, mesenchymal stem cells (MSCs) differentiate into the cell lineages that are responsible for bone and fat formation (1–3). In humans, it has been suggested that aging alters the expression of peroxisome proliferator-activated receptor- γ and runt-related transcription factor by lowering the levels of osteoblast differentiation and enhancing adipogenesis (4). Increasing amounts of bone marrow fat

may also affect bone turnover through the inhibition of osteoblast function and survival and the promotion of osteoclast differentiation and activation (5).

Using magnetic resonance (MR) imaging or spectroscopy, several studies in elderly subjects report marrow adiposity to be an independent predictor of osteoporosis and fractures (6–12). Patients with osteoporosis have greater marrow fat than subjects with osteopenia, who in turn have increased marrow fat compared with those with normal bone density (9). The fraction of marrow fat in the lumbar vertebrae has also been reported to be an independent risk factor for fracture (12). We previously found that bone acquisition is reciprocally related to marrow adiposity in young subjects (13, 14), and a similar relation has been observed in patients with anorexia nervosa (15). The aim of the current study was to examine whether the reciprocal

relation between adipose and skeletal tissue occurs at every site of the bone and whether it is present in both young and old men and women. Because MSCs are closely bound to endosteal surfaces (16), the marrow fat-bone link is likely regulated by a complex process, involving multiple growth factors and activation of lineage-specific transcription factors, at a local level at all ages, regardless of gender. We used MR imaging to obtain measures of marrow fat and cortical bone throughout the entire femoral diaphysis of 17 healthy young and elderly males and females.

Subjects and Methods

The institutional review board approved the investigational protocol, and informed consent was obtained for all participants. Study subjects were eight healthy young adults, aged 18–25 yr, and nine healthy older subjects, aged 55–70 yr, nine females and eight males. The participants were either friends or relatives of employees at our institution. Candidates for this study were excluded if they had a diagnosis of any underlying disease or chronic illness, they had been admitted to the hospital at any time during the previous 3 yr, or they were taking any medications regularly that are known to affect bone metabolism. None of the study subjects had smoked more than a pack of cigarettes in the past 5 yr. Measures of height, weight, and body mass index (BMI) were determined in all participants.

MR imaging studies of the middle third of 34 femurs (determined by an image localizer) were acquired on a 1.5-T system (LX Cvi; GE Healthcare Systems, Milwaukee, WI) and a knee coil using an identical protocol. The protocol included two axial sequences: 1) a T1-weighted spin echo sequence with a repetition time of 600 msec, echo time of 14 msec, field of view of 20 cm, 256×256 matrix, and a slice thickness of 5 mm for measurements of bone structure; and 2) three-point Dixon sequence with repetition time = 4000 msec. Field of view, matrix size, slice thickness, and anatomical region matched the one chosen for T1-weighted imaging. The total amount of time required for the MR imaging examination was approximately 30 min, and no contrast material was used. Care was taken to position the subjects and to adjust the number of slices so that the same anatomic extent for each subject and the left and right femurs were scanned independently, with the same slice number obtained for both legs; typically, between 20 and 35 anatomical locations were examined. Output images from the three-point Dixon sequence were installed directly into the image database for viewing and calculations. Fat percentage was calculated using data from the intramedullary canal in the Dixon images. Average intensities in the canals from the two Dixon fat and water images were used to calculate percent marrow fat [%MF; %MF = $I_F / (I_F + I_W)$, where I_F and I_W are the signal intensities in the fat and water images, respectively].

To calculate femoral cortical bone area (CBA), we developed a graphical user interface with Matlab 2006b (Mathworks, Natick, MA) using custom algorithms. First, the user selects a MR image and then crops a rectangular region of interest containing the femur. An image histogram is generated and a peak detection function finds the most probable peaks for adipose and lean tissue. The image is recontrasted such that all voxels with intensities greater than the lean tissue peak are set to one, whereas the voxels with

lesser intensities remain dampened. A median filter is applied to the image to remove pixel noise, and bone contours are then extracted via a contour algorithm. The voxels contained within the periosteum and endosteum contours are counted and defined as CBA. The coefficients of variation for three repeated measures of %MF and CBA in six subjects were determined to be 2.3 and 1.3%, respectively.

Statistical analysis

Simple linear regression analyses were used to investigate the associations between MR imaging measures of %MF and values for CBA using StatView statistical software (SAS Institute Inc., Cary, NC). Multiple linear regressions were used to assess the relationships between the correlation values as the outcome measure and age, sex, height, and weight.

Results

Table 1 describes the gender, age, and anthropometric measures of the participants in the young and old groups. The mean age of subjects in the young group was 21.1 ± 2.4 yr, whereas that of the old group was 62.6 ± 4.2 yr. There were no statistically significant differences between the mean weight, height, or BMI of the two age groups (all P values >0.05).

Table 2 shows the range of %MF and the CBA for the midthird of the shaft of the left and right femurs in all 17 subjects. Regardless of age or gender, the correlations between %MF and CBA values for all femurs were strongly negative ($r = -0.54$ to -0.97 ; all P values = 0.01–0.0001). Figure 1A depicts the midthird of the right femur in a 19-yr-old male, and Fig. 1B shows the values for %MF and CBA at all slices along the midshaft of the bone and their overall relationship in the same subject. Figure 2, A–C, depicts the similar outcome measures in a 65-yr-old male, a 22-yr-old female, and a 58-yr-old female, respectively; a similar configuration in the association was seen in all four femurs.

Table 3 shows the multiple linear regressions assessing the predictive values of age, sex, height, and weight on the strength of the relation between %MF and CBA in the right and left femurs of all subjects. Age, gender, and anthropometric measures did not affect the association (all P values >0.05).

Discussion

Recent evidence suggests that increases in marrow fat and decreases in bone formation are immutably coupled and that increased marrow fat, like low bone density, could be a predictor of osteoporotic fractures (12). However, whether the relation between adipose and bone tissues in elderly humans is the unintended consequence of a passive accumulation of fat as bone is lost and marrow space increases is a matter of debate. In the current study, to avoid the confounding effects of age-related changes in the mar-

TABLE 1. Age and anthropometric measures in eight young and nine elderly males and females

	Sex	Age (yr)	Weight (kg)	Height (cm)	BMI
18–25 yr old					
1	F	18.7	46.0	154	19.5
2	M	19.0	91.6	202	22.5
3	M	19.0	75.7	170	26.2
4	F	19.1	72.0	167	25.7
5	F	22.1	49.0	158	19.8
6	M	23.2	64.9	174	21.4
7	M	23.3	71.0	175	23.2
8	M	24.5	74.7	172	25.3
Mean ± sd		21.1 ± 2.4	68.1 ± 14.8	171.4 ± 14.6	22.9 ± 2.6
57–69 yr old					
1	M	57.6	89.8	175	29.3
2	F	58.3	64.0	162	24.4
3	F	58.8	54.5	162	20.8
4	F	61.1	68.0	166	24.7
5	F	62.4	95.0	158	38.1
6	F	62.9	62.7	150	27.9
7	M	65.3	85.7	167	30.7
8	F	68.6	72.0	164	26.8
9	M	68.6	66.2	176	21.4
Mean ± sd		62.6 ± 4.2	73.1 ± 13.8	164.4 ± 8.1	27.1 ± 5.3

M, Male; F, female.

row canal, we used MR imaging, a technique that allows the determination of %MF independently of bone size, to examine the relation between %MF and cortical bone in the appendicular skeleton. We found strong, inverse relationships between the amounts of cortical bone and marrow fat at all locations along the midshaft of all femurs, a relationship that was present in young and old subjects, regardless of gender or anthropometric measures. This

result extends our previous finding that bone acquisition is reciprocally related to marrow adiposity in young subjects (13, 14). Our current results underscore the strength of the local connections between bone and fat and provide additional evidence for the emerging role of bone marrow adiposity on osteoblastogenesis and bone strength.

The mechanism(s) for the striking inverse association between adipogenesis and osteoblastogenesis have yet to be

TABLE 2. MR imaging measures of %MF and CBA in eight young and nine elderly males and females

	Left femur					Right femur				
	%MF	CBA (mm ²)	β	σ	r^2	%MF	CBA (mm ²)	β	σ	r^2
18–25 yr old										
1	68–86	214–364	–5.72	1.05	–0.74	69–86	214–353	–6.83	1.25	–0.73
2	74–81	261–447	–23.36	4.91	–0.74	72–82	273–447	–15.64	3.06	–0.76
3	65–84	212–346	–5.17	0.62	–0.86	65–84	216–353	–5.71	0.67	–0.86
4	69–87	293–386	–4.87	0.66	–0.83	72–87	307–390	–4.14	0.80	–0.71
5	67–86	255–337	–4.30	0.51	–0.86	66–84	275–349	–4.40	0.77	–0.75
6	83–87	321–449	–36.86	8.32	–0.66	84–88	303–444	–26.63	7.42	–0.58
7	79–87	244–408	–14.99	2.48	–0.77	79–87	251–390	–17.71	3.59	–0.70
8	81–87	223–311	–8.48	3.05	–0.56	78–86	227–321	–9.47	2.21	–0.65
57–69 yr old										
1	75–86	266–383	–8.13	2.84	–0.56	77–76	255–406	–11.45	3.52	–0.60
2	64–87	245–300	–2.10	0.37	–0.76	66–87	230–322	–4.37	0.39	–0.95
3	77–86	218–343	–13.63	1.03	–0.95	77–86	221–351	–14.47	0.82	–0.97
4	77–85	255–380	–13.47	1.32	–0.92	76–85	244–353	–8.60	1.35	–0.81
5	81–85	226–388	–18.63	4.46	–0.78	67–85	246–398	–15.82	2.91	–0.79
6	71–86	172–309	–7.15	0.93	–0.87	67–85	163–306	–5.97	0.55	–0.93
7	71–87	233–456	–7.24	0.67	–0.93	68–87	236–408	–4.99	0.79	–0.84
8	66–83	222–276	–2.35	0.63	–0.64	76–86	187–275	–6.44	1.30	–0.75
9	81–87	265–423	–17.73	5.36	–0.54	82–88	273–444	–28.61	6.42	–0.67

β represents unstandardized coefficients, σ standard error, and r standardized coefficients.

^a All P values ≤ 0.01 .

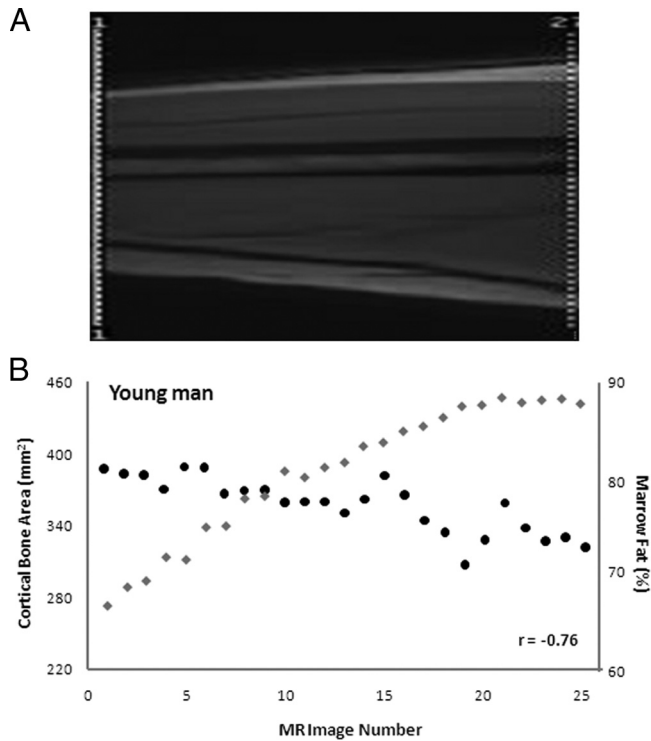


FIG. 1. A, Depiction of the localizer image. B, Values for %MF (black circles) and CBA (gray diamonds) at all slices along the midthird of the right femoral shaft in a 19-yr-old male.

elucidated. The process of MSC differentiation into either osteoblasts or adipocytes is regulated by a complex process that requires the recruitment of MSCs, the presence of multiple growth factors, and activation of lineage-specific transcription factors (17, 18). Additionally, progressive infiltration of bone marrow by fat is associated with the paracrine secretion of toxic fatty acids and adipokines that would affect osteoblast function and survival. In contrast, high levels of peroxisome proliferator-activator- γ expression due to the increasing number of bone marrow adipocytes would promote osteoclast differentiation and bone resorption. Future studies are needed to clarify whether stem cell differentiation is a mutually exclusive process with a commitment to either adipocytes or osteoblasts and whether bone marrow fat influences the formation of bone in a paracrine manner through the release of adipokines.

Regardless of the mechanism by which marrow fat is related to bone, adipose tissue in the marrow cavity negatively affects the biomechanical strength of bone because fatty marrow is a weaker biomechanical support medium than hematopoietic marrow. Pathological data of the iliac crest and vertebral bodies showed greater marrow fat content in elderly subjects with reduced cancellous bone density when compared with aged-matched controls (7, 19, 20). Previous imaging investigations have also shown higher vertebral marrow fat content in subjects with morphological evidence of bone weakness, such as end plate

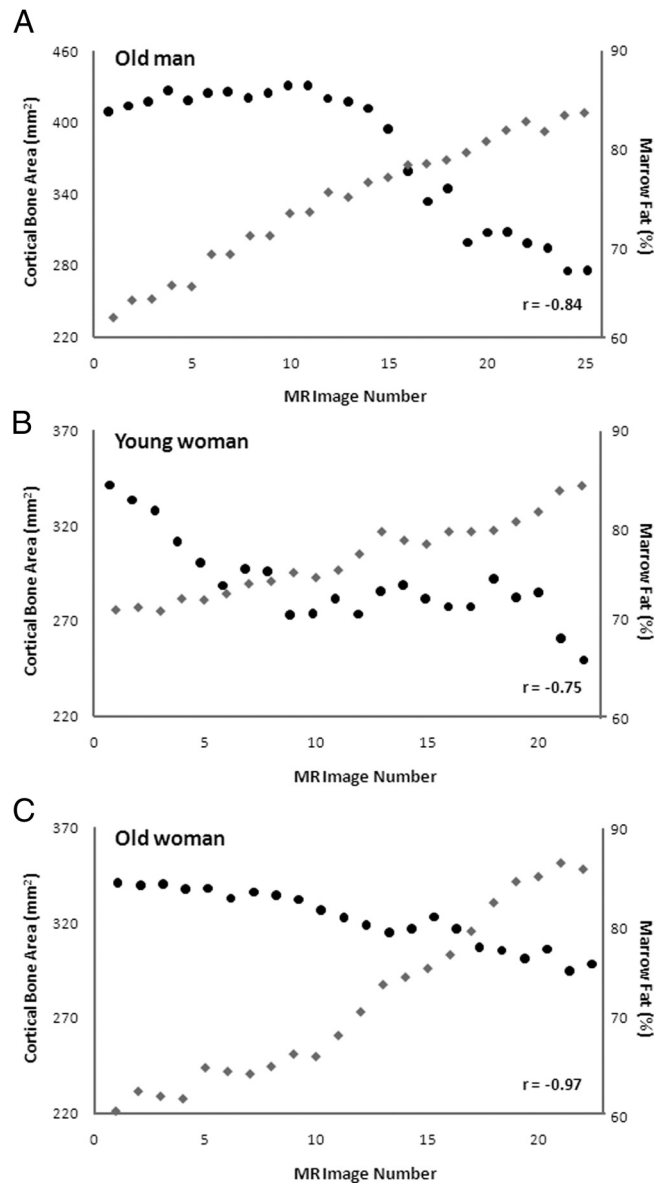


FIG. 2. A, Values for %MF (black circles) and CBA (gray diamonds) at all 5-mm slices along the midthird of the femur in a 65-yr-old male. B, A 22-yr-old female. C, A 58-yr-old female.

depression or compression fractures (21). Indeed, increased marrow adiposity, like low bone density, is a predictor of vertebral osteoporotic fractures (12, 22). In the past, the analysis of bone marrow fat required invasive bone biopsies, but we can now noninvasively quantify bone marrow fat using different MR imaging techniques. These measures could be valuable aides in studies deciphering the mechanism underlying the local bone-fat connection. They could also become a surrogate marker for the assessment of osteoporosis and fracture risk.

There are several limitations to the current cross-sectional study. We studied only a small number of subjects, and our results are pertinent only to the long bones of the appendicular skeletons of healthy adults and cannot be extrapolated to the axial skeleton or sick patients. For exam-

TABLE 3. Multiple regression analysis for the prediction of the correlation between %MF and CBA

%MF-CBA correlation	β	SE	P value	R ²
Left femur				
Age	0.001	0.002	0.621	0.000
Gender	-0.103	0.096	0.301	
Weight	-0.001	0.003	0.763	
Height	0.002	0.005	0.671	
Right femur				
Age	-0.001	0.001	0.362	0.090
Gender	-0.092	0.078	0.259	
Weight	0.000	0.003	0.909	
Height	0.000	0.004	0.963	

β represents unstandardized coefficients, and R² represents the coefficient of multiple determination.

ple, the fat-bone relation may be completely obliterated in patients with hematological disorders and myelodysplastic syndromes and those receiving medical treatments such as corticosteroid therapy. Additionally, the cohort was too small to allow for the reliable assessment of differences between subjects, and future studies in larger cohorts are needed to determine how age and sex influence the strength of this relation. Moreover, pertinent information regarding other possible determinants of this association, such as vitamin D status, exercise habits, and diet is lacking. Studies are also needed to corroborate this reciprocal association at other skeletal sites. Most importantly, although our findings provide evidence of a local reciprocal relationship between marrow fat and cortical bone, they do not shed light on whether this relation is due to osteoblast proliferation or osteoclast activation or both. Increasing our understanding of the mechanism for this connection could lead to better diagnosis and treatment approaches for osteoporosis.

Acknowledgments

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Disclosure Summary: The authors have nothing to disclose.

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