


# Marrow Fat Content and Composition in $\beta$ -Thalassemia: A Study using $^1\text{H}$ -MRS

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**Background:**  $\beta$ -thalassemia is a genetic disease that causes abnormal production of red blood cells (ineffective erythropoiesis, IE). IE is a condition known to change bone marrow composition.

**Purpose:** To evaluate the effect of IE on the marrow fat content and fat unsaturation levels in the proximal femur using  $^1\text{H}$ -MRS.

**Study Type:** Prospective.

**Subjects:** Twenty-three subjects were included in this study, seven control and 16  $\beta$ -thalassemia subjects.

**Field Strength/Sequence:** 3.0T; stimulated echo acquisition Mode (STEAM); magnetic resonance spectroscopy (MRS) sequence.

**Assessment:** Multiecho MRS scans were performed in four regions of the proximal left femur of each subject, that is, diaphysis, femoral neck, femoral head, and greater trochanter. The examined regions were grouped into red (diaphysis and femoral neck) and yellow marrow regions (femoral head and greater trochanter).

**Statistical Tests:** The Jonckheere–Terpstra test was used to evaluate the impact of increasing disease severity on bone marrow fat fraction (BMFF), marrow conversion index, and fat unsaturation index (UI). Pairwise comparison analysis was performed when a significant trend ( $P < 0.05$ ) was found. K-means clustering analysis was used to examine the clusters observed when BMFF in the red and yellow regions were studied (diaphysis against greater trochanter).

**Results:** BMFF showed a significant decreasing trend with increasing disease severity in both red ( $T_{JT} = 109.00$ ,  $z = -4.414$ ,  $P < 0.05$ ) and yellow marrow regions ( $T_{JT} = 108.00$ ,  $z = -4.438$ ,  $P < 0.05$ ). The opposite trend was observed in UI in both bone marrow regions (red marrow:  $T_{JT} = 180.5$ ,  $z = 3.515$ ,  $P < 0.05$ ; yellow marrow:  $T_{JT} = 155.0$ ,  $z = 2.282$ ,  $P = 0.05$ ). Three distinct forms of marrow adipogenesis were found when plotting BMFF diaphysis against BMFF greater trochanter: 1) normal (centroid: 80.4%, 66.6%), 2) partial disruption (centroid: 51.1%, 16.6%), and 3) total disruption (centroid: 2.6%, 1.6%).

**Data Conclusion:**  $\beta$ -thalassemia is associated with decreased marrow fat, and increased marrow fat unsaturation level.

**Level of Evidence:** 2

**Technical Efficacy Stage:** 3

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$\beta$ -THALASSEMIA, a genetic hemoglobin disorder, is defined by impaired synthesis of the  $\beta$ -globin chain of hemoglobin.<sup>1</sup> It is characterized by the premature destruction of developing red blood cells (ineffective erythropoiesis, IE), chronic hemolytic anemia, and subsequent clinical complications.<sup>2</sup> There is a wide range of clinical severities within the  $\beta$ -thalassemia syndromes.  $\beta$ -thalassemia minor ( $\beta$ -TMI) is

characterized by mild microcytic anemia only,<sup>1</sup> while at the other end of the spectrum is  $\beta$ -thalassemia major ( $\beta$ -TMA) for which regular transfusions are required to reduce the complications from chronic anemia and IE.<sup>1</sup> Those with anemia too severe to be considered as minor, but who occasionally require transfusion, are termed as having  $\beta$ -thalassemia intermedia ( $\beta$ -TI) or nontransfusion-dependent  $\beta$ -thalassemia.<sup>1,2</sup>

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The consequence of anemia in  $\beta$ -thalassemia patients can be observed by changes to their bone marrow composition. Normally, bone marrow is predominantly hematopoietic (red) marrow at birth, but is gradually replaced by fatty (yellow) marrow as the person ages.<sup>3,4</sup> However, bone marrow reconversion, that is, a decrease in marrow adipose tissue (MAT), and an increase in red marrow, has been observed in  $\beta$ -thalassemia patients and other diseases characterized by increased erythropoietic demand.<sup>3</sup>

Optimal hematopoiesis requires an intact and functional bone marrow microenvironment.<sup>5</sup> MAT is one of the main components of the bone marrow stroma and plays an essential role in the maintenance of hematopoiesis.<sup>6</sup> In recent years, MAT has been described as a fine-tuned expandable/contractible fat depot that works to maintain optimal hematopoiesis.<sup>7</sup> The plasticity of MAT in responding to hematopoietic demands implies that MAT levels could be used as a measure of the efficacy of thalassemia treatment in suppressing IE. This is especially important in  $\beta$ -thalassemia, where the suggested serum biomarkers of IE (eg, serum transferrin receptor or erythropoietin levels<sup>8–10</sup>) are susceptible to alteration according to transfusion and body iron stores.

In addition to the size and amount of marrow fat, the fatty acid composition of MAT may also have clinical relevance. For example, changes in bone marrow composition, assessed using magnetic resonance imaging (MRI), have been observed in osteoporosis, obesity, anorexia nervosa, and type 2 diabetes mellitus.<sup>11–13</sup> These studies revealed significantly lower marrow fat unsaturation levels in patients than controls. An erythropoiesis study, on the other hand, revealed increases in marrow fat unsaturation levels as erythropoiesis increases.<sup>14</sup> It has been suggested in an osteoporosis study that marrow fatty acid composition is better correlated with bone marrow density than marrow fat content.<sup>12,15,16</sup> The clinical relevance of MAT levels and composition in relation to erythropoiesis, however, is still unclear.

The spectrum of disease severity in  $\beta$ -thalassemia provides an opportunity to study the impact of increasing the requirement of hematopoiesis (due to IE) on marrow adipogenesis. The ability of proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) to resolve the signal of each individual lipid peak makes it valuable in studying bone marrow fat content and fatty acid composition. This technique has been commonly used to assess and quantify body fat composition *in vivo*.<sup>17,18</sup> Thus, the purpose of this study was to utilize <sup>1</sup>H-MRS noninvasively to study the impact of IE on bone marrow fat content and fatty acid composition in  $\beta$ -thalassemia patients.

## Materials and Methods

### Selection of Participants

The study protocol and study design were approved by the Research Ethics Committee of our institute. Written informed consent was obtained from all subjects.

The study population consisted of  $\beta$ -thalassemia patients from our institution's hematology unit, and control subjects who were healthy volunteers. Subjects were aged between 18 and 40 years.  $\beta$ -thalassemia patients had their diagnosis confirmed by the standard laboratory criteria, that is, high-performance liquid chromatography (HPLC) and gel electrophoresis.<sup>19,20</sup> The differentiation of  $\beta$ -thalassemia subjects into  $\beta$ TMi,  $\beta$ TI, and  $\beta$ TMa categories was in accordance with clinical criteria, and the results of blood count, HPLC, and Hb gel electrophoresis tests.<sup>21</sup> Control subjects had normal blood counts. The exclusion criteria were the possible covariates that may modify bone marrow composition<sup>22</sup>: 1) pregnancy, 2) smoking ( $\geq 10$  pack-year), 3) frequent alcohol consumption ( $\geq 10$  units/week), 4) involvement in competitive athletics, 5) other concomitant hemoglobinopathies (eg, sickle cell disease or  $\alpha$ -thalassemia), 6) hemolytic disorders (eg, coexistent ovalocytosis, G6PD deficiency), 7) active bleeding, 8) use of erythropoietin or other drugs that might affect erythropoiesis (eg, hydroxyurea, luspatercept), or 9) a history of serious leg injuries. Control subjects with known osteopenia or osteoporosis were also excluded from the study, while no such exclusion criterion was placed on the  $\beta$ -thalassemia patients, as osteopenia or osteoporosis are known to be associated with this disease.<sup>23</sup>

### Scanning Protocol

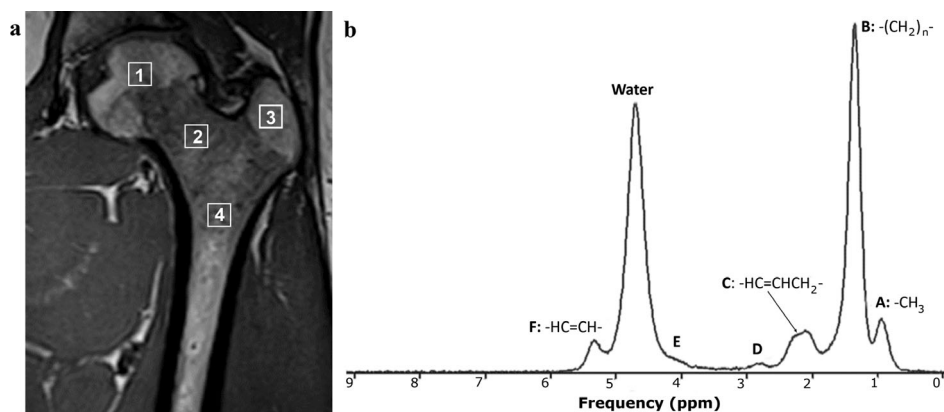
All participants underwent MRI using a 3.0T whole-body scanner (Magnetom Prisma, Siemens Healthcare, Erlangen Germany) with a body 18-channel phased-array coil system. The subjects were positioned supine and feet-first on the MRI table.

A T<sub>1</sub>-weighted 3D "sampling perfection with application optimized contrasts using different flip angle evolution" (SPACE) scan sequence was performed in coronal planes with the following parameters: repetition time / echo time (TR/TE): 600/3.4 msec; slice thickness: 1.5 mm; field of view (FOV): 300 × 300 × 144 mm<sup>3</sup>; matrix size: 256 × 256 × 96. The FOV was chosen to cover the left lower limb from the hip joint to the middle third of the femoral shaft. The images obtained from the coronal SPACE sequence were reconstructed to obtain images in both axial and sagittal planes. These images were then used for optimal positioning of MRS volumes of interest.

MRS acquisitions were performed using a single voxel stimulated echo acquisition mode (STEAM) sequence with TR: 3500 msec; mixing time (TM): 10 msec; TE varying from 20–40 msec in 5-msec increments in separate acquisitions. Other parameters were: water suppression: off; voxel size: 10 × 10 × 10 mm<sup>3</sup>; number of averages: 12; number of points: 1024; and bandwidth: 1200 Hz. The scanning was performed at four regions of the proximal femur: 1) femoral head, 2) femoral neck, 3) greater trochanter, and 4) proximal diaphysis (Fig. 1a).

### Spectral Fitting

Acquired spectra were processed using an offline AMARES algorithm included in the Java-based MRUI (jMRUI) software package.<sup>24,25</sup> The preprocessing steps included calibration according to the main CH<sub>2</sub> peak (1.3 ppm). Spectral assignments of the water peak (4.7 ppm) and six fat peaks were based on previous studies.<sup>17,18</sup> Peaks were assigned as follows: A, methyl protons (observed at 0.90 ppm); B, bulk methylene and  $\beta$ -carboxyl protons (observed at



**FIGURE 1: (a)** Location of the voxels of interest: 1) femoral head, 2) femoral neck, 3) greater trochanter, and 4) diaphysis, shown on a coronal  $T_1$ -weighted SPACE image of a subject from the control group (24-year-old female). **(b)** The spectrum shown was obtained from the proximal diaphysis of this subject. The lipids that were commonly resolved *in vivo* were terminal methyl protons (A: 0.9 ppm), bulk methylene protons (B: 1.3 ppm), methylene protons (C: 2.1 ppm), and olefinic protons (F: 5.3 ppm).

1.3 ppm); C,  $\alpha$ -carboxyl, and  $\alpha$ -olefinic (observed at 2.1 ppm); D, diacyl (observed at 2.75 ppm); E, glycerol (observed at 4.2 ppm); and F, olefinic (observed at 5.3 ppm) (Fig. 1b).

The rules applied to the spectral fitting procedure were as follows: Gaussian model function was assumed for all peaks; peak B was fitted using three Gaussians; the fat peaks A and C were each fitted using two Gaussians, while peak D was modeled by a single Gaussian. The water peak and peaks E and F were fitted using five Gaussians.<sup>17</sup> The resonance frequencies were constrained to lie within  $\pm 0.05$  ppm of the observed frequencies. The linewidths of the water and fat peaks were constrained to  $\leq 100$  Hz.

Given that spectral broadening effects were observed in iron overloaded patients, the fat signal,  $I_{fat}$ , was defined as the total signal amplitude of peaks A–D, while the water signal,  $I_{water}$ , was defined as the total signal amplitude of the water peak and peaks E–F. The signals underwent a  $T_2$ -relaxation correction process by applying nonlinear least-square fitting to account for the presence of iron.<sup>26</sup>

To evaluate the relationship of bone marrow fat content with IE, bone marrow fat fraction (BMFF) was calculated according to the equation:

$$\text{Bone marrow fat fraction, BMFF (\%)} = \frac{I_{fat}}{I_{fat} + I_{water}} \times 100\%.$$

To evaluate the impact of IE on marrow adipogenesis, the BMFF measured in the diaphysis (in healthy adults, this is the region in the proximal femur that will develop MAT last) was compared with the BMFF obtained from the greater trochanter (in healthy adults, this is the region in the proximal femur that will first develop MAT). A marrow conversion index (MCI), the ratio of BMFF in diaphysis to BMFF in greater trochanter, was also calculated and expressed as a percentage.<sup>22</sup>

To evaluate fatty acid composition, a bone marrow unsaturation index (UI)<sup>12</sup> was calculated for each marrow region. UI was only calculated for high-quality spectra, where peaks A, B, C, and F were clearly identifiable (Fig. 1b). UI was calculated from the spectra obtained at each TE and the values were then averaged. The equation used was:

$$\text{Unsaturation Index, UI} = \frac{I_F}{I_A + I_B + I_C + I_F}$$

where  $I_A$ ,  $I_B$ ,  $I_C$ , and  $I_F$  are the signal amplitudes of lipid peaks A, B, C, and F, respectively.

### Statistical Analysis

The data obtained from the four regions examined were grouped into two categories: 1) red marrow regions (diaphysis and femoral neck), and 2) yellow marrow regions (femoral head and greater trochanter). The grouping was based on the usual anatomic distribution of marrow fat found in healthy adults.<sup>4</sup> The Jonckheere–Tersprtra test was used to analyze the impact of increasing severity of IE on UI and BMFF in both red and yellow marrow regions. When a significant trend was observed (two-sided  $P < 0.05$ ), pairwise multiple comparisons were performed. The impact of IE on bone marrow adipogenesis was studied using Jonckheere–Tersprtra trend analysis and k-means clustering analysis. To test for the correlation between UI and marrow fat content, a Pearson’s correlation test was employed. Data were analyzed using IBM SPSS Statistics for Windows, v. 23 (Armonk, NY) and MatLab R2019b (MathWorks, Natick, MA).

## Results

### Subjects and Spectral Results

Overall, 23 subjects were examined, of which seven were healthy, while the rest were  $\beta$ -thalassemia patients (four  $\beta$ TMi, five  $\beta$ TI, and seven  $\beta$ TMa) (Table 1). The mean body mass index (BMI) of all participants was  $22.7 \pm 3.2$  kg/m<sup>2</sup>; except for two subjects (one  $\beta$ TMi and one  $\beta$ TMa) who were slightly overweight, the rest of the participants had a normal BMI. One  $\beta$ TMa subject had hypogonadism.

Spectra obtained from three of the subjects (two  $\beta$ TI and one  $\beta$ TMa) were excluded from the calculation of BMFF as they were not interpretable due to excessive iron level (they were all found to have ferritin level  $\geq 4000$   $\mu$ g/L). UI values

**TABLE 1. Subjects Demographics and MR Spectroscopy Results for All Subjects**

Characteristics	Control	$\beta$ TMi	$\beta$ TI	$\beta$ TMa
<i>n</i> (M/F)	7(2/5)	4(2/2)	5(2/3)	7 (4/3)
Age (yrs)	28.5 $\pm$ 2.4	31.5 $\pm$ 5.7	33.3 $\pm$ 7.3	26.8 $\pm$ 6.6
Height (cm)	155.0 $\pm$ 8.7	163.8 $\pm$ 7.8	161.7 $\pm$ 7.5	159.7 $\pm$ 9.0
Weight (kg)	57.1 $\pm$ 9.8	62.1 $\pm$ 18.6	52.5 $\pm$ 3.4	59.2 $\pm$ 9.5
BMI (kg/m <sup>2</sup> )	22.5 $\pm$ 1.4	22.9 $\pm$ 5.3	20.0 $\pm$ 1.1	23.2 $\pm$ 2.8
Bone marrow fat fraction (BMFF)				
Femoral neck	67.18 $\pm$ 4.13	66.63 $\pm$ 14.03	28.61 $\pm$ 11.03	26.40 $\pm$ 27.94
Diaphysis	70.40 $\pm$ 3.54	55.67 $\pm$ 16.03	20.82 $\pm$ 14.93	27.27 $\pm$ 32.77
Femoral head	84.93 $\pm$ 6.63	85.21 $\pm$ 4.11	43.45 $\pm$ 7.72	35.72 $\pm$ 37.05
Greater trochanter	80.36 $\pm$ 6.43	82.30 $\pm$ 2.89	45.90 $\pm$ 10.68	33.53 $\pm$ 33.71
Red marrow	68.79 $\pm$ 7.09	61.15 $\pm$ 15.13	24.72 $\pm$ 12.49	26.83 $\pm$ 29.04
Yellow marrow	82.64 $\pm$ 6.71	83.76 $\pm$ 3.64	44.68 $\pm$ 8.44	34.62 $\pm$ 33.79
MCI	82.56 $\pm$ 13.06	67.87 $\pm$ 20.95	45.18 $\pm$ 26.63	90.38 $\pm$ 48.04
Unsaturation index (UI)				
Femoral neck	0.065 $\pm$ 0.017	0.095 $\pm$ 0.026	0.094 $\pm$ 0.039	N/A
Diaphysis	0.060 $\pm$ 0.017	0.097 $\pm$ 0.010	0.101 $\pm$ 0.015	N/A
Femoral head	0.070 $\pm$ 0.020	0.091 $\pm$ 0.015	0.092 $\pm$ 0.029	N/A
Greater trochanter	0.057 $\pm$ 0.013	0.062 $\pm$ 0.009	0.086 $\pm$ 0.019	N/A
Red marrow	0.062 $\pm$ 0.002	0.096 $\pm$ 0.018	0.100 $\pm$ 0.027	N/A
Yellow marrow	0.064 $\pm$ 0.017	0.077 $\pm$ 0.019	0.089 $\pm$ 0.022	N/A

$\beta$ TMi =  $\beta$ -thalassemia minor;  $\beta$ TI =  $\beta$ -thalassemia intermedia;  $\beta$ TMa =  $\beta$ -thalassemia major; BMI = body mass index; MCI = marrow conversion index.

were only calculated from high-quality spectra obtained from the subjects (for example, spectra obtained from two  $\beta$ TI and all  $\beta$ TMa subjects were excluded due to excessive spectral broadening).

Figure 2 demonstrates examples of marrow spectra obtained from two  $\beta$ -thalassemia patients (one  $\beta$ TMa and one  $\beta$ TI), with an age difference of 10 years. Figure 2a shows a patient with a total absence of fat peaks in bone marrow (proximal diaphysis). Figure 2b depicts a spectrum when iron is present.

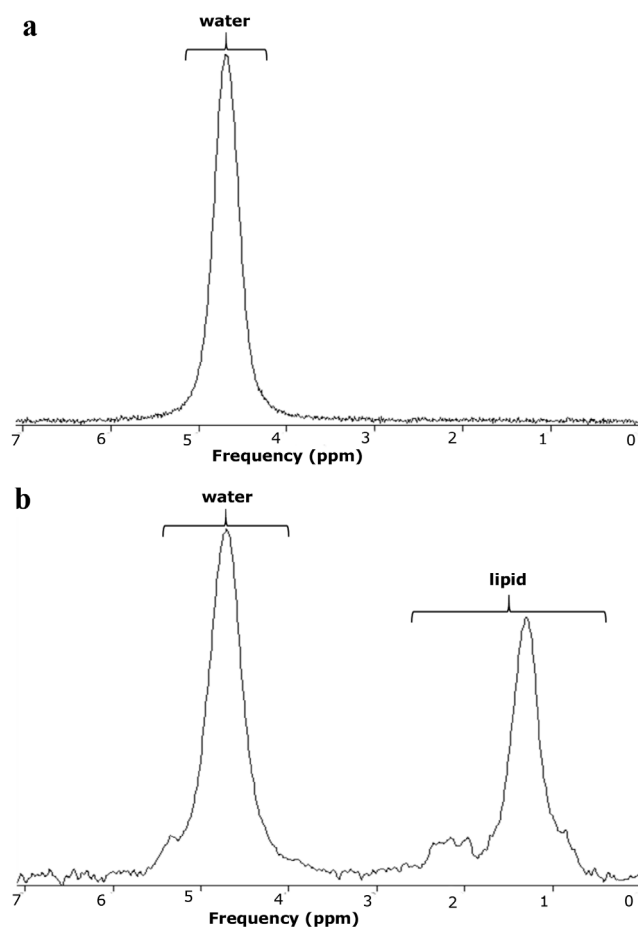
An example of the change in <sup>1</sup>H-MR spectra for water and lipid with echo time in the bone marrow of subjects with iron-overload is shown in Fig. 3a. All spectra acquired show the expected monoexponential decay. The results from  $T_2$  curve fitting and calculation (Fig. 3b) were used to calculate BMFF and the subsequent MCI.

The calculated BMFF, MCI, and UI for each group are presented in Table 1.

### **Bone Marrow Fat Fraction with IE**

Figure 4a depicts the changes in proximal femur BMFF as the severity of the thalassemia increased. The Jonckheere–Terpstra trend analysis test showed that in both red and yellow marrow regions there were statistically significant decreasing trends in BMFF as the severity of thalassemia increased (red marrow:  $T_{JT} = 109.00$ ,  $z = -4.414$ ,  $P < 0.05$ ; yellow marrow:  $T_{JT} = 108.00$ ,  $z = -4.438$ ,  $P < 0.05$ ).

Post-hoc analysis (Table 2) showed that, of the six possible pairs of subject groups, the three subject group pairs that had significant differences ( $P < 0.05$ ) in BMFF in both red and yellow marrow regions were: control vs.  $\beta$ TI, control vs.  $\beta$ TMa, and  $\beta$ TMi vs.  $\beta$ TI. One pair ( $\beta$ TMi vs.  $\beta$ TMa) showed a significant difference in the BMFF only in yellow marrow ( $P < 0.05$ ), and not in red marrow ( $P = 0.094$ ). The wide range of BMFF in  $\beta$ TMa subjects was clearly visible in both red (BMFF: 1.00–72.30%) and yellow (BMFF: 0.77–85.41%) marrow regions (Table 1, Fig. 4a).



**FIGURE 2: (a)** A spectrum obtained from the left proximal diaphysis of a 28-year-old male  $\beta$ TMa subject. The spectrum shows an absolute absence of lipid peak, indicating a total displacement of MAT, in favor of hematopoietic red bone marrow. **(b)** A spectrum obtained from the left proximal diaphysis of a 38-year-old male  $\beta$ TI subject. The spectrum shows a partial displacement of marrow adipose tissue in favor of hematopoietic red bone marrow. A slight spectral broadening effect in the spectrum is due to an increase in gastrointestinal iron absorption and sporadic blood transfusion. To account for the broadened spectra, lipid signals near the water peak (4.2 and 5.3 ppm) were assumed to be part of the water signal during the quantification of marrow fat content.

### Bone Marrow Adipogenesis in IE

MCI showed no significant trend with increasing disease severity ( $T_{JT} = 65.5$ ,  $z = -0.476$ ,  $P = 0.634$ ) (Table 1, Fig. 4b). However, three distinct clusters were found when the BMFF in the diaphysis was plotted against the BMFF in the greater trochanter (Fig. 5). The first cluster contained 13 observations (all seven controls, four  $\beta$ TMi, and two  $\beta$ TMa) in which both regions had high levels of BMFF (centroid: 80.4%, 66.6%). The second cluster consisted of four subjects (three  $\beta$ TI and one  $\beta$ TMa) with the centroid in between the first and the third cluster (centroid: 51.1%, 16.6%). The third cluster, with very low levels of BMFF in both regions (centroid: 2.6%, 1.6%), contained three subjects (all three being  $\beta$ TMa).

### Composition of Bone Marrow Fatty Acid With IE

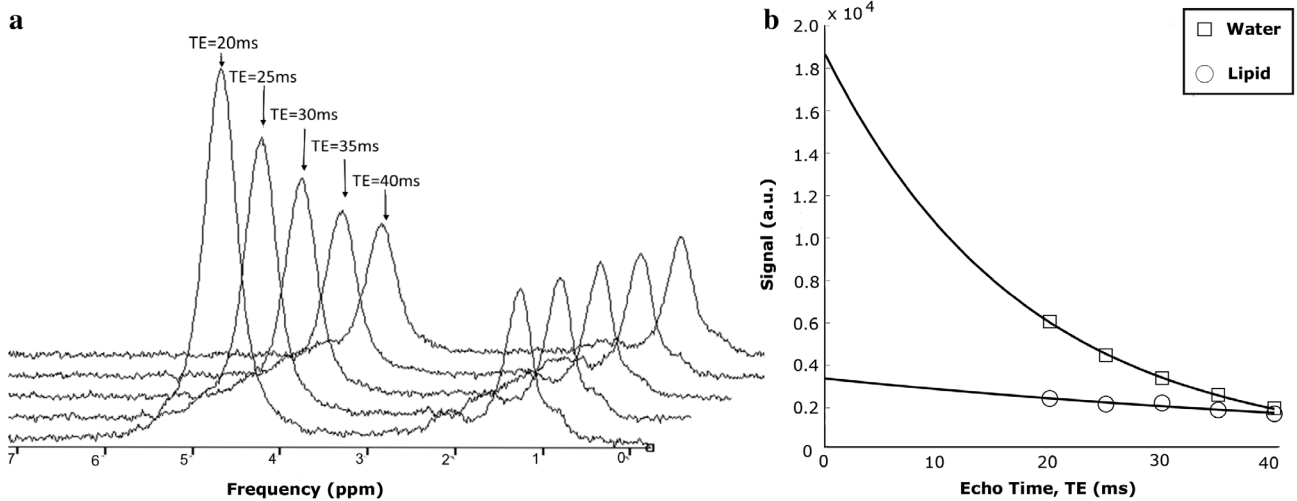
The UI increased significantly as the disease severity increased, with red marrow regions more affected than yellow marrow regions (red marrow:  $T_{JT} = 180.5$ ,  $z = 3.515$ ,  $P < 0.05$ ; yellow marrow:  $T_{JT} = 155.0$ ,  $z = 2.282$ ,  $P = 0.05$ ) (Fig. 4c). In red marrow regions, subjects with  $\beta$ -thalassemia (ie,  $\beta$ TMi and  $\beta$ TI) were found to have significantly higher ( $P < 0.05$ ) UI values compared to controls (Table 2). For yellow marrow regions, a statistically significant difference ( $P < 0.05$ ) was only found between controls and  $\beta$ TI. Pearson's correlation analysis indicated a negative correlation between the UI and BMFF ( $r = -0.486$ ,  $P < 0.05$ ).

### Discussion

The adapted  $^1\text{H}$ -MRS technique and analyses employed in this study demonstrated differences in BMFF, MCI, and UI between different regions of the proximal femur, in both control and thalassemia patients, which may reflect differences in the proportion of hematopoiesis in these subject groups. Overall, this study demonstrated a significantly lower BMFF and a higher UI occurring with increasing severity of thalassemia, a condition associated with expanded erythroid marrow due to IE. BMFF was more affected by disease severity than UI.

A strong inverse relationship between MAT content and the body's hematopoietic demands has been well established.<sup>3,23</sup> In our study we found that there were three distinct responses of MAT to hematopoietic stress. The first cluster (consisting of controls,  $\beta$ TMi, and two  $\beta$ TMa subjects) demonstrates normal marrow adipogenesis in both red and yellow marrow regions, signifying normal hematopoiesis. Although  $\beta$ TMi subjects showed a slight decrease in the BMFF in the red marrow regions as compared to the control group, in keeping with their known mild IE,<sup>1</sup> the differences were not statistically significant. The second cluster (consisting of  $\beta$ TI and one  $\beta$ TMa subjects) showed a marked reduction of BMFF in both red and yellow marrow regions compared to cluster 1, implying a partial disruption of the bone marrow conversion process, and a greater increase in hematopoiesis than in  $\beta$ TMi or normal subjects. The third cluster (consisting only of  $\beta$ TMa subjects) showed a total suppression of marrow adipogenesis. The negligible amount of marrow fat indicates the effect of very marked ineffective erythropoiesis, which would be expected if the  $\beta$ TMa patient is not adequately transfused. The three distinct forms of marrow adipogenesis may summarize the response of MAT as the body's hematopoietic needs increase, ie, MAT decreases as hematopoiesis increases. These results suggest that quantitative information on MAT could be an indicator of body erythropoietic activity.

The strong plasticity properties of adipose tissue, changing in amount in response to physiological stresses, may be



**FIGURE 3:** (a) The spectral peaks of water and lipid obtained from a diaphysis of 38-year-old subject (male,  $\beta$ TI subject) using a single-voxel STEAM sequence. Each spectrum was obtained separately using TEs ranging from 20–40 msec. The relaxation of signals with increasing time is observed here, emphasizing the importance of  $T_2$ -relaxation correction in the calculation of marrow fat content. (b) The relaxation curves of the spectra shown in (a). The signal decay for water is more rapid than lipid.  $T_2$ -relaxation correction eliminates the TE-dependency in marrow fat content calculation.

the explanation for the three distinct responses found in this study.<sup>27</sup>  $\beta$ TMi subjects, who are mildly anemic and asymptomatic, show only minimal nonsignificant differences in MAT compared to the control group, and thus they are in the same cluster. Treatments given to  $\beta$ TI, if any, aim to support the patients’ hematopoiesis (eg, folic acid supplements), and transfusions are given only in situations of severe anemia due to stresses such as infections or pregnancy.<sup>28</sup> Hence, there is a partial disruption of MAT in  $\beta$ TI patients (seen in the second cluster) as they will remain anemic, with increased erythropoiesis. The objective of regular transfusion in  $\beta$ TMa patients is to suppress their increased (but largely ineffective) erythropoiesis, so the high variability in BMFF in these subjects may reflect the intensity of their transfusion regimen,

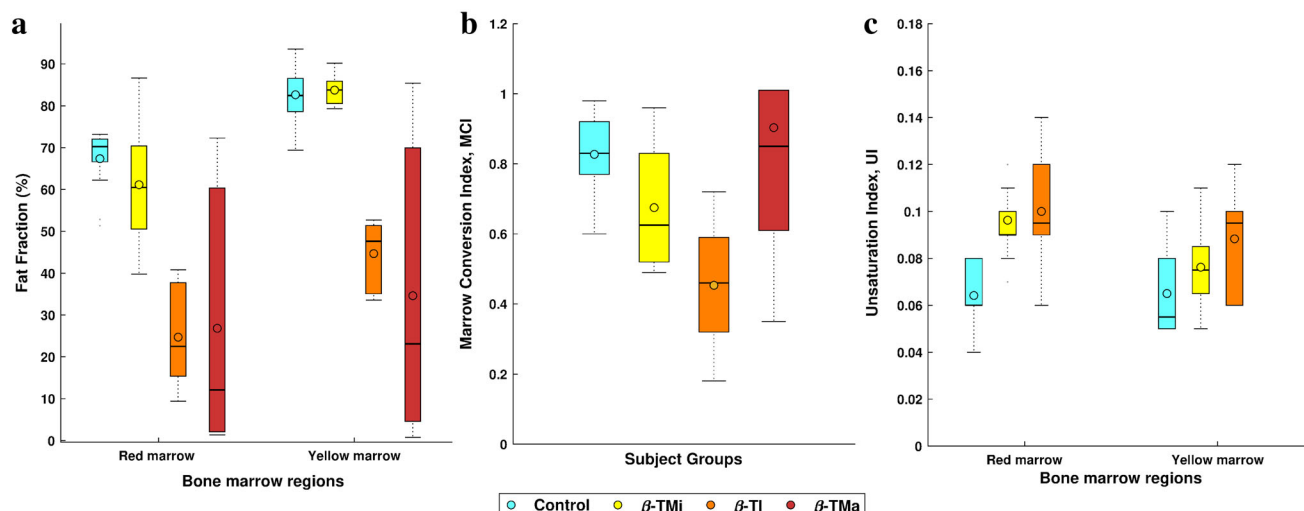
and/or their individual compliance or response to the transfusions. Impaired differentiation of mesenchymal stem cells due to severe hypoxia may also be a contributory cause to reduced MAT in these  $\beta$ TMa patients.<sup>29</sup> We found that hypogonadism and being overweight did not appear to affect marrow adipogenesis in the subjects with these complications.

The increased unsaturation of lipids in  $\beta$ -thalassemia subjects may be due to the working of the key enzyme involved in lipid metabolism (i.e., stearoyl-CoA desaturase-1).<sup>30,31</sup> This enzyme catalyzes the synthesis of monounsaturated lipids by adding one double bond to the saturated lipids. Lipolysis and remodeling of MAT with highly saturated lipids when facing physiological stress can also be a contributory factor to the increase in UI.<sup>31,32</sup> Indeed, increased

**TABLE 2. Statistical Test Results for Bone Marrow Fat Fraction and Unsaturation Index of  $\beta$ -Thalassemia and Control Subjects**

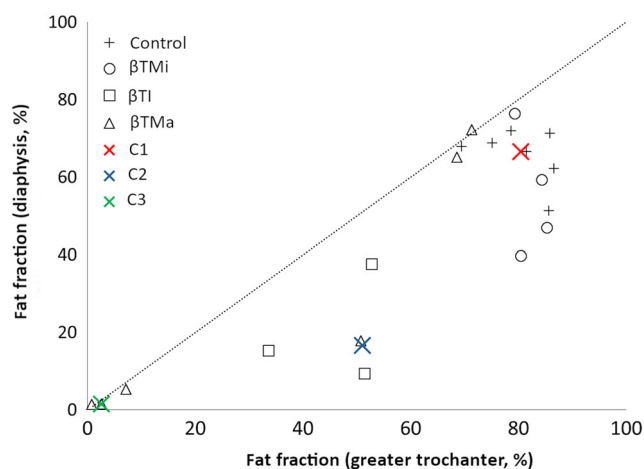
	Bone marrow fat fraction						Unsaturation index					
	Red marrow			Yellow marrow			Red marrow			Yellow marrow		
	$T_{JT}$	$z$	$P$	$T_{JT}$	$z$	$P$	$T_{JT}$	$z$	$P$	$T_{JT}$	$z$	$P$
Control vs $\beta$ TMi	37.0	-1.297	0.584	62.0	0.410	1.000	88.5	3.186	<0.05	65.0	1.349	0.266
Control vs $\beta$ TI	0.0	-3.464	<0.05	0.0	-3.464	<0.05	65.0	2.777	<0.05	58.5	2.158	<0.05
Control vs $\beta$ TMa	21.0	-3.240	<0.05	10.0	-3.806	<0.05	N/A					
$\beta$ TMi vs $\beta$ TI	1.0	-2.969	<0.05	0.0	-3.098	<0.05	27.0	0.398	1.000	31.5	0.977	0.493
$\beta$ TMi vs $\beta$ TMa	20.0	-2.160	0.092	6.0	-3.240	<0.05	N/A					
$\beta$ TI vs $\beta$ TMa	30.0	-0.562	1.000	30	-0.562	1.000	N/A					

$\beta$ TMi =  $\beta$ -thalassemia minor;  $\beta$ TI =  $\beta$ -thalassemia intermedia;  $\beta$ TMa =  $\beta$ -thalassemia major.



**FIGURE 4:** Boxplots of proximal femur BMFF, MCI, and UI grouped according to the increasing severity of the disease to show the impact of IE on bone marrow composition. (a) BMFF for red marrow regions (proximal diaphysis and femoral neck) and yellow marrow regions (femoral head and greater trochanter). (b) MCI for red marrow regions (proximal diaphysis and femoral neck) and yellow marrow regions (femoral head and greater trochanter). (c) Marrow UI for red marrow regions (proximal diaphysis and femoral neck) and yellow marrow regions (femoral head and greater trochanter). (d) The horizontal line indicates the median, while the bottom and top edges of the box indicate the 25th and 75th percentiles. The whiskers extend to the outliers. The circle in the box indicates the average value.

unsaturated lipids and greater lipolysis of MAT, as erythropoiesis increases, has also been observed in a previous study.<sup>14</sup> The decreased level of saturated lipid may relate to an increased supply of energy to marrow cells,<sup>33</sup> while unsaturated lipids may play a role in cell proliferation.<sup>34</sup> As an increase in lipid unsaturation has been observed in the condition of increased erythropoiesis and lipid metabolism,<sup>14,30,31</sup> it will be of interest to study longitudinally how the MAT unsaturation index changes in individual patients in relation to therapies aimed at suppressing overactive erythropoietic activity. Unfortunately, we were unable to evaluate the



**FIGURE 5:** A scatterplot with the line of equality to show the changes of BMFF in proximal diaphysis in relation to bone marrow fat fraction in greater trochanter in different subject groups. Each centroid of a cluster obtained from the simple k-means clustering method is labeled with 'x'. C1, C2, and C3 represent three forms of marrow adipogenesis: 1) normal (centroid: 80.4%, 66.6%), 2) partial disruption (centroid: 55.1%, 16.6%), and total disruption (centroid: 2.6%, 1.6%), respectively.

unsaturation index in  $\beta$ TMa patients due to the excessive spectrum broadening due to very high levels of tissue iron.

In iron-overloading conditions, in which spectral broadening and rapid signal relaxation are expected, several methods have been used to obtain more accurate estimates of each metabolite. For example, the STEAM sequence was used in this study due to it being less sensitive to short  $T_2$  relaxation times and homonuclear scalar coupling.<sup>35</sup> Moreover, to resolve for signal ambiguity, signals obtained at 4–5.3 ppm were defined as water signals regardless of the spectral quality. Furthermore, our data showed that water and fat peaks have different  $T_2$  values and the transverse relaxation of water is more affected by iron compared to fat. Hence, to correct for  $T_2$  decay, we obtained spectra at multiple TEs and the fitting for water and fat relaxation times was done separately.

### Limitations

1)  $\beta$ -thalassemia subjects were divided according to their severity, resulting in the number of subjects in each group being limited. 2) This study did not correct for age and gender-related factors, although they are known to be able to affect bone marrow composition.<sup>22</sup> However, as evidenced by the magnitude of the differences in BMFF, and the fact that all our subjects were within the age range 18–40 years and there were males and females in all groups, the effect of IE on bone marrow appeared to exceed the effect of age and gender on bone marrow composition. 3) While this  $^1$ H-MRS method is promising as a research and clinical tool to evaluate MAT, the low spectral quality due to the presence of iron will complicate and cause inaccuracies in the assessment of MAT in thalassemia. As signals obtained at 4–5.3 ppm were defined

as water signals, the quantified BMFF may not reflect the exact amount of fat in the bone marrow. Underestimation of olefinic protons at 5.30 ppm may also occur due to strong J-coupling interactions. While water suppression techniques may be able to resolve water and olefinic peaks, due to time constraints during scanning, we were unable to implement such a technique. Other than that, this technique has a very poor spatial resolution, which is a significant limitation, as bone marrow has a spatially heterogeneous fat distribution. Chemical shift-encoded (CSE) fat–water imaging techniques have the potential to overcome the limitations of this technique,<sup>15,36,37</sup> but this technique would require validation and optimization to improve accuracy in quantifying fat in the iron-overloaded bone marrow. The use of an MR system of higher field strength may improve the resolution of the peaks.

## Conclusion

<sup>1</sup>H-MRS promises to be a valuable tool in noninvasively assessing marrow fat composition and to study the role of IE in changing marrow composition. There is an inverse relationship between marrow fat content and IE and an increase in the unsaturation index. Further studies are required to illuminate the strength of the relationships between marrow fat content and fatty acid composition with erythropoietic activity. The existence of other factors that may affect marrow fat content and the unsaturation index in IE conditions should also be studied. The plasticity properties of MAT in response to the body's hematopoietic activity need further study, especially in  $\beta$ TMa subjects.

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## References

- Rund D, Rachmilewitz E. Beta-thalassemia. *N Engl J Med* 2005;353(11):1135-1146.
- Ribeil JA, Arlet JB, Dussiot M, Moura IC, Courtois G, Hermine O. Ineffective erythropoiesis in beta -thalassemia. *Sci World J* 2013;2013:394295.
- Malkiewicz A, Dziedzic M. Bone marrow reconversion — Imaging of physiological changes in bone marrow. *Pol J Radiol* 2012;77(4):45-50.
- Moore SG, Dawson KL. Red and yellow marrow in the femur: Age-related changes in appearance at MR imaging. *Radiology* 1990;175(1):219-223.
- Tikhonova AN, Dolgalev I, Hu H, et al. The bone marrow microenvironment at single-cell resolution. *Nature* 2019;569(7755):222-228.
- Li Z, MacDougald OA. Stem cell factor: The bridge between bone marrow adipocytes and hematopoietic cells. *Haematologica* 2019;104(9):1689-1691.
- Turner RT, Martin SA, Iwaniec UT. Metabolic coupling between bone marrow adipose tissue and hematopoiesis. *Curr Osteoporos Rep* 2018;16(2):95-104.
- Guimaraes JS, Cominal JG, Silva-Pinto AC, et al. Altered erythropoiesis and iron metabolism in carriers of thalassemia. *Eur J Haematol* 2015;94(6):511-518.
- Kautz L, Jung G, Du X, et al. Erythroferrone contributes to hepcidin suppression and iron overload in a mouse model of beta-thalassemia. *Blood* 2015;126(17):2031-2037.
- Porter JB, Cappellini MD, Kattamis A, et al. Iron overload across the spectrum of non-transfusion-dependent thalassaemias: Role of erythropoiesis, splenectomy and transfusions. *Br J Haematol* 2017;176(2):288-299.
- Kim TY, Schafer AL. Diabetes and bone marrow adiposity. *Curr Osteoporos Rep* 2016;14(6):337-344.
- Yeung DK, Griffith JF, Antonio GE, Lee FK, Woo J, Leung PC. Osteoporosis is associated with increased marrow fat content and decreased marrow fat unsaturation: A proton MR spectroscopy study. *J Magn Reson Imaging* 2005;22(2):279-285.
- Bredella MA, Fazeli PK, Daley SM, et al. Marrow fat composition in anorexia nervosa. *Bone* 2014;66:199-204.
- Bathija A, Davis S, Trubowitz S. Marrow adipose tissue: Response to erythropoiesis. *Am J Hematol* 1978;5(4):315-321.
- Martel D, Leporq B, Bruno M, Regatte RR, Honig S, Chang G. Chemical shift-encoded MRI for assessment of bone marrow adipose tissue fat composition: Pilot study in premenopausal versus postmenopausal women. *Magn Reson Imaging* 2018;53:148-155.
- Patsch JM, Li X, Baum T, et al. Bone marrow fat composition as a novel imaging biomarker in postmenopausal women with prevalent fragility fractures. *J Bone Miner Res* 2013;28(8):1721-1728.
- Hamilton G, Yokoo T, Bydder M, et al. In vivo characterization of the liver fat (1)H MR spectrum. *NMR Biomed* 2011;24(7):784-790.
- Ren J, Dimitrov I, Sherry AD, Malloy CR. Composition of adipose tissue and marrow fat in humans by 1H NMR at 7 Tesla. *J Lipid Res* 2008;49(9):2055-2062.
- Trent RJ. Diagnosis of the haemoglobinopathies. *Clin Biochem Rev* 2006;27(1):27-38.
- The laboratory diagnosis of haemoglobinopathies. *Br J Haematol* 1998;101(4):783-792.
- Sripichai O, Makarasara W, Munkongdee T, et al. A scoring system for the classification of beta-thalassemia/Hb E disease severity. *Am J Hematol* 2008;83(6):482-484.
- Pansini V, Monnet A, Salleron J, Hardouin P, Cortet B, Cotten A. 3 Tesla (1) H MR spectroscopy of hip bone marrow in a healthy population, assessment of normal fat content values and influence of age and sex. *J Magn Reson Imaging* 2014;39(2):369-376.
- Steer K, Stavnychuk M, Morris M, Komarova SV. Bone health in patients with hematopoietic disorders of bone marrow origin: Systematic review and meta-analysis. *J Bone Miner Res* 2017;32(4):731-742.
- Naressi A, Couturier C, Devos JM, et al. Java-based graphical user interface for the MRUI quantitation package. *Magma* 2001;12(2-3):141-152.
- Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 1997;129(1):35-43.
- Sharma P, Martin DR, Pineda N, et al. Quantitative analysis of T2-correction in single-voxel magnetic resonance spectroscopy of hepatic lipid fraction. *J Magn Reson Imaging* 2009;29(3):629-635.
- Pellegrinelli V, Carobbio S, Vidal-Puig A. Adipose tissue plasticity: How fat depots respond differently to pathophysiological cues. *Diabetologia* 2016;59(6):1075-1088.
- Taher AT, Musallam KM, Cappellini MD, Weatherall DJ. Optimal management of beta thalassaemia intermedia. *Br J Haematol* 2011;152(5):512-523.



29. Cicione C, Muinos-Lopez E, Hermida-Gomez T, Fuentes-Boquete I, Diaz-Prado S, Blanco FJ. Effects of severe hypoxia on bone marrow mesenchymal stem cells differentiation potential. *Stem Cells Int* 2013; 2013:232896.
30. Li J, Condello S, Thomes-Pepin J, et al. Lipid desaturation is a metabolic marker and therapeutic target of ovarian cancer stem cells. *Cell Stem Cell* 2017;20(3):303-314.
31. Scheller EL, Doucette CR, Learman BS, et al. Region-specific variation in the properties of skeletal adipocytes reveals regulated and constitutive marrow adipose tissues. *Nat Commun* 2015;6:7808.
32. Scheller EL, Khandaker S, Learman BS, et al. Bone marrow adipocytes resist lipolysis and remodeling in response to beta-adrenergic stimulation. *Bone* 2019;118:32-41.
33. Miranda M, Pino AM, Fuenzalida K, Rosen CJ, Seitz G, Rodriguez JP. Characterization of fatty acid composition in bone marrow fluid from postmenopausal women: Modification after hip fracture. *J Cell Biochem* 2016;117(10):2370-2376.
34. Maedler K, Spinas GA, Dyntar D, Moritz W, Kaiser N, Donath MY. Distinct effects of saturated and monounsaturated fatty acids on beta-cell turnover and function. *Diabetes* 2001;50(1):69-76.
35. Hamilton G, Middleton MS, Bydder M, et al. Effect of PRESS and STEAM sequences on magnetic resonance spectroscopic liver fat quantification. *J Magn Reson Imaging* 2009;30(1):145-152.
36. Hernando D, Hines CD, Yu H, Reeder SB. Addressing phase errors in fat-water imaging using a mixed magnitude/complex fitting method. *Magn Reson Med* 2012;67(3):638-644.
37. Hernando D, Kellman P, Haldar JP, Liang ZP. Robust water/fat separation in the presence of large field inhomogeneities using a graph cut algorithm. *Magn Reson Med* 2010;63(1):79-90.