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Bone marrow fat content in 70 adolescent girls with anorexia nervosa: Magnetic resonance imaging and magnetic resonance spectroscopy assessment

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Abstract

Background—Adolescents and women with anorexia nervosa have increased bone marrow fat and decreased bone formation, at least in part due to hormonal changes leading to preferential stem cell differentiation to adipocytes over osteoblasts.

Objective—The purpose of this study was to evaluate marrow fat content and correlate with age and disease severity using knee MRI with T1 relaxometry (T1-R) and MR spectroscopy (MRS) in 70 adolescents with anorexia nervosa.

Materials and methods—We enrolled 70 girls with anorexia nervosa who underwent 3-T knee MRI with coronal T1-W images, T1-R and single-voxel proton MRS at 30 and 60 ms TE. Metaphyses were scored visually on the T1-W images for red marrow. Visual T1 score, T1 relaxometry values, MRS lipid indices and fat fractions were analyzed by regression on age, body mass index (BMI) and bone mineral density (BMD) as disease severity markers. MRS measures included unsaturated fat index, T2 water, unsaturated and saturated fat fractions.

Results—All red marrow measures declined significantly with age. T1-R values were associated negatively with BMI and BMD for girls 16 years ($P=0.03$ and $P=0.002$, respectively) and positively for those 17 years ($P=0.05$ and $P=0.003$, respectively). MRS identified a strong inverse association between T2 water and saturated fat fraction from 60 ms TE data ($r=-0.85$, $P<0.0001$). There was no association between unsaturated fat index and BMI or BMD.

Conclusions—The association between T1 and BMI and BMD among older girls suggests more marrow fat in those with severe anorexia nervosa. In contrast, the physiological association

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between marrow fat content and age remained dominant in younger patients. The strong association between T2 water and saturated fat may relate to the restricted mobility of water with increasing marrow fat.

Keywords

Adolescents; Anorexia nervosa; Bone marrow; Fat; Magnetic resonance imaging; Magnetic resonance spectroscopy

Introduction

Bone marrow fat composition is increasingly recognized as a contributor to overall bone quality. Women with osteoporosis and fragility fractures have decreased bone mineral density (BMD) and increased bone marrow fat [1, 2]. Adolescents and young women with anorexia nervosa, a disorder characterized by extreme subcutaneous fat depletion [3], demonstrate increased marrow fat in all but the most malnourished patients [4, 5]. These patients are known to have suppression of bone formation, diminished bone density and increased fracture risk [6–9].

The skeletal problems associated with eating disorders may be mechanistically linked to abnormalities in osteoblast and osteoclast progenitor cells within bone marrow [10–13]. Hormonal abnormalities such as estrogen deficiency and hypercortisolemia in affected patients likely mediate adipocyte over osteoblast differentiation in the mesenchymal stem cell pool, resulting in premature conversion from red marrow to yellow marrow and, thus, increased marrow fat content [14]. Increasing evidence suggests that bone marrow activity alternates between osteoblast and adipocyte formation [10, 15]. A recent investigation of marrow fat composition in women with anorexia nervosa identified an inverse relationship between marrow fatty acid saturation and BMD [16]. However, an inverse relationship between total marrow fat content and BMD has not been consistently demonstrated [17].

Magnetic resonance imaging (MRI), computed tomography (CT) and bone marrow biopsy have been used to document marrow hypoplasia and increased levels of fatty marrow in patients with anorexia nervosa [5, 18, 19], similar to the marrow lipid accumulation observed in aging individuals (ages 52–92) who have osteoporosis [1, 2, 20–25]. The investigation of young adolescents with anorexia nervosa is complicated by developmental maturation during the peri-pubertal years. Both bone marrow fat and bone density are normally increasing rapidly at this time [26, 27]. As girls achieve peak bone mass during adolescence, disease-associated alterations in bone marrow composition and subsequent inhibition of bone formation during this important developmental period may contribute to increased fracture risk. The goal of this work was to evaluate the relationship between bone marrow composition and disease severity in girls with anorexia nervosa with onset in early to mid-adolescence. We utilized knee MR with spectroscopy and T1 relaxometry in a large cohort of adolescent girls with anorexia nervosa to evaluate marrow fat composition, including saturation and unsaturation, and correlate findings with age, body mass index (BMI), and BMD, with BMI and BMD serving as markers of disease severity.

Materials and methods

Patients

From 2012 to 2015, we prospectively enrolled 70 girls with anorexia nervosa, ages 11–18 years, mean 15.5 ± 1.9 years, at two large adolescent medicine eating disorder clinics ($n=13$ from Boston Children's Hospital, Boston, MA, and $n=57$ from Hasbro Children's Hospital, Providence, RI). Human subjects committee approval was obtained at both institutions, and written informed consent and assent were obtained for each subject. Inclusion required a diagnosis of anorexia nervosa based on Diagnostic and Statistical Manual of Mental Disorders (DSM) 5 criteria [28] and ages 11–18 years. Patients with concomitant chronic diseases (cystic fibrosis, celiac disease and neurofibromatosis), those taking medications known to affect bone metabolism (including glucocorticoids, depot medroxyprogesterone acetate or other hormonal contraception) or with hemodynamic instability were excluded.

BMI and BMD measurements

Clinical data collected from each patient included height and weight for BMI calculation, duration of anorexia nervosa, duration of amenorrhea and prior fracture history. All patients had a tibial volumetric BMD (mg/cm^3) measured by peripheral quantitative CT (pQCT) using a Stratec XCT 3000 device (Orthometrix, White Plains, NY) with a 12-detector unit, voxel size of 0.4 mm, slice thickness of 2.3 mm and scan speed of 25 mm/s.

Magnetic resonance imaging

All subjects underwent MR imaging of the knee with 15-channel receive coils on one of three 3-T scanners all with identical software (Trio systems; Siemens Medical Inc., Erlangen, Germany). During the course of the study period, a fat/water phantom consisting of Hellmann's Real Mayonnaise™ was used to provide calibration measurements for the three scanners utilized. The spectroscopic studies found methylene to water signal amplitude ratios to be within 3% of each other for the 30 ms echo time spectra and within 7% of each other for the longer echo time of 60 ms. Similarly, T1 values as evaluated with the T1 measurement protocol were found to be within 3% of each other on the three different scanners. Coronal T1-weighted spin echo images (repetition time [TR] 600 ms, echo time [TE] 9.7 ms, matrix 384×288 , echo train length (ETL) 166, number of excitations 1, field of view 140 mm, 3-mm slice thickness, 0.3-mm gap) were obtained through the knee with a field of view of 16 cm to include the distal femoral and proximal tibial metaphyses. We also performed spin-lattice relaxation (T1) relaxometry consisting of nine fast spin echo (FSE) acquisitions of varying TR (TR=250, 350, 500, 750, 1,000, 1,500, 2,000, 3,000 and 5,000 ms, TE=17 ms, matrix 128×128 , ETL 3, flip angle 180 degrees, field of view 140 mm, 4-mm slice thickness, 1-mm gap) [29, 30]. MR spectroscopy was performed using single voxel point resolved spectroscopy (PRESS) acquisitions with voxels placed in two locations, the distal medial femoral metaphysis and the distal lateral femoral metaphysis. The distal femoral metaphysis was selected for spectroscopic and relaxometry evaluation based upon the results of our prior study in which this location demonstrated the most significant increased marrow fat in girls with anorexia nervosa relative to normal controls [29]. Voxel volumes were $1 \times 1 \times 1 \text{ cm}^3$, and two spectra were acquired from each voxel at two separate echo times of 30 ms and 60 ms to allow for spectral T2 estimates of the major resonances.

The TR for each PRESS acquisition was 2.5 s and 32 signal averages were employed for a scan time of approximately 90 s per spectrum. Spectral readout employed 2,048 time points with a bandwidth of 2,500 Hz.

Image analysis

Visual assessment—Two pediatric radiologists (K.E. and a non-author, with 21 years and 3 years of experience, respectively), blinded to subject age and disease severity, visually assessed all of the images for red marrow content, designated as areas of low signal intensity. The distal femoral and proximal tibial metaphyses were graded according to the following scale, validated in our previous study [29]: 0=homogeneous hyperintensity, no red marrow; 1=few hypointense striations, mild red marrow; 2=scattered hypointense areas, moderate red marrow; 3=more diffuse hypointense regions throughout the metaphysis, extensive red marrow (Fig. 1). Scores were decided by consensus. Distal femoral and proximal tibial physes were assessed as open or closed as a marker of skeletal maturation.

T1 relaxometry—Voxelwise T1 maps were generated from the variable TR scans using the following equation: $S_{TR} = S_{max}(1 - e^{-TR/T1})$ where S_{TR} is the signal intensity of the voxel for a particular TR and S_{max} is the signal intensity for a TR of infinity. S_{max} and T1 were calculated at each voxel and the corresponding maps generated using IDL software (Harris Geospatial Solutions, Broomfield, CO) developed in-house. Mean T1 values for the distal femoral metaphyses were recorded (ImageJ; National Institutes of Health, Bethesda, MD). The anatomical locations of these regions were consistent for all subjects and all regions of interest were consistently 72 square pixels (approximately 25 mm²) in size (Fig. 2).

MR spectroscopy (MRS)—Spectra were manually phased and baseline corrected using vendor-supplied spectroscopic analysis software to achieve absorption mode spectra for spectral peak quantitation. Spectral fits with Gaussian functions were then performed using the vendor-supplied software to obtain peak areas for five lipid resonances and the water resonance (w) visually present in the bone marrow spectra. The lipid resonances were assigned as follows [16, 31]: olefinic protons from the unsaturated portions of the hydrocarbon chain around 5.4 ppm (lipid 5), the primary methylene proton signal around 1.3 ppm (lipid 2), terminal methyl protons around 0.9 ppm (lipid 1), methylene protons two bonds removed from the glycerol head group around 1.6 ppm (lipid 3) and a combined signal from methylene protons adjacent to the polar head group and to double bonds along the hydrocarbon chain around 2.1 ppm (lipid 4) (Fig. 3). Smaller lipid resonances from the polar head group around 4.3 ppm were generally observed as a right-hand shoulder of the water peak in the 30 ms TE spectra, but were not generally observed in the 60 ms TE and were not quantified.

Ratio indices from the peak quantifications at each echo time were calculated. The unsaturation index was measured as the olefinic peak area divided by total lipid peak areas, as previously described [1, 16]:

$$\text{Unsaturated fat index} = (\text{lipid 5}) / (\text{lipid 1} + \text{lipid 2} + \text{lipid 3} + \text{lipid 4} + \text{lipid 5}).$$

The ratios of unsaturated (R_{unsat}) and saturated (R_{sat}) fat to water were generated by dividing the relevant lipid area by the water area:

$$R_{\text{unsat}} = \text{lipid 5} / \text{water}$$

$$R_{\text{sat}} = \text{lipid 2} / \text{water}$$

Transverse (T_2) relaxation estimates of the olefinic protons ($T_{2\text{lipid5}}$), the water protons (T_{2w}) and the primary methylene protons ($T_{2\text{lipid2}}$) were estimated from the ratio of relevant peak areas at $TE=30$ and 60 ms, S_{30}/S_{60} , assuming a monoexponential decay using the equation:

$$T_2(\text{ms}) = 30 / \ln(S_{30}/S_{60})$$

where $\ln$ denotes the natural log.

Spectral fits were quite consistent in all patients with the exception of three data sets degraded by motion. The resonances are from chemical moieties within trabecular bone where susceptibility differences between bone and water and lipid cause broadening of the resonances, in turn causing overlap between adjacent peaks, particularly for the two resonances on either side of the primary methylene peak. These resonances are not sharp peaks as would be found in pure oils but rather appear as shoulders off the main peak. We noted that slightly different phasing of the spectra could shift the areas of lipid 1 into lipid 3 and vice versa. Importantly, the sum of lipid 1 + lipid 2 + lipid 3 was always consistent and only this sum was used in the calculations of the unsaturation index.

Statistical analysis

All marrow measures were continuous variables and were, therefore, analyzed by linear regression or analysis of variance (ANOVA). We assessed the simple association of each marrow measure with age (continuous) by linear regression, and with physeal closure (trichotomous) by one-way ANOVA. We assessed the influence of clinical covariates (BMI, BMD and duration of anorexia) on each marrow measure using a multi-step model-building procedure. We first conducted simple linear regression of the marrow measure on the clinical covariate. This analysis was followed by multiple linear regression analysis adjusted simultaneously for age and physeal closure, to determine whether age mediated the association with closure or vice versa. Finally, we tested whether the relation of the marrow measure to the clinical variable, if any, was modified by age (expressed as a continuous variable) or physeal closure (a trichotomy); this step was accomplished by adding interaction terms to the regression model. Where significant interaction with physeal closure was detected (e.g., $\text{BMD} \times \text{closure}$), we estimated the rate of change of the marrow measure per unit covariate in each closure category using parameters of the fitted model. Where significant age interaction was detected (e.g., $\text{BMI} \times \text{age}$), we estimated the rate of change of

the marrow measure per unit covariate for three specific ages, chosen from the continuous age range to represent the minimum (12 years), median (15 years) and maximum (19 years). Marrow measures with severely skewed distribution were log-transformed for analysis. For reporting, we retransformed mean $\pm$ standard error on the $\log_{10}$ scale ($\pm$ SE) to mean $\pm$ percentage standard error on the original scale ($10 \pm 100\% \times [10^{\text{SE}} - 1]$) and coefficients on the $\log_{10}$ scale (b) to percentage change on the original scale ($100\% \times [10^b - 1]$). Among the clinical covariates, duration of anorexia alone showed a strongly skewed distribution and was log-transformed for use as a regression predictor. Regression coefficients for duration of anorexia are expressed per 50% (1.5-fold) increase, corresponding to 0.1761 units on the $\log_{10}$ scale. Correlations between marrow measures were assessed with the Pearson correlation coefficient. $P < 0.05$ was considered a significant result. All computations were performed with SAS software (version 9.4, Cary, NC).

Results

The clinical and anthropometric data for the 70 patients are summarized in Table 1. Subjects had a mean BMI of $18.7 \pm 1.7 \text{ kg/m}^2$ (range: 12.8–22.4). Duration of anorexia nervosa symptoms and amenorrhea showed skewed distributions. The duration of anorexia nervosa symptoms ranged from 1 to 60 months, with a median of 4.5 months, and range of amenorrhea was 1–18 months, with a median of 4 months. Twenty-two of the 70 girls had a history of fracture. BMI and BMD both increased with age in our patients ($P = 0.004$ and $P = 0.002$, respectively), as in the normal population [27, 32].

MRI marrow assessment

The MRI and MRS results, described in detail in the following sections, are summarized in Tables 2 and 3.

Qualitative assessment

Assessment of physeal closure revealed 17 girls with both distal femoral and proximal tibial physes open, 15 girls with femur open and tibia closed, and 38 girls with both closed. Physeal closure and age were strongly associated ($P < 0.0001$ by ANOVA). No patients younger than 14 years had both physes closed, and no patients older than 16 years had both physes open.

According to the T1 visual scoring system, higher values indicate less fatty marrow. The femoral T1 qualitative visual score was strongly inversely associated with age ($P = 0.0008$) and with physeal closure ($P = 0.0004$), with a consistently lower score in those with closed physes. When adjusted for age and physeal closure, no statistically significant association was seen between visual T1 score and clinical measures.

T1 relaxometry

Higher T1 relaxometry values indicate less marrow fat due to T1 shortening associated with fat. T1 relaxometry values declined with age ($P < 0.0001$) and with physeal closure ($P = 0.0002$) (Table 2). The relation of T1 to BMI and BMD was significantly modified by age (Table 3). Whereas T1 values were associated negatively with BMI and BMD for the

youngest girls ($P=0.03$ and $P=0.002$, respectively), the association changed significantly in the positive direction with increasing age (Fig. 4; $P=0.05$ and $P=0.003$ for BMI and BMD age interaction, respectively). No relationship was demonstrated between T1 value and duration of anorexia nervosa.

MR spectroscopy

The spectroscopy data showed a relationship between BMD and saturated (R_{sat}) and unsaturated (R_{unsat}) fat fractions that varied according to physeal closure, as indicated by statistically significant interactions ($P=0.02$ for saturated fat and $P=0.01$ for unsaturated fat; Table 3, Fig. 5). The skeletally immature girls (both physes open) demonstrated the normal increase in marrow fat as BMD increased, while the skeletally mature girls (both physes closed) showed a decrease in marrow fat MRS markers as BMD increased. The data showed no relationships between saturated or unsaturated fat and BMI (>0.20) or duration of anorexia nervosa ($P>0.80$).

T2 water as calculated from the MRS data showed a strong inverse correlation with age and physeal closure, both $P<0.0001$ (Table 2, Fig. 6). T2 of olefin likewise declined with age and physeal closure. T2 of methylene showed an increase with physeal closure but no age trend (Table 2). None of the T2 parameters showed an association with BMI, BMD or duration of anorexia nervosa (typically >0.30 adjusted for age and physeal closure).

MRS identified a strong correlation between T2 water and saturated fat fraction (methylene/water) as evaluated at the TE of at 60 ms, $r=-0.85$, $P<0.0001$.

We found no relationships between MRS values (unsaturated index, saturated fat [methylene], unsaturated fat [olefin] and T2 water) and duration of anorexia nervosa. There was no association between unsaturated fat index and BMD ($P>0.40$) or BMI ($P>0.90$).

Discussion

Bone health in adolescent girls with anorexia nervosa is compromised by nutritional deprivation and altered hormonal milieu (e.g., hypercortisolemia, estrogen and androgen deficiency, etc.) that impair pubertal development, as well as the delicate balance between osteoblast and adipocyte differentiation within bone marrow [5, 29]. Our results confirm that the strong physiological association between marrow fat content and age remains dominant even in patients with anorexia nervosa. Because the hormonal milieu in girls with this disease may impact skeletal age [33–35], we compared chronological age with distal femoral and proximal tibial physeal closure and found preservation of the normal relationship across our study population.

The relationship between T1 and both BMI and BMD among older girls suggests the presence of more marrow fat ($\downarrow$ T1) in those with more severe disease ($\downarrow$ BMI). The increased red marrow normally present in younger girls and strong age-related changes in marrow fat may obscure the effect of disease severity in younger patients with anorexia nervosa. The strong correlation observed between T2 water and saturated fat has not previously been reported and may relate to restricted motion of water with increasing marrow fat. MR/MRS

techniques can be used to assess qualitatively and quantitatively marrow fat content in patients with this disease.

The relationship between bone density and bone marrow fat content is complex and remains incompletely understood. Within marrow, there is a balance between adipogenesis and osteoblastogenesis that favors bone development during growth. The predictable, physiological conversion of red to yellow marrow accompanies this process. This balance between marrow fat and bone becomes even more complicated during puberty when hormonally mediated acceleration of marrow conversion occurs at the time of growth spurt [27]. Women and girls with anorexia nervosa are known to have diminished BMD and an increased risk of fracture [36, 37]. Recent studies have demonstrated increased marrow fat by MRI and MRS in mild to moderate anorexia nervosa [5, 16, 29], but the relationship between disease severity and marrow fat content is unknown. The most accurate method for marrow fat assessment by MR is also controversial, with some advocating MRS [16] and others T1 imaging [38]. Our results from this large cohort of adolescent girls with anorexia nervosa indicate that qualitative visual scoring of T1-weighted images, T1 relaxometry and MRS all provide similar assessment of marrow fat, although single-voxel MRS is subject to sampling bias in younger patients with greater proportions of red marrow.

All of our measures of marrow fat showed the strongest association with age. A trend of increasing marrow fat throughout childhood, adolescence and adulthood has been well documented [39]. It has not been previously demonstrated, however, that this correlation is maintained in the face of anorexia nervosa, despite the known relative increase in marrow fat compared to age-matched controls, including both in the appendicular skeleton in adolescents [29] and axial skeleton in young adults [5]. This relationship is evident upon visual inspection of MR images, as well as with quantitative MRS and relaxometry techniques.

A comparison of marrow fat and markers of disease severity yielded interesting results. In our skeletally mature girls (17 years and closed physes), marrow fat content, as measured by relaxometry, was inversely correlated with BMI and BMD, when adjusted for age-related marrow effect. Patients with higher BMI had lower marrow fat content relative to those with lower BMI and more severe disease. These findings suggest that the accelerated red to yellow marrow conversion seen in women with anorexia nervosa is related to the degree of nutritional deprivation. The inverse correlation between marrow fat and BMD supports the link between marrow fat and bone health. In contrast, this relationship was reversed in our younger, skeletally immature girls (16 years) in whom a positive correlation was seen between marrow fat and both BMI and BMD. This finding is likely due to the physiological maturation within marrow and bone that dominates the relatively minor effects associated with mild to moderate disease. Newton et al. [27] demonstrated a positive relationship between bone marrow adipose tissue and bone mineral content, which increased with age in pre-pubertal girls. The authors postulated additive effects of the metabolic and reproductive hormonal milieu as girls approach puberty. At puberty, both marrow fat and BMI are increasing rapidly. The inverse relationship between marrow fat and BMI, seen in older adolescents and women with anorexia nervosa, may thus be obscured in our younger

patients by their higher quantities of red marrow undergoing physiological conversion, even in the setting of anorexia nervosa.

MRS evaluation of marrow composition in post-pubertal girls is complicated by the predominance of fat relative to limited water. We chose to examine saturated and unsaturated fat fractions, as well as the T2 value of water employing dual-echo PRESS acquisitions to understand more definitively the relationship between fat content and bone health in anorexia nervosa. We note that similar quantitation of bone marrow spectra (5 lipid peaks and water) was performed by Bredella et al. [16] who, however, utilized an LCMODEL (linear combination of model spectra) approach (presumably time domain analyses) for spectral analysis and acquired data at only one TE of 30 ms. Thus, in addition to the marrow composition indices reported in that prior study, we also report indices associated with 60 ms TE data, and T2 values for the resonances of water, olefinic and methylene protons. The use of two echo times allowed us to identify the decline in marrow T2 water resonance with age and physeal closure.

The clinical significance of spectral T2 relaxation data in marrow is, as yet, unknown as it has not been broadly applied to clinical tasks. Similarly, the use of a monoexponential T2 decay model for estimating T2 values from dual echo data, as performed in this study, is somewhat limited as fat resonances are known to be subject to J-coupling modulations and possibly other non-monoexponential decay mechanisms. Unfortunately, exploring more involved spectral acquisitions with more echoes was not feasible within the confines of a 50-min MR exam that already included two-voxel sampling at two echo times with multiple signal averages in addition to a full T1 mapping protocol and more routine clinical sequences for marrow evaluation. The spectral T2 relaxation times we have estimated, however, should provide reasonable estimates of the relative signal intensities one might anticipate for the major resonances in marrow for different echo times. This information should, in turn, prove useful in the design of studies examining dietary effects on the relative levels of, for example, unsaturated vs. saturated fats in marrow.

Study limitations merit acknowledgment and consideration. Our study population included girls with mild to moderate disease. Therefore, our results may not apply to extremely underweight adolescents with anorexia nervosa. Young women with severe anorexia nervosa and extremely low BMI have been shown to have a reduction in both fatty and hematopoietic marrow, an accumulation of extracellular hyaluronic acid in marrow spaces and increased free water content [40, 41]. This hyperhydration and reduced bone marrow fat fraction, observed via spectroscopy and relaxometry techniques, is suspected to be a physiological response to reduced hematopoietic requirements as a result of severely decreased body mass [40, 42–44]. Additionally, we did not enroll healthy control subjects who underwent parallel assessments of bone density and marrow composition. Therefore, we are limited in our ability to draw definitive conclusions. We previously reported increased marrow fat in young girls with mild to moderate anorexia nervosa relative to age-matched normal controls [29], and the normal physiological pattern of red to yellow marrow transformation is well recognized on MR imaging. Although the absence of histological confirmation of the accuracy of the MR fat content assessment could be considered another limitation, T1 relaxometry and MRS evaluation of tissue fat content are generally validated

techniques. Finally, MRS of marrow spectra is inherently challenging and small changes in fat content are difficult to assess, particularly in the high fat regime of yellow marrow due to difficulties quantifying the small water peak. Furthermore, the smaller lipid resonances are sensitive to manual spectral phasing, particularly those in proximity to the large methylene peak.

Conclusion

This study aimed to determine whether a positive correlation existed between marrow fat and clinical markers of disease, BMI and BMD, in adolescent girls with anorexia nervosa, with the hope of strengthening the utility of quantitative marrow fat evaluations as a novel skeletal health assessment in these patients. We found that the age-related impact of red to yellow marrow conversion is preserved in this population of moderately ill girls, and there was no consistent correlation between marrow fat and BMI or BMD. The relationship between marrow fat, as measured by T1 relaxometry and proton MR spectroscopy, and both BMI and BMD varied depending upon the status of physeal closure. We demonstrated that marrow fat content is inversely associated with BMI and BMD in skeletally mature adolescents with anorexia nervosa, while the reverse was seen in skeletally immature subjects. The inverse of this association in younger girls is a reminder that the predictable, physiological conversion of hematopoietic to yellow marrow remains strong, even in the face of nutritional deprivation. Finally, the strong association between T2 water and marrow fat saturation in this patient population is newly recognized and may relate to restricted mobility of water with increasing marrow fat.

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Fig. 1. Qualitative bone marrow scoring system on coronal T1-weighted images. 0=homogeneous hyperintensity, no red marrow (**a**). 1=few hypointense striations (*arrow*), mild red marrow (**b**). 2=scattered hypointense areas, moderate red marrow (**c**). 3=more diffuse hypointense regions, extensive red marrow (**d**). Marrow patterns (**c**) and (**d**) are expected in similarly aged healthy girls, as we determined in our original study comparing girls with anorexia nervosa to those with age-matched control subjects [29]

Fig. 2.
T1 map obtained from the variable repetition time spin-lattice (T1) relaxometry acquisition in a 14-year-old girl. For calculation of T1 values, 25 mm² regions of interest were placed on the T1 maps in the medial and lateral distal femoral metaphyses

Fig. 3. MR spectroscopy in the same 14-year-old girl as in Fig. 2. Manually phased spectral fits from the medial distal femoral metaphysis at echo times 30 ms (left) and 60 ms (right). Saturated methylene represents the largest peak (lipid 2) while the unsaturated olefinic peak is the smaller, left-most peak (lipid 5). Water is to the right of and larger than the olefinic peak in this case. Inserts show coronal and axial localizer images used to position the spectroscopy voxels

Fig. 4.

The relationship of marrow relaxometry T1 value to body mass index (BMI) (a) and bone mineral density (BMD) (b), analyzed by multiple linear regression with effect modification by age. Age was modeled as a continuous variable; displayed lines correspond to subjects at selected ages near the sample minimum (12 years, ·····), median (15 years, - - -), and maximum (19 years, ———). Association of T1 with BMI and BMD was negative at the youngest ages ($P=0.03$ and $P=0.002$, respectively) and changed significantly toward positive association with increasing age ($P=0.05$ and $P=0.003$ for age interaction, respectively)

Fig. 5.

The relationship of bone mineral density (BMD) to bone marrow saturated fat fraction (R_{sat} , in **a**) and unsaturated fat fraction (R_{unsat} , in **b**) is analyzed by multiple linear regression with effect modification by skeletal maturity. Association of R_{sat} and R_{unsat} with BMD differed among subjects with open physes ($\circ \cdots \cdots$), subjects with open femoral but closed tibial physes ($\triangle \cdots \cdots$), and subjects with both physes closed ($\blacksquare \cdots \cdots$); $P=0.02$ and $P=0.01$ for $\text{BMD} \times \text{closure}$ interaction effect on R_{sat} and R_{unsat} , respectively

Fig. 6.

Marrow T₂ of water was inversely related to skeletal maturity as indicated by physeal closure (**a**) ($P < 0.0001$ by one-way analysis of variance), as well as age (**b**) (-2.48 ± 0.55 ms/yr, linear regression coefficient $\pm$ standard error, $P < 0.0001$). Box plots (**a**) summarize the distribution of T₂ for each category of physeal closure. Cross (+) indicates the mean; center line the median; top and bottom the interquartile range (IQR). Vertical lines extend to the farthest data point within $1.5 \times$ IQR above or below the box. Data points farther than $1.5 \times$ IQR from the box are considered outliers (●). Fitted line (**b**) from simple regression analysis

Table 1
Characteristics of 70 adolescent girls with anorexia nervosa

Characteristic	Mean $\pm$ SD	Median	Min, Max
Age, yr	15.5 $\pm$ 1.9	15.6	11.5, 19.0
Height, cm	160.5 $\pm$ 7.9	159.2	142.0, 178.0
Weight, kg	48.4 $\pm$ 6.5	49.0	25.9, 59.4
BMI, kg/m ²	18.7 $\pm$ 1.7	18.9	12.8, 22.4
BMD, mg/cm ³	305 $\pm$ 49	306.0	164.0, 391.0
Duration of AN, months	8 $\pm$ 10	4.5	1, 60
Duration of amenorrhea, months *	5 $\pm$ 4	4.0	1, 18

* Rounded down; premenarchal participants excluded

AN anorexia nervosa, BMD bone mineral density, BMI body mass index, Max maximum, Min minimum, SD standard deviation

Table 2
Variability of lateral femoral marrow measures with age and physical closure status in 70 young women with anorexia nervosa

Measure	Coefficient of age $\pm$ SE*	P	Physical closure status	Mean $\pm$ SD [†]	P
Visual score	-0.17 $\pm$ 0.05	0.0008	Both open	1.88 $\pm$ 0.60	0.0004
			Femur open, tibia closed	1.80 $\pm$ 0.94	
			Both closed	1.08 $\pm$ 0.75	
T ₁ , ms	-21.9 $\pm$ 5.1	<0.0001	Both open	665 $\pm$ 84	0.0002
			Femur open, tibia closed	613 $\pm$ 79	
			Both closed	564 $\pm$ 73	
T ₂ water, ms	-2.48 $\pm$ 0.55	<0.0001	Both open	33.2 $\pm$ 10.1	<0.0001
			Femur open, tibia closed	21.8 $\pm$ 7.8	
			Both closed	17.5 $\pm$ 4.7	
T ₂ olefin, ms	-3.02 $\pm$ 1.26	0.02	Both open	69.9 $\pm$ 20.5	0.006
			Femur open, tibia closed	56.7 $\pm$ 22.7	
			Both closed	51.1 $\pm$ 14.9	
T ₂ methylene, ms	1.45 $\pm$ 1.20	0.23	Both open	66.8 $\pm$ 19.0	0.003
			Femur open, tibia closed	85.8 $\pm$ 23.1	
			Both closed	85.0 $\pm$ 12.7	
UI at 30 ms	0.0041 $\pm$ 0.0010	<0.0001	Both open	0.057 $\pm$ 0.011	<0.0001
			Femur open, tibia closed	0.071 $\pm$ 0.015	
			Both closed	0.081 $\pm$ 0.014	
UI at 60 ms	0.0012 $\pm$ 0.0008	0.14	Both open	0.071 $\pm$ 0.018	0.25
			Femur open, tibia closed	0.064 $\pm$ 0.007	
			Both closed	0.070 $\pm$ 0.010	
R _{sat} at 30 ms [‡]	0.039 $\pm$ 0.009	0.0001	Both open	0.55 $\pm$ 0.14	<0.0001
			Femur open, tibia closed	0.67 $\pm$ 0.13	
			Both closed	0.77 $\pm$ 0.13	
R _{sat} at 60 msec [‡]	0.072 $\pm$ 0.021	0.001	Both open	0.81 $\pm$ 0.32	<0.0001
			Femur open, tibia closed	1.18 $\pm$ 0.26	
			Both closed	1.33 $\pm$ 0.23	
R _{unsat} at 30 msec [‡]	0.055 $\pm$ 0.013	<0.0001	Both open	-0.41 $\pm$ 0.26	0.0004

Measure	Coefficient of age ± SE*	P	Physcal closure status	Mean ± SD†	P
R _{unsat} at 60 msec‡	0.085 ± 0.018	<0.0001	Femur open, tibia closed	-0.27 ± 0.19	<0.0001
			Both closed	-0.16 ± 0.16	
			Both open	-0.17 ± 0.28	
			Femur open, tibia closed	0.11 ± 0.23	
			Both closed	0.30 ± 0.21	

UI unsaturation index, *R_{sat}* saturated fat fraction, *R_{unsat}* unsaturated fat fraction

* Change in measure per year of age, from simple linear regression analysis, with standard error (SE). *P* tests for zero coefficient (no relation to age)

† Unadjusted mean and standard deviation (SD) in each physcal closure group. *P* from one-way analysis of variance, testing for equal mean in the three groups

‡ *R_{sat}* and *R_{unsat}* showed strongly skewed distribution and were log10-transformed for analysis; coefficient and means displayed are in log10 units (1 unit corresponding to 10-fold difference; 0.3 units corresponding to 2-fold difference)

Table 3
Relation of relaxometry and MR spectroscopy (MRS) measures to clinical variables, as modified by age or physéal closure status*

Marrow measure	Effect modifier	Effect modifier level	Regression coefficient (95% CI)		
			BMD, mg/cm ³	BMD, mg/cm ³	Duration of anorexia, 50% increase
T ₁ , ms	Age, yr	12	-19.3 (-36.3, -2.3)	-1.19 (-1.93, -0.46)	3.7 (-13.1, 20.4)
		15	-2.6 (-14.5, 9.4)	-0.39 (-0.79, 0.00)	-2.7 (-11.1, 5.8)
		19	19.7 (-8.3, 47.8)	0.68 (-0.02, 1.38)	-11.1 (-25.2, 3.0)
R _{air} , 60 ms	Physéal closure	<i>P</i> _{Interaction}	0.05	0.003	0.27
		Both open	8.6 (-5.1, 24.1)	0.87 (0.17, 1.58)	1.1 (-9.2, 12.6)
		Femur open, tibia closed	0.1 (-18.1, 22.4)	0.12 (-0.60, 0.86)	5.2 (-8.2, 20.6)
		Both closed	-8.7 (-27.7, 15.4)	-0.33 (-0.76, 0.11)	4.2 (-4.1, 13.4)
R _{unsat} , 60 ms	Physéal closure	<i>P</i> _{Interaction}	0.42	0.02	0.87
		Both open	11.2 (-1.0, 24.9)	0.77 (0.15, 1.39)	1.6 (-7.6, 11.7)
		Femur open, tibia closed	-1.70 (-17.4, 17.0)	0.13 (-0.51, 0.77)	4.5 (-7.3, 17.9)
		Both closed	-8.5 (-25.3, 12.1)	-0.33 (-0.71, 0.05)	3.9 (-3.5, 11.8)
		<i>P</i> _{Interaction}	0.20	0.01	0.91

BMD bone mineral density, BMI body mass index, R_{sat} saturated fat fraction, R_{unsat} unsaturated fat fraction

* Multiple linear regression analysis relating each marrow measure to clinical variables. Interaction terms were included to test for modification of relationship by age (expressed as a continuous variable) or by skeletal maturity (three categories according to physéal closure). Regression coefficients with 95% confidence intervals (CI) are derived from parameters of fitted model and describe change in the marrow measure per unit change in the clinical variable. Distinct coefficients were calculated for each category of physéal closure or for three specific ages, chosen from the continuous age range to represent the sample minimum (12 yr), median (15 yr) and maximum (19 yr). Coefficients for T₁ are in ms per indicated unit of clinical variable. R_{sat} and R_{unsat} were log-transformed for analysis; regression coefficients (b) are transformed to units of percentage ($100\% \times [10^b - 1]$) per indicated unit of clinical variable. Duration of anorexia was log-transformed for use in regression models; 50% increase corresponds to increment of 0.1761 on log10 scale. *P*_{Interaction} tests for effect modification, i.e. uniformity of regression coefficients over the continuous range of age or among the three classes of physéal closure