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Running title: Bone marrow fat fraction, T1 and T2*

Breath-hold MR measurements of fat fraction, T1 and T2* of water and fat in vertebral bone marrow

Purpose: To assess the feasibility of measuring the fat fraction, T1 and T2* relaxation times of water and fat signals in vertebral bone marrow using breath-hold MR gradient echo images of the spine.

Materials and Methods: MRI experiments were performed at 1.5 T on eight healthy volunteers (35.1 ± 15.7 years, 5 men and 3 women) using two sagittal four-echo 3D gradient echo Volumetric Interpolated Breath-hold Examination (VIBE Dixon) sequences acquired at two different flip angles (5° and 15°). The water/fat decomposition was performed in the vertebral bodies of L1 to L5 by fitting the signal to a function that depends on the echo time and the flip angle to calculate the fat fraction (FF) and T1 and T2* relaxation times of water and fat signals. Repeatability was assessed by scanning one volunteer six times.

Results: The mean fat fraction over L1 to L5 was 33±8%. The mean T1 and T2* of water and fat signals were respectively $T1_w$=701±151 ms, $T2^*_w$=13.7±2.9 ms, $T1_f$=334±113 ms and $T2^*_f$=11.4±2.7 ms. When considering each vertebra separately, the fat fraction increased from L1 to L5 and the $T1_w$ decreased from L1 to L5. The mean coefficients of variation obtained from the repeatability study were 8% (FF), 11% ($T1_w$), 17% ($T1_f$), 8% ($T2^*_w$) and 27% ($T2^*_f$).

Conclusion: The method introduced in the current study allows for the measurement of the fat fraction and water and fat relaxation times, with the overall imaging protocol being less than 40 seconds.

Key words: Magnetic Resonance Imaging; Chemical Shift Imaging; Vertebral Bone Marrow; VIBE Dixon; Vertebral Fat Fraction; Relaxation Times.

INTRODUCTION

Vertebral bone marrow is a composite tissue containing adipocytes and hematopoietic cells surrounded by trabecular bone. There are two types of bone marrow: yellow and red marrow. The yellow marrow is mostly composed of adipocytes and chemically composed of ~80% of fat and 15% of water (1). The red marrow is mostly composed of hematopoietic cells and chemically composed of ~40% of water, 40% of fat and 20% of proteins (1). With age, the red marrow tends to be replaced by yellow marrow so that the red bone marrow in adults only remains in parts of the axial skeleton (1).

There is a growing interest in measuring the fat fraction in vertebral bone marrow as it has been shown to be a biomarker of the physiopathological status of this tissue. For instance, the fat fraction in vertebral bone marrow is lower in patients suffering from symptomatic myeloma than in patients suffering from asymptomatic myeloma (2). Moreover, the vertebral fat fraction of patients who received chemotherapy increased after treatment (3). Also of relevance is the assessment of bone marrow T1 and T2* relaxation times. T2* has been shown to be a moderate biomarker for osteoporosis (4) and T1 of the water signal has been shown to significantly differ between pathologic and normal bone marrow (5).

In 1984, Dixon proposed a method based on the chemical shift difference between water and fat signals to estimate the fat fraction in the liver (6). More sophisticated models were then introduced to perform chemical shift encoded imaging. Yu et al. (7) proposed a method to simultaneously measure the fat fraction and T2*. Later, Chebrolu et al. (8) proposed a method to separately measure the T2* of the water and fat signals. Bydder et al. (9) introduced a model where multi-gradient echo

acquisitions are performed at multiple flip angles to measure the fat fraction, T1 and T2* of the water and fat signals.

Chemical shift encoded imaging has recently been validated as a method to quantify vertebral bone marrow fat (2-4, 10-14). More specifically, Baum et al. (13) used it with a T2* correction to measure the fat fraction over the whole spine and Takasu et al. (14) used the IDEAL algorithm (15) to measure the fat fraction and discriminate between patients suffering from symptomatic and asymptomatic myeloma.

Given the importance of fat fraction, T1 and T2* relaxation times as biomarkers of disease, in the current study we sought to assess the feasibility and repeatability of measuring the fat fraction as well as the T1 and T2* relaxation times of the water and fat signals in vertebral bone marrow.

MATERIALS AND METHODS

MR Imaging

All experiments were conducted according to the procedures approved by the local institutional review board. A group of eight healthy volunteers (age range 19-57 years, mean age 35.1 ± 15.7 years, 5 men and 3 women) were enrolled in the current study. One volunteer (a 50-year-old man) was scanned six times to perform a repeatability study. After each examination, the volunteer exited the scanner, the scanner landmark was reset and then the volunteer was repositioned in the scanner for the following measurement.

The lumbar spines of the volunteers were scanned on a 1.5 T MR system (Aera, Siemens Healthcare, Erlangen, Germany) using the spine array receiver coil and a standard body array coil. The imaging protocol included a T1-weighted sequence, a T2-weighted sequence with fat saturation and a chemical shift encoded sequence

(3D gradient echo Volumetric Interpolated Breath-hold Examination, VIBE Dixon) acquired at two different flip angles. All images were acquired in sagittal planes.

Pilot experiments were performed using the VIBE Dixon with two different TRs (TR = 8.21 ms or 11 ms) and four or five echo times (TE1/TE2/TE3/TE4/TE5 = 1.18/2.34/4.4/6.8/9.2 ms). Preliminary data analysis indicated that the best results were obtained with TR = 8.21 ms and four echoes. Thus, four echoes (TE1 to TE4) were employed for the final protocol. Other scanning parameters were: bandwidth 1220 Hz/pixel, matrix 256 x 192, field of view 315 x 420 mm², partial Fourier 75% in both phase encoding directions, parallel imaging with GRAPPA (GeneRalized Autocalibrating Partially Parallel Acquisitions) using an acceleration factor R = 2 in the phase direction and R = 2 in the slice encoding direction resulting in a total acceleration factor of R = 4, 60 slices, a slice thickness of 4 mm and an acquisition time of 16 s (or 20 s for the acquisition at TR = 11 ms). Two sets of images were acquired with flip angles of 5° and 15° resulting in a total of two 16 s (or 20 s) breath-hold acquisitions.

Data Analysis

Regions of interest (ROIs) were drawn with ImageJ (Rasband WS, ImageJ, US National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/) in the vertebral bodies of the five lumbar vertebrae (L1 to L5) using the central slice of the image stack. In all volunteers, 52-pixel-area ROIs were placed by a radiologist (J.L.; 15 years of experience) in the anterior part of vertebral bodies to avoid vascular artefacts. The mean signal intensities and standard deviations were calculated at the four echo times and two flip angles using the same ROIs.

Signal Analysis

Taking into account the presence of multiple peaks in the fat spectrum and a separate T2* for water and fat signals, the signal intensity of a spoiled gradient echo sequence can be written as (16):

$$S(TE_i, TR, \alpha) = S_0 \left[(1-f)\, W + f\, F \sum_{p=1}^{P} A_p e^{i\left(\frac{2\pi\, TE_i}{t_{2\pi,p}}\right)} \right]$$

with

$$W = \frac{[1 - \exp(-TR/T1_w)] \sin\alpha}{1 - \exp(-TR/T1_w)\cos\alpha} e^{-TE_i/T2_w^*}$$

$$F = \frac{[1 - \exp(-TR/T1_f)] \sin\alpha}{1 - \exp(-TR/T1_f)\cos\alpha} e^{-TE_i/T2_f^*}$$

where *S(TE,TR,α)* is the signal intensity, TE_i the echo time of the ith echo, *TR* the repetition time, *α* the flip angle, S_0 a term including the initial magnetization and the receiver gain, *f* the fat fraction, *W* and *F* the water and fat signals, *P* the number of peaks in the fat spectrum, A_p the relative abundance of the pth fat peak, $t_{2\pi,p}$ the difference between the period of the water peak and the pth fat peak, *T1* the longitudinal relaxation time, and *T2** the transverse relaxation time. The subscripts *w* and *f* refer to the water and fat signals, respectively. In the current study, magnitude data acquired at the two flip angles and four echo times were jointly fitted to the following equation:

$$S(TE_i, TR, \alpha) =$$

$$S_0 \sqrt{\left[(1-f)W + fF\sum_{p=1}^{P} A_p \cos\left(\frac{2\pi\, TE_i}{t_{2\pi,p}}\right)\right]^2 + \left[fF\sum_{p=1}^{P} A_p \sin\left(\frac{2\pi\, TE_i}{t_{2\pi,p}}\right)\right]^2}$$

This data fitting provides the fat fraction and the T1 and T2* relaxation times of the water and fat signals in the ROI. The use of magnitude data can create ambiguity between the water and fat signals. This ambiguity was resolved by assuming that the

T1 of the water signal is higher than the T1 of the fat signal. The signal with the highest T1 was then assigned to water (9). The fat spectrum was modelled using the liver fat spectrum characterized by Hamilton et al. (17) and only the three main peaks (0.9, 1.3 and 2-2.2 ppm) were considered. The choice of the liver fat spectrum instead of the bone marrow fat spectrum acquired in the bone marrow of the proximal femur by Karampinos et al. (10) was motivated by the negligible difference between the three main peaks of the two spectra (87% of the total signal in bone marrow fat spectrum compared to 90% in the liver fat spectrum). Furthermore the quantification of fat resonances in bone marrow suffers from the short T2* which results in large resonance peaks. Data analysis was performed with in-house built scripts written in Mathematica (Wolfram Research, Champaign, IL, USA).

Repeatability

The repeatability was assessed by measuring the mean and standard deviation obtained for each parameter on the five lumbar vertebrae of one volunteer.

RESULTS

MR images corresponding to the four echoes of the chemical shift encoded acquisition on one volunteer are shown in Figure 1. The ROIs used to sample the signal in L1 to L5 are also indicated. Figure 2 shows the evolution of the signal in a ROI as a function of time for the sequences acquired at the flip angles equal to 5° and 15°. The fat fraction, T1 and T2* relaxation times were fitted in the lumbar vertebrae of every subject. The mean values and standard deviations averaged over L1 to L5 are presented in Table 1.

Figure 3 shows the fat fraction, T1 and T2* relaxation times of each individual vertebra. There was an increase of the fat fraction from L1 to L5 (around 2% per vertebra) and a decrease of T1 of the water signal from L1 to L5. The T1 of the fat signal and the T2* of the water and fat signals did not show any specific variation over the lumbar vertebrae.

The results of the repeatability study are summarised in Table 2. The repeatability obtained for the fat fraction was high (coefficient of variation (CV) from 3 to 8%) with exception of one vertebra (L4 17%). The coefficients of variation of the T1 and T2* of water ranged from 3% to 17%, with the majority of the values around 10%. For the relaxation times of fat, the coefficients of variation ranged from 8 to 46% with the majority of the values around 20%. Furthermore, the fat fraction measured in the volunteer (50-year-old man) was higher than the mean fat fraction measured within the cohort and the T1 of water was lower. The T1 of fat, T2* of water and T2* of fat were in the range of the parameters measured within the cohort.

DISCUSSION

In the current study we proposed a fast method to measure the fat fraction, T1 and T2* relaxation times of water and fat in bone marrow. The acquisition protocol uses two breath-hold acquisitions, with a total acquisition time of less than 40 s. In previous studies the fat fraction (13; 18-20), the T1 of the water signal and of the fat signal (5, 21) and a common T2* for water and fat signals (4) were measured with different methods in a total scan time of 10 min. For instance, Kugel et al. measured a fat fraction of 36.9±10.7% in L3 by MR spectroscopy in a population of 31-40 years of age (18) and Baum et al. found a mean fat fraction of 36.4±10.2% in L1 to L5 in a population of 26±4 years of age (13). The fat fraction in the vertebral bone marrow

measured in the current study is close to the values found in these previous studies. Moreover, the increase of the fat fraction from L1 to L5 observed in the current study is in agreement with the results of Baum et al. (13) and Martin et al. (19).

In addition to fat fraction quantification, T1 measurements are of interest in bone marrow for investigations of osteoporosis and malignant infiltrations (5). De Bazelaire et al. measured a common T1 of 549±52 ms in bone marrow at 1.5 T using an inversion recovery sequence with multiple inversion times (22). The separate T1 relaxation times of the water and fat signals in vertebral bone marrow were measured at 1.5 T with MR spectroscopy by Träber et al. (21) and with a saturation recovery sequence by Biffar et al. (5) on the normal-appearing bone marrow of patients with benign and malignant lesions. The results obtained in these studies are summarised in Table 3. The T1 relaxation times measured in the current study are slightly shorter than those obtained by Träber et al. and Biffar et al..

Kühn et al. (4) showed that the measure of both the fat fraction and the multi-peak fat corrected T2* relaxation time is of interest for the diagnosis of osteoporosis. They measured a common T2* of 9.3 ms at 3.0 T for the water and fat signals. Our results ($T2^*_w$=13.7 ms and $T2^*_f$=11.4 ms) are consistent with the functional dependence of the T2* on the magnetic field strength, that is, an increase of T2* with decreasing field strength.

The standard deviations measured in the repeatability study were smaller than in the cohort study. These results suggest that the relatively large standard deviations measured in the cohort study were partially due to inter-subject variability. Inter-subject variability of fat fraction and T1 could be explained by the changes of the vertebral fat fraction with age and sex (18, 20) and by the change of the T1 with the fat fraction (23), respectively. It can also be noted that the fat fraction measured in

the repeatability study was higher than the mean fat fraction measured within the cohort. This could be expected as the volunteer scanned for the repeatability study was older than the mean cohort and the fat fraction in vertebrae increases with age (18, 20).

In the current study, the fat fraction, T1 and T2* relaxation times of water and fat signals were measured in healthy volunteers. In previous studies, the fat fraction and relaxation times were measured in patients with various pathologies. For instance, Takasu et al. (2) measured a vertebral fat fraction of 73% in healthy volunteers versus 44% in patients with multiple myeloma. Biffar et al. (5) measured a $T1_w$ = 828 ms in normal appearing vertebrae of patients with osteoporotic lesions and a $T1_w$ = 1252 ms in the pathological lesions of the same patients. Kühn et al. (4) measured a common water-fat T2* of 9.3 ms in the vertebrae of healthy volunteers versus a T2* of 18.2 ms in the vertebrae of patients suffering from osteoporosis. Given the results of our repeatability study and the differences in fat fraction and relaxation times observed in pathological conditions, we can conclude that the method described here could allow for the detection of changes in fat fraction and relaxation times that occur in these pathologies.

In the current study we used a method based on a dual flip angle (DFA) acquisition where the echoes are acquired at two different flip angles. The DFA method was initially proposed by Liu et al. (24) to suppress the T1-weighting of the signal. Wang et al. (25) recently proposed a joint DFA method where data are simulated at two flip angles and jointly fitted to the signal equation to simultaneously determine the fat fraction and the T1 of the water and fat signals using a common T2*. They showed a good agreement between Monte Carlo simulations and Cramer-Rao lower bound analysis. Bydder et al. (9) previously introduced the model used in the current study

where the fat fraction, T1 and T2* of the water and fat signals are jointly determined using a DFA approach. They obtained good results in vitro and a poorer accuracy on the liver probably due to mis-registration problems between the two breath-hold scans (9) and low values of fat fraction. Here we applied this method to lumbar spine imaging which is robust to movement. Images acquired at 5° and 15° were super-imposable without registration. Moreover we used flip angles close to the Ernst angles of the water and fat signals, which are, given our acquisition parameters and the T1 we measured, 8° for the water signal and 13° for the fat signal. By using flip angles close to the Ernst angles, the use of a DFA approach allows for an increase of the SNR compared to the small flip angle approach, where low flip angles are used to reduce the T1-weighting.

Chemical shift encoded imaging in bone marrow is challenging because of the presence of trabecular bone and high fat fractions. Trabecular bone creates local inhomogeneities in the magnetic field which shorten T2* (10). Gee et al. showed that T2* should be taken into account for a proper separation of the water and fat signals in bone marrow (12). In chemical shift encoded imaging, the T2* decay of the water and fat signals is often considered to be identical. This assumption is valid for liver applications where fat fractions are low but it is no longer valid in bone marrow where fat fractions can be up to 50% (8, 26). In other words, given the high fat content of bone marrow the water and fat signals contribute almost equivalently to the total signal. The error on the minority component is thus reduced. Therefore, when applying chemical shift encoded sequences to bone marrow imaging, a dual T2* decay should be modelled for a proper signal analysis. In the current study, in order to measure the T2* of the water and fat signals, the echo times were set for the water and fat signals to be either in phase or opposed phase. The first echo time

was chosen as short as possible to have a better T2* estimate (26) and the other echoes were set to be in the range of the T2* measured. The repetition time was then set as short as possible to reduce the breath-hold duration.

The current study has some limitations. We used magnitude data in our fitting. Bydder et al. (9) stated that there is no reason to prefer complex or amplitude data for fat quantification, whereas Hernando et al. (27) showed that the fitting is more accurate with complex data when the phase signal is reliable. As we aim here at developing an easily implementable method for clinical imaging, we chose to use magnitude images that are readily available on MR clinical scanners. On the other hand, it is well known that the use of magnitude data can create an ambiguity between the water and fat signals. We resolved this ambiguity by assuming that the T1 of the water signal is always superior to the T1 of the fat signal (9). Furthermore, our results could have been affected by flip angle inhomogeneities. This limitation can be circumvented by measuring the flip angles with B1 maps. Other limitations of our study included the small number of volunteers, and no measurements performed on patients.

In conclusion, we have shown that the measurement of the fat fraction, T1 and T2* relaxation times of the water and fat signals in the vertebral bone marrow of healthy volunteers is feasible in less than 40 seconds using two chemical shift encoded sequences. Previous studies showed that the fat fraction and relaxation parameters are modified in specific pathologies. The application of this method to patients with bone marrow disorders could help to investigate the role of fat fraction, T1 and T2* as biomarkers of disease.

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Table 1

Results of the fitting algorithm corresponding to the mean and standard deviation of bone marrow fat fraction, T1 and T2* for the water and fat signals averaged over L1 to L5 for all volunteers (n=8)

	Mean±std
FF	33±8%
$T1_w$	701±151 ms
$T1_f$	334±113 ms
$T2^*_w$	13.7±2.9 ms
$T2^*_f$	11.4±2.7 ms

Table 2

Results of the repeatability study (n=6) performed on one volunteer with the mean values and standard deviations measured on the five lumbar vertebrae.

	L1	L2	L3	L4	L5
FF (%)	33±2	36±1	38±2	41±7	36±3
$T1_w$ (ms)	607±39	574±61	620±74	496±80	538±62
$T1_f$ (ms)	321±27	362±45	407±102	379±86	324±47
$T2^*_w$ (ms)	11.4±0.8	11.7±0.4	10.7±0.7	10.5±1.8	10.4±0.8
$T2^*_f$ (ms)	14.4±4.5	12.2±2.1	9.6±1.9	15.1±7.0	11.2±2.5

Table 3

T1 of the water and fat signals measured in the present study and in the studies of Träber et al. (21) and Biffar et al. (5) at 1.5 T

	Current study	Träber et al.	Biffar et al.	
			Group 1[a]	Group 2[b]
$T1_w$ (ms)	701±151	901±13	872±129	878±177
$T1_f$ (ms)	334±113	266±2	324±81	386±144

[a] Normal-appearing bone marrow of patients with benign osteoporotic fractures

[b] Normal-appearing bone marrow of patients with malignant infiltrations

Figure 1. Sagittal MR images of a 23-year-old woman. Images correspond to the chemical shift encoded acquisitions obtained at the four echo times with a flip angle of 5°. From the left to the right the water and fat signals are dephased / almost opposed phase / in phase / opposed phase. The regions of interest used in the lumbar vertebrae L1 to L5 are displayed in the four images.

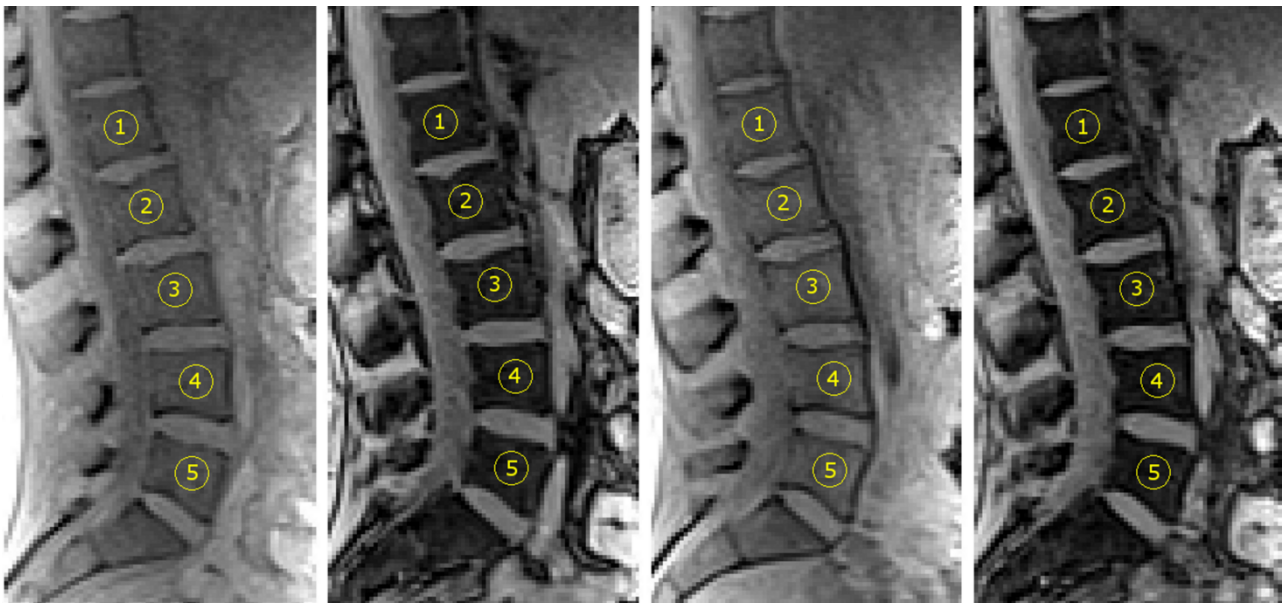

Figure 2. Example of one data set and its corresponding fitting for the acquisitions at 5° and 15°. The points correspond to the mean signal measured in a ROI in the vertebral bone marrow of L3 of one volunteer at 5° (green) and 15° (red). The lines represent the corresponding fitting at 5° (line) and 15° (dashed line).

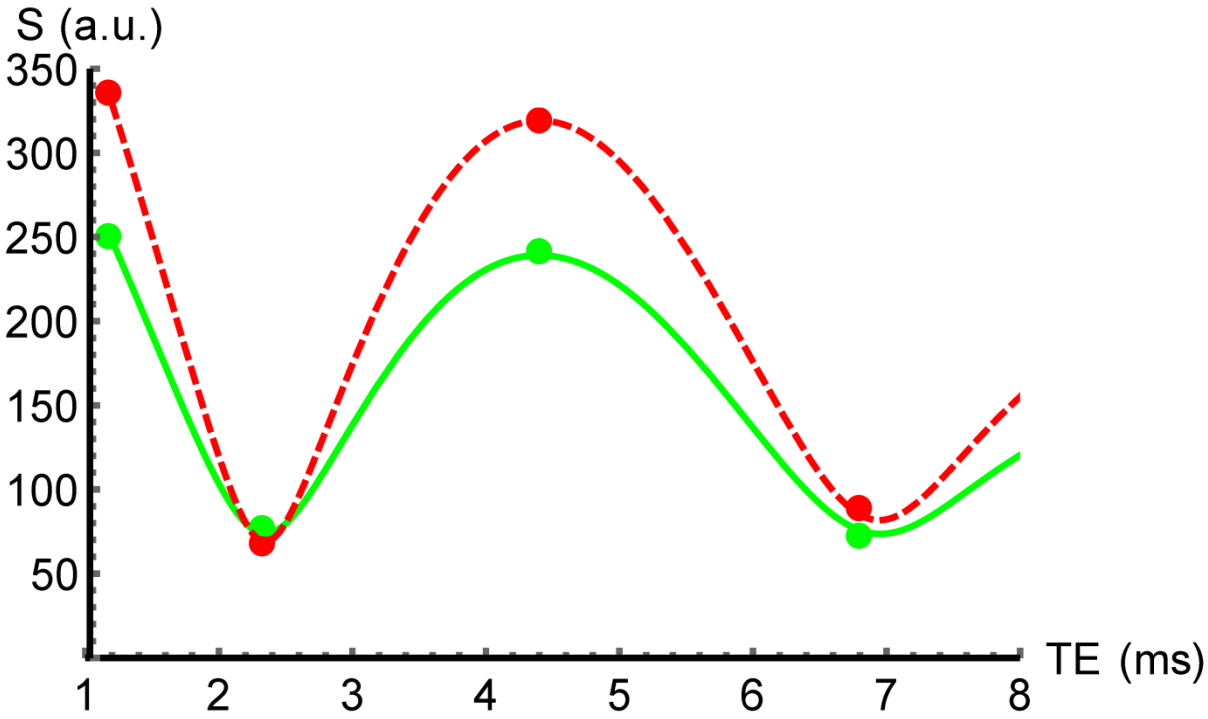

Figure 3. Mean and standard deviation of bone marrow fat fraction, T1 and T2* for the fat and water signals for each vertebrae (L1 to L5).