Received: 2 July 2020 | Revised: 9 October 2020 | Accepted: 14 October 2020

DOI: 10.1002/nbm.4439

RESEARCH ARTICLE

NMR IN BIOMEDICINE WILEY

Physiological variation of the vertebral bone marrow water T2 relaxation time

Stefan Ruschke[1] | Jan Syväri[1] | Michael Dieckmeyer[2] | Daniela Junker[1] | Marcus R. Makowski[1] | Thomas Baum[2] | Dimitrios C. Karampinos[1]

[1]Department of Diagnostic and Interventional Radiology, School of Medicine, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany

[2]Department of Diagnostic and Interventional Neuroradiology, School of Medicine, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany

Correspondence
Stefan Ruschke, Department of Diagnostic and Interventional Radiology, Klinikum rechts der Isar, School of Medicine, Technical University of Munich, Ismaninger Street 22, 81675, Munich, Germany.
Email: stefan.ruschke@tum.de

Funding information
H2020 European Research Council, Grant/Award Number: grant agreement No 677661 (ProFatMRI); Philips Healthcare

The aim of this study was to investigate physiological variations of the water T2 relaxation time in vertebral bone marrow with respect to age, body mass index (BMI), sex and proton density fat fraction (PDFF) based on single-voxel magnetic resonance spectroscopy (MRS) at 3 T. Multi-TE single-voxel STEAM MRS data of a single lumbar vertebra (L4 or L5) from 260 subjects (160/100 female/male, age: 0.7/37.1/77.7 years, BMI: 13.6/26.2/44.5 kg/m^2 [min./median/max.]) with no history of vertebral bone marrow pathologies were retrospectively included. All data were processed using a joint series T2-constrained time domain-based water–fat model. Water T2 and PDFF data were analyzed using (a) Pearson's correlation r and (b) multiple linear regression without interactions of the independent variables. Min./median/max. water T2 and PDFF were 11.2/21.1/42.5 ms and 4.0%/36.8%/82.0%, respectively. Pearson's correlation coefficients were significant ($P < .05$) for water T2 versus age ($r = -0.429/-0.210$ female/male) and for water T2 versus PDFF ($r = -0.580/-0.546$ female/male) for females and males, respectively. Females showed significant higher water T2 values compared with males ($P < .001$). Multiple linear regression for water T2 without interactions revealed a $R^2 = 0.407$ with PDFF ($P < .001$) and sex ($P < .001$) as significant predictors. The current study suggests that under physiological conditions vertebral bone marrow water T2 is negatively correlated with age and PDFF and shows significant differences between females and males. The observed systematic trends are of relevance for the evaluation of T2 values and T2-weighted bone marrow parameters. Further research on the exact mechanisms and drivers of the observed water T2 behavior is required.

KEYWORDS
3 T, age, magnetic resonance spectroscopy, proton density fat fraction, sex, STEAM, vertebral bone marrow, water T2

Abbreviations used: a.u., arbitrary unit; BMI, body mass index; BOLD, blood oxygenation level-dependent; CI, confidence interval; PDFF, proton density fat fraction; PDWF, proton density water fraction; PRESS, point-resolved spectroscopy; STEAM, stimulated echo acquisition mode; SVD, singular-value decomposition.

[Correction added on 5 December 2020, after first online publication: The spelling of the fifth named author's surname was corrected.]

1 | INTRODUCTION

The bone marrow residing within the trabecular bone matrix is traditionally subclassified as red and yellow bone marrow. Red bone marrow is rich in hematopoietic cells and important for hematopoiesis and the lymphatic system, whereas yellow bone marrow consists mainly of adipocytes, which play an active role in the endocrine system.[1] Vertebral bone marrow is considered one of the most typical red bone marrow regions and a target tissue for studying bone marrow alternations in health and disease. Specifically, assessment of the quantitative magnetic resonance parameters of the vertebral bone marrow environment[2] utilizing a chemical shift-based differentiation between a water and fat component has been shown to be of relevance in many pathophysiological processes, including metabolic disorders and hematopoietic disorders.[3] For example, bone marrow fat content variations, characterized by the bone marrow fat fraction, have been demonstrated to correlate with obesity,[4] type 2 diabetes mellitus,[5] osteoporosis[6] and multiple myeloma.[7]

The characterization of the vertebral bone marrow environment using proton magnetic resonance enables the spectral analysis of the tissue components based on their chemical shift difference. Spectral analysis based on chemical shift differences allows differentiation between signals arising from water molecules and fat molecules. Furthermore, the spectral water component is mainly associated with the hematopoietic bone marrow cells while the spectral fat component is considered as mainly associated with the bone marrow adipocytes.

Over recent decades, primarily the spectral fat component has been investigated using magnetic resonance spectroscopy (MRS),[8] including the characterization of the proton density fat fraction (PDFF)[9] and triglyceride unsaturation.[10] However, very little is known about systematic trends of the water component in the vertebral bone marrow spectrum, except for some observed correlation of T2 with age.[9,11]

Since vertebral bone marrow is highly active in hematopoiesis, differences in the hematopoietic processes between females and males (e.g., due to hormone levels[12]) would also suggest systematic effects in the spectral water component. Furthermore, the tissue's iron content is well known to affect the transverse relaxation time T2 and is physiologically mainly driven by the presence of hemoglobin and myoglobin-producing cells as well as ferritin in bone marrow.[13] Bone marrow T2 has therefore been assessed after blood transfusions for iron deposition.[14–16] However, fat suppression is required for quantitatively tracking bone marrow iron overload and to measure water T2.[17] Consequently, single-voxel MRS is a well-suited method to extract water T2 values without a bias from the fat component.

Therefore, the purpose of the current study is to provide normative data on physiological variations of vertebral bone marrow water T2 with respect to age, body mass index (BMI), sex and PDFF. Specifically, a retrospective pooled analysis of single-voxel MRS data of the fourth/fifth lumbar vertebral body (L4/L5) was performed to investigate potential correlations between age, sex, BMI and the MRS-derived parameters, water T2 and PDFF.

2 | METHODS

2.1 | Study design and subjects

For the current exploratory study, primary data were pooled from previous studies[6,9,18-23] and retrospectively reanalyzed in a single batch.

Exclusion criteria were a history of vertebral bone marrow pathologies, vertebral fractures, spine surgery and medication affecting bone metabolism (eg, bisphosphonates).

All the included studies had been approved by their respective institutional review boards in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the studies.

2.2 | MRS acquisition

All data were acquired using a single-voxel stimulated echo acquisition mode (STEAM) spectroscopy sequence with the following parameters: TR = 6 seconds (to minimize any T_1-weighting effects); TE = 11/15/20/25 ms; TM = 16 ms (set to the shortest TM to minimize T_1-weighting effects and *J*-coupling effects[24]); eight repetitions with four phase cycles; 4096 sampling points; a spectral acquisition bandwidth of 5 kHz; no water suppression; and no regional saturation bands. Data from subjects aged under 18 years deviated with respect to the following acquisition parameters to keep the scan time to a minimum: TR = 5 seconds; TE = 12/16/20/24 ms; TM = 18 ms; four repetitions with four phase cycles; and a spectral acquisition bandwidth of 3 kHz.

The MRS measurement was performed in the L5 vertebral body based on a localizing scan. The L4 vertebral body was measured in case the L5 was subject to degenerative changes. The default MRS voxel size of 15 × 15 × 15 mm was adjusted if necessary to fit inside the vertebral bone marrow region. Voxel localization-induced chemical shift displacements were acknowledged during the voxel placement by leaving a margin between the voxel and the surfaces of the inner cortex and the surfaces of the endplates.

All measurements were performed on 3 T scanners (Ingenia, Philips Healthcare, the Netherlands) at the Klinikum rechts der Isar (Munich, Germany) and at Phoenix Children's Hospital (Phoenix, AZ, USA) using the built-in, 12-channel posterior table coil array for signal reception. In some studies the 16-channel anterior coil array was additionally used.

2.3 | MRS data processing and quantification

In-house developed routines written in MATLAB 2018b (MathWorks Inc., Natick, MA, USA) were used for the data processing and subsequent signal quantification. The data processing consisted of the following main steps: SVD-based coil combination,[25] simple signal averaging, zero-order phase correction, frequency offset correction and frequency referencing based on the methylene signal. The signal quantification was performed in the time domain using a joint series T2-constrained water–fat model based on MATLAB's Levenberg–Marquardt optimization algorithm using the following signal model equation:

$$S(t,TE) = \sum_i \rho_i e^{i\phi_i} e^{(j2\pi\omega_i - d_i)t} e^{-\frac{TE}{T_{2,i}}}$$

where ρ_i is the proton density, φ_i is an initial phase term, d_i is the Lorentzian damping factor, $T_{2,i}$ is the transverse relaxation time and ω_i is the precession frequency of the ith frequency component, respectively; t is the readout time and TE is the echo time. The quantification ruleset included the following degrees of freedom: 11 x ρ_i (1 x water peak and 10 x fat peaks), 3 x d_i (1 x water peak, 1 x methylene peak, 1 x all other fat peaks), ω_i (1 x water [4.67 ppm], 1 x fat peaks [0.90, 1.30, 1.59, 2.04, 2.25, 2.78, 4.10, 4.30, 5.19 and 5.30 ppm]), 1 x φ_i (common phase for all components) and 2 x $T_{2,i}$ (1 x water peak, 1 x all fat peaks). The parameter ranges were limited in the follow way: ρ_i, $d_i \in \mathbb{R}^+$, $\omega_i \in$ {$\mathbb{R}$ |]- 0.2 ppm; 0.2 ppm [}, $\varphi_i \in$ {$\mathbb{R}$ |]-0.2 rad; 0.2 rad[}, $T_{2,i} \in$ {$\mathbb{R}$|]0; 1 s[}. The PDFF was determined as the ratio of the sum of the proton density of all fat peaks over the sum of the proton density of the water and all fat peaks.

2.4 | Statistical analysis

All tests were performed using a two-sided 0.05 level of significance.

Statistical analysis was performed in Python v.3.6.10 using the scipy package v.1.4.1. Wilcoxon rank-sum tests were used to analyze differences of the obtained not-normally distributed parameters between females and males. Pearson's correlation coefficients (r) were computed to investigate the correlation between the obtained parameters.

The statsmodels package v.0.11.0 was used for the multiple linear regression analysis. PDFF was entered as a dependent variable and age, BMI and sex were entered as independent variables without interactions between independent variables. In an analogous manner, water T2 was entered as a dependent variable and age, BMI, PDFF and sex were entered as independent variables. Independent variables were dropped in a backward elimination process. Parameters were kept in the regression models if P was smaller than the chosen level of significance ($P < .05$), and the coefficient of determination (R^2) was calculated.

3 | RESULTS

3.1 | Cohort characteristics

The final data pool included MRS datasets from 260 subjects of an initial 269 datasets. The data displayed a large range in age and BMI (Table **1**). In total, nine datasets were discarded due to low spectral quality or corrupted measurements. Wilcoxon rank sum tests were not significant for the distributions of age (P = .328) and BMI (P = .602) between females (n = 160) and males (n = 100), respectively (see Table **1** for details).

3.2 | MRS acquisition, data processing and quantification

In Figure 1, two example spectra are shown, including the fitted quantification model for A, a 27-year-old female, and B, a 74-year-old male with representative water T2 and PDFF values. Overall, a tendency of increasing PDFF with age and decreasing water T2 with age and PDFF was observed (see also Figure S1). The included 260 MRS datasets showed the following parameter distribution characteristics (min./median/max.):

TABLE 1 Cohort characteristics of the pooled data. *P*-values (differences between female and male groups) were calculated using the Wilcoxon rank-sum test

	All subjects (*n* = 260) Min./median/ max. (number of missing values)	Female (*n* = 160) Min./median/max. (number of missing values)	Male (*n* = 100) Min./median/max. (number of missing values)	*P*-value
Age (years)	0.7/37.1/77.7 (0)	0.7/40.5/77.7 (0)	1.6/32.9/74.4 (0)	.328
BMI (kg/m^2)	13.6/26.2/44.5 (14)	13.6/26.3/44.5 (11)	13.8/26.1/38.6 (3)	.602
Weight (kg)	8.0 /77.0/159.0 (2)	8.0/73.9/159.0 (2)	12.1/84.8/123.7 (0)	.008
Height (cm)	68.2/ 168.5/195.5 (13)	68.2/ 164.3/195.5 (10)	92.0 / 178.0/192.0 (3)	<.001
Water T2 (ms)	11.2/21.1/42.5 (0)	14.0/23.1/42.5 (0)	11.2/18.4/36.9 (0)	<.001
PDFF (%)	4.0/36.8/82.0 (0)	7.0/34.8/69.1 (0)	4.0/37.9/82.0 (0)	.104

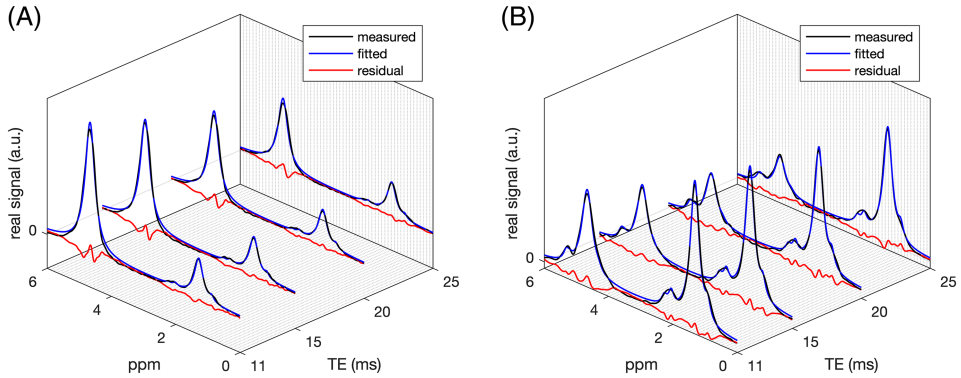

FIGURE 1 Exemplary multi-TE STEAM series fitting for A, a 27-year-old female (BMI: 22.6 kg/m^2, water T2: 22.6 ms, PDFF: 18%), and B, a 74-year-old male (BMI: 25.1 kg/m^2, water T2: 16.4 ms, PDFF: 51%). The water peak at around 4.67 ppm is more pronounced in spectra with lower PDFF, as can be seen in A in comparison with B. a.u., arbitrary unit

11.2/21.1/42.5 ms water T2; 4.0/36.8/82.0% PDFF (see also the diagonal in Figure 2 displaying the underlying distribution estimated using univariate kernel density estimations for water T2 and PDFF for the female and male groups, respectively).

3.3 | PDFF

The Wilcoxon rank-sum test (Table **1**) indicated that PDFF was not statistically significantly different (*P* = .104) between females and males. Pearson's correlation (see Figure 2, off-diagonal) yielded positive correlations for PDFF versus age for females (*r* = 0.716) and males (*r* = 0.581), respectively.

The multiple linear regression analysis for the dependent variable PDFF yielded R^2 = 0.456 against the independent variables age (*P* < .001) and sex (*P* = .002) (Table 2).

3.4 | Water T2

Unlike PDFF, the Wilcoxon rank-sum test (Table **1**) indicated water T2 was statistically significant different between females and males (*P* < .001). Using Pearson's correlation (see Figure 2, off-diagonal), negative correlations were observed for water T2 versus age (*r* = −0.429/−0.210 females/males) as well as for water T2 versus PDFF (*r* = −0.580/−0.546 females/males) for the female group and male group, respectively.

The multiple linear regression analysis for the dependent variable water T2 yielded R^2 = 0.407 against the independent variables PDFF (*P* < .001) and sex (*P* < .001) (Table 2).

4 | DISCUSSION

The current study investigated the physiological variations of water T2 in vertebral bone marrow with respect to age, BMI, sex and PDFF. The current findings suggest that vertebral bone marrow water T2 is physiologically negatively correlated with age and PDFF and showed significant differences between females and males.

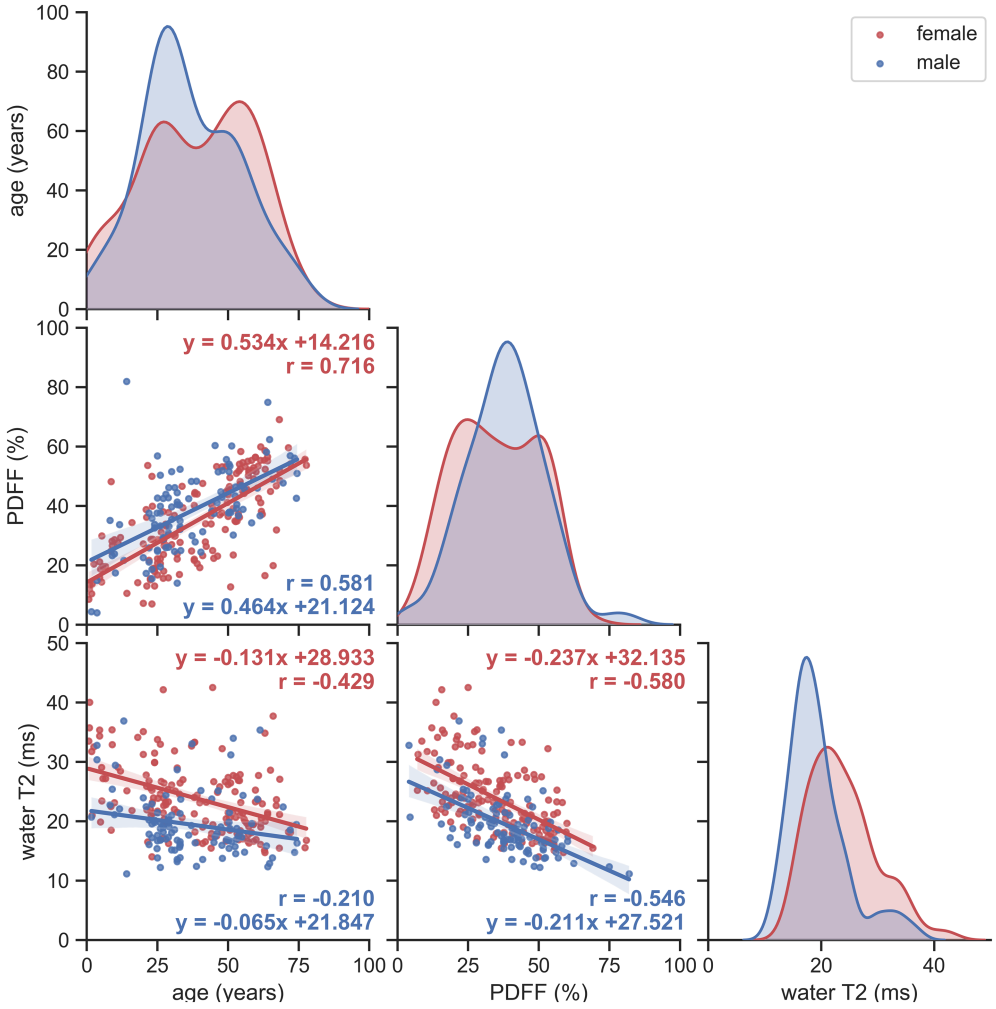

FIGURE 2 Pairs plot and Pearson's correlations for the parameters age, PDFF and water T2 grouped by female (red) and male (blue) subjects, respectively. The diagonal displays univariate kernel density estimations of the sample distribution for the parameters age, PDFF and water T2 (from upper left to lower right) for female and male subjects, respectively. On the lower off-diagonal, scatter plots and linear regression analysis for PDFF and water T2 versus age, respectively, and water T2 versus PDFF, are presented. PDFF, proton density fat fraction

TABLE 2 Results from the multiple-linear regression analysis for water T2 and PDFF. R^2 and adjusted R^2 are given for each analysis together with the coefficient, *P*-value and 95% confidence interval (CI) ranges of the coefficient for each independent variable, respectively. The coefficient indicates the effect strength of the respective parameter. Sex was encoded as binary dummy variable (0 = female, 1 = male)

	R^2/adjusted R^2: 0.407/0.402			
			coefficient CI	
Dependent variable: water T2 (ms)	**coefficient**	***P*** **> |t|**	**[0.025**	**0.975]**
intercept	31.8159	<.001	30.221	33.411
PDFF (%)	−0.2277	<.001	−0.268	−0.187
sex (male)	−3.6535	<.001	−4.838	−2.469
	R^2/adjusted R^2: 0.456/0.451			
			coefficient CI	
Dependent variable: PDFF (%)	**coefficient**	***P*** **> |t|**	**[0.025**	**0.975]**
intercept	15.0979	<.001	11.930	18.266
age (years)	0.5116	<.001	0.442	0.581
sex (male)	4.2674	.002	1.614	6.921

The measured vertebral bone marrow PDFF showed the known correlation with age and differences between females and males.[19,20] The Pearson's correlation for the dependent variable PDFF revealed the known stronger positive correlation with age for the female (r = 0.716) group compared with the male (r = 0.581) group. The multiple linear regression analysis yielded a model fit of R^2 = 0.456. To summarize the analysis for the dependent variable PDFF, the regression analyses suggested that PDFF variations could be explained by age and sex.

The measured water T2 showed a physiological variation with age, sex and PDFF. The Pearson's correlation for the dependent variable water T2 showed a negative correlation with both age and PDFF for both the female and the male group, respectively. Interestingly, female subjects

showed a higher water T2 value for the same age and PDFF. Furthermore, water T2 in the female group appeared to be higher in younger subjects but then decreased strongly with age (compared with the male group).

The multiple linear regression analysis for the dependent variable water T2 against the independent variables sex and PDFF (all $P < .001$) is in agreement with the Pearson's correlation. Adding age as an additional independent variable did not improve the model fit (data not shown), which is likely due to the already present correlation of age with the independent variable PDFF.

The multiple linear regression analysis for the dependent variable water T2 yielded an R^2 of 0.407. To summarize the analysis for the dependent variable water T2, the regression analyses indicated a correlation with PDFF and also showed a negative effect for the male sex.

4.1 | Previous work

4.1.1 | PDFF

The vertebral bone marrow fat fraction is a well-studied topic, although many previous studies assessed T2-weighted fat fraction parameters[26,27] and not quantitative PDFF values. Many studies investigating normative fat fraction values at the spine assessed PDFF values with chemical shift encoding-based multi-echo gradient-echo imaging and used single-voxel MRS for the purpose of method validation.[20,28] Overall, the observed PDFF in vertebral bone marrow with their pronounced variation with age and sex are in good agreement with reported absolute values and trends in the literature.[2,19,20,28]

4.1.2 | Water T2

Voxel-selective spectroscopic methods were previously proposed[29] for the probing of the vertebral bone marrow environment due to their capability to differentiate water and fat signals based on their chemical shift difference at a given region of interest. The first bone marrow water T2 measurements—although in the iliac bone marrow—using STEAM at 1.5 T were reported by Jensen et al[30] to range between 25 and 46 ms and 34 and 55 ms in normal controls and polycythaemia vera patients, respectively. Schick et al[31] reported the first quantitative water T2 relaxation measurements in vertebral bone marrow using PRESS at 1.5 T as ranging between 32 and 65 ms in 14 healthy volunteers. Other studies using PRESS at 1.5 T followed, reporting mean water T2 values of 39.7[32] and 46.9 ms.[26]

The currently obtained water T2 values are in good agreement with previously reported values in the literature. At 3 T, Barber et al[33] reported a mean ± SD water T2 of 23.6 ± 5.3 ms in the L3 vertebrae of 19 volunteers using PRESS. Also at 3 T but using STEAM, in a cohort of 86 subjects (that is a subset of the current data), Dieckmeyer et al[9] reported mean ± SD water T2 of 22.5 ± 5.1 (range: 14.5-35.0) ms.

Neumayer et al[11] investigated the reproducibility of relaxation measurements in the L2 and L3 vertebrae using STEAM at 3 T in 46 healthy volunteers. Neumayer et al also obtained a smaller mean water T2 in male subjects compared with female subjects (21 vs. 25 ms) and, similar to the current study, also reported a negative correlation of water T2 with age ($r = -0.54$, $P < .001$) for all subjects.

While Kugel et al[26] found that water T2 values were independent of the subject's age and sex, Dieckmeyer et al[9] reported the correlation of water T2 with PDFF in women, and later Neumayer et al[11] showed a correlation of water T2 with PDFF in men.

4.2 | Interpretation

The main observations of the current study with respect to the physiological variation of water T2 values are the difference between female and male subjects and the correlation with PDFF. The observed difference between female and male subjects can potentially be explained by differences in the hematopoietic processes[12] and resulting differences in the composition of the hematopoietic components, especially with respect to iron-containing components; for example, on average, male blood samples exhibit higher serum iron levels[34] and a higher hematocrit.[35] Furthermore, ferritin levels are expected to be similar in males and postmenopausal females, but may be reduced in premenopausal females.[36]

The effect of tissue iron content on T2 is well known and has been described in the context of iron quantification in multiple organs, including bone marrow.[14-16,37] However, previous studies investigating T2 relaxation values with respect to bone marrow iron concentration did not consider general differences in blood parameters, including the hematocrit. Furthermore, many previously applied imaging-based methods for the study of bone marrow T2 may be biased due to the presence of the fat component, which usually cannot be fully suppressed.

The influence of whole blood composition on T2 has also been investigated independently from the bone marrow perspective. Bryant et al[38] investigated ex vivo relaxation parameters of blood samples and found a linear correlation for T2 versus hematocrit of ~ -5.5 ms/% hematocrit at 1.4 T and 24°C (value was visually estimated from Figure 1[38]). Furthermore, relaxation and susceptibility effects in blood were also intensely studied in the context of blood oxygenation level-dependent (BOLD) imaging, where the blood oxygenation level[39,40] and the hematocrit[41,42] are

considered to be central contributors to T2 relaxation effects. For example, Qin et al[43] reported mean T2 values in venous blood at 3 T of 63.4 ± 7.0 and 61.4 ± 5.3 ms in female and male subjects, respectively. Thus, the mean difference in T2 of venous blood between female and male subjects of 2 ms as previously reported is smaller but of the same order as the current difference of 3.6 ms in vertebral bone marrow water T2 values between the two sexes (see sex coefficient, multiple-linear regression analysis for water T2 in Table 2).

The increased water T2 relaxation time in subjects with lower PDFF has previously been attributed to potential differences in the populations of the extracellular and intracellular water pools within the red marrow hematopoietic component.[44] Specifically, Schick et al[44] concluded that increased water T2 relaxation time in subjects with a lower PDFF could be related to the long T2 of intracellular water in hematopoietic cells.

A potential explanation of why vertebral bone marrow water T2 is better correlating with PDFF than with age could be that the bone marrow water–fat composition—as characterized by PDFF—is better correlating with T2 relaxation modulating effects. Furthermore, the physiological variation of PDFF may already comprise age effects since PDFF is already correlating with age. Bone marrow PDFF measurements were shown to correlate well with histology-assessed marrow fat fractions[45] and adipocyte density and volume.[46] In addition, MacEwan et al[47] reported that the proton density water fraction (PDWF = 1 – PDFF) correlated well with histology-assessed bone marrow cellularity. In an earlier study, Ballon et al[48] established that the water fraction—although not corrected for T2 weighting (STEAM at 1.5 T, TE = 30 ms)—correlated well with cellularity measurements from bone marrow biopsies, and also in the presence of pathologies including leukemia, melanoma, neutropenia and aplastic anemia.

4.3 | Implications

The established correlations of vertebral bone marrow water T2 with age and sex have some direct implications for the investigation of T2 relaxation variations. The reported underlying physiological variations have to be considered in the interpretation of bone marrow water T2 values, especially in the assessment of bone marrow cellular composition changes in patients with hematological diseases[44] and in the assessment of bone marrow iron deposition in subjects undergoing repetitive blood transfusions.[14]

The relaxation properties of vertebral bone marrow are also of importance in the measurement of other quantitative parameters using imaging-based MR techniques as well as volume-selective MRS techniques. In volume-selective MRS, during the process of volume selection the magnetization usually experiences relaxation weighting, of which T2 weighting usually has a strong nonneglectable effect. As a consequence, these weighting effects have to be corrected for to obtain quantitative parameters such as the PDFF, otherwise the measured values are biased by—in the case of vertebral bone marrow—age, sex and PDFF-dependent effects.

The importance of relaxation weighting effects for the assessment of PDFF values in vertebral bone marrow is becoming increasingly observed in the literature. While Kugel et al[26] found the water T2 to be independent of the subject's age and sex, Dieckmeyer et al[9] reported the correlation of water T2 in women, and later Neumayer et al[11] confirmed the findings of Dieckmeyer et al, as well as being able to show a similar trend in men.

4.4 | Limitations

The current study has some limitations. First, the data were retrospectively pooled from previous studies. We acknowledge that a prospective cohort study would have been more representative. However, the overall cohort characteristics are sufficient for the purpose of the current study.

Second, the current study provides no method of validation for the measured T2 values. However, multi-TE STEAM MRS is widely considered to be the gold standard for the in vivo measurement of T2 relaxation properties and therefore potential measurement errors are expected to be small.

Third, the offered hypothesis for the found relationship between sex and water T2 is not supported by validation measurements (eg, by blood sample testing). The included datasets did not provide sufficient further clinical information, including blood tests. Furthermore, due to the retrospective design of the analysis, we were neither able to obtain any additional information nor perform additional testing.

Fourth, although neglectable, the reported PDFF values are subject to slight T1-weighting (during the mixing time) and to the assumption of a common T2 relaxation time for all fat peaks except the methylene peak. Furthermore, the fat signal was not corrected for *J*-modulations.

Fifth, the current study assumed only a single water T2 relaxation component and did not investigate any bi-compartment modeling or more advanced relaxation models. However, advanced modeling of higher order effects was not feasible with the limited number of echoes available and would have required more information in the echo time dimension.

5 | CONCLUSION

The current data suggest vertebral bone marrow water T2 to be physiologically decreasing with age and PDFF, and to be lower in males. Further research on the exact mechanisms and drivers of the observed relationships is required. The observed dependence of bone marrow water T2 on

PDFF and age has to be considered in the interpretation of vertebral bone marrow composition parameters that are affected by T2 and in the application of bone marrow water T2 for the characterization of bone marrow iron deposition.

ACKNOWLEDGEMENTS

The authors would like to thank Houchun H. Hu (Nationwide Children's Hospital, Columbus, OH, USA) and Jeffrey H. Miller (Phoenix Children's Hospital, Phoenix, AZ, USA) for data courtesy, respectively, and Birgit Waschulzik (Institut für Medizinische Informatik, Statistik und Epidemiologie, Klinikum rechts der Isar der TU München, Munich, Germany) for statistical consultation.

The current work was supported by the European Research Council (grant agreement no. 677661, ProFatMRI). This work only reflects the authors' view and the EU is not responsible for any use that may be made of the information it contains. The authors acknowledge research support from Philips Healthcare. Open access funding enabled and organized by Projekt DEAL.

ORCID

Stefan Ruschke https://orcid.org/0000-0001-9658-6541

REFERENCES

1. Veldhuis-Vlug AG, Rosen CJ. Clinical implications of bone marrow adiposity. *J Intern Med*. 2017;283(2):121-139.
2. Karampinos DC, Ruschke S, Dieckmeyer M, et al. Quantitative MRI and spectroscopy of bone marrow. *J Magn Reson Imaging*. 2018;47(2):332-353.
3. Cordes C, Baum T, Dieckmeyer M, et al. MR-based assessment of bone marrow fat in osteoporosis, diabetes, and obesity. *Front Endocrinol*. 2016;7(6):74.
4. Bredella MA, Gill CM, Gerweck AV, et al. Ectopic and serum lipid levels are positively associated with bone marrow fat in obesity. *Radiology*. 2013;269(2):534-541.
5. Baum T, Yap SP, Karampinos DC, et al. Does vertebral bone marrow fat content correlate with abdominal adipose tissue, lumbar spine bone mineral density, and blood biomarkers in women with type 2 diabetes mellitus? *J Magn Reson Imaging*. 2012;35(1):117-124.
6. Dieckmeyer M, Ruschke S, Rohrmeier A, et al. Vertebral bone marrow fat fraction changes in postmenopausal women with breast cancer receiving combined aromatase inhibitor and bisphosphonate therapy. *BMC Musculoskelet Disord*. 2019;20(1):1-7.
7. Oriol A, Valverde D, Capellades J, Cabañas ME, Ribera J-M, Arús C. In vivo quantification of response to treatment in patients with multiple myeloma by 1H magnetic resonance spectroscopy of bone marrow. *Magn Reson Mater Phy*. 2007;20(2):93-101.
8. Hu HH, Kan HE. Quantitative proton MR techniques for measuring fat. *NMR Biomed*. 2013;26(12):1609-1629.
9. Dieckmeyer M, Ruschke S, Cordes C, et al. The need for T_2correction on MRS-based vertebral bone marrow fat quantification: implications for bone marrow fat fraction age dependence. *NMR Biomed*. 2015;28(4):432-439.
10. 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.
11. Neumayer B, Widek T, Stollberger R, Scheurer E. Reproducibility of relaxometry of human lumbar vertebrae at 3 Tesla using 1H MR spectroscopy. *J Magn Reson Imaging*. 2018;48(1):153-159.
12. 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(1):7808.
13. Harrison PM, Arosio P. The ferritins: molecular properties, iron storage function and cellular regulation. *Biochim Biophys Acta Bioenerg*. 1996;1275(3):161-203.
14. Papakonstantinou O, Alexopoulou E, Economopoulos N, et al. Assessment of iron distribution between liver, spleen, pancreas, bone marrow, and myocardium by means of R2 relaxometry with MRI in patients with β-thalassemia major. *J Magn Reson Imaging*. 2009;29(4):853-859.
15. Elias EJ, Liao JH, Jara H, et al. Quantitative MRI analysis of craniofacial bone marrow in patients with sickle cell disease. *Am J Neuroradiol*. 2013;34(3):622-627.
16. Sirignano M, Dillman JR, Weiss BD, et al. Change in liver, spleen and bone marrow magnetic resonance imaging signal intensity over time in children with solid abdominal tumors. *Pediatr Radiol*. 2018;48(3):325-332.
17. Papakonstantinou O, Foufa K, Benekos O, et al. Use of fat suppression in R2 relaxometry with MRI for the quantification of tissue iron overload in beta-thalassemic patients. *Magn Reson Imaging*. 2012;30(7):926-933.
18. Franz D, Weidlich D, Freitag F, et al. Association of proton density fat fraction in adipose tissue with imaging-based and anthropometric obesity markers in adults. *Int J Obes*. 2017;42(2):175-182.
19. Baum T, Rohrmeier A, Syväri J, et al. Anatomical variation of age-related changes in vertebral bone marrow composition using chemical shift encoding-based water-fat magnetic resonance imaging. *Front Endocrinol*. 2018;9:141.
20. Ruschke S, Pokorney A, Baum T, et al. Measurement of vertebral bone marrow proton density fat fraction in children using quantitative water-fat MRI. *Magn Reson Mater Phys Bio Med*. 2017;30(5):449-460.
21. Baum T, Inhuber S, Dieckmeyer M, et al. Association of quadriceps muscle fat with isometric strength measurements in healthy males using chemical shift encoding-based water-fat magnetic resonance imaging. *J Comput Assist Tomogr*. 2016;40(3):447-451.
22. Schlaeger S, Inhuber S, Rohrmeier A, et al. Association of paraspinal muscle water–fat MRI-based measurements with isometric strength measurements. *Eur Radiol*. 2018;60:1-10.
23. Cordes C, Dieckmeyer M, Ott B, et al. MR-detected changes in liver fat, abdominal fat, and vertebral bone marrow fat after a four-week calorie restriction in obese women. *J Magn Reson Imaging*. 2015;42(5):1272-1280.
24. 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.
25. Bydder M, Hamilton G, Yokoo T, Sirlin CB. Optimal phased-array combination for spectroscopy. *Magn Reson Imaging*. 2008;26(6):847-850.

26. Kugel H, Jung C, Schulte O, Heindel W. Age- and sex-specific differences in the 1H-spectrum of vertebral bone marrow. *J Magn Reson Imaging*. 2001; 13(2):263-268.
27. Griffith JF, Yeung DKW, Ma HT, Leung JCS, Kwok TCY, Leung PC. Bone marrow fat content in the elderly: A reversal of sex difference seen in younger subjects. *J Magn Reson Imaging*. 2012;36(1):225-230.
28. Baum T, Yap SP, Dieckmeyer M, et al. Assessment of whole spine vertebral bone marrow fat using chemical shift-encoding based water-fat MRI. *J Magn Reson Imaging*. 2015;42(4):1018-1023.
29. Schick F, Bongers H, Jung W-I, Skalej M, Lutz O, Claussen CD. Volume-selective proton MRS in vertebral bodies. *Magn Reson Med*. 1992;26(2): 207-217.
30. Jensen KE, Jensen M, Grundtvig P, Thomsen C, Karle H, Henriksen O. Localized in vivo proton spectroscopy of the bone marrow in patients with leukemia. *Magn Reson Imaging*. 1990;8(6):779-789.
31. Schick F, Bongers H, Jung WI, et al. Proton relaxation times in human red bone marrow by volume selective magnetic resonance spectroscopy. *Appl Magn Reson*. 1992;3(6):947-963.
32. Träber F, Block W, Layer G, et al. Determination of H relaxation times of water in human bone marrow by fat-suppressed turbo spin echo in comparison to MR spectroscopic methods. *J Magn Reson Imaging*. 1996;6(3):541-548.
33. Barber QM, Yahya A. Aspects of spinal bone marrow fat to water quantification with magnetic resonance spectroscopy at 3 T. *Biomed Phys Eng Express*. 2015;1(4):047001.
34. Ritchie RF, Palomaki GE, Neveux LM, Navolotskaia O, Ledue TB, Craig WY. Reference distributions for serum iron and transferrin saturation: a practical, simple, and clinically relevant approach in a large cohort. *J Clin Lab Anal*. 2002;16(5):237-245.
35. Adeli K, Raizman JE, Chen Y, et al. Complex biological profile of hematologic markers across pediatric, adult, and geriatric ages: establishment of robust pediatric and adult reference intervals on the basis of the Canadian health measures survey. *Clin Chem*. 2015;61(8):1075-1086.
36. Van den Bosch G, Van den Bossche J, Wagner C, De Schouwer P, Van De Vyvere M, Neels H. Determination of iron metabolism-related reference values in a healthy adult population. *Clin Chem*. 2001;47(8):1465-1467.
37. Isokawa M, Kimura F, Matsuki T, et al. Evaluation of bone marrow iron by magnetic resonance imaging. *Ann Hematol*. 1997;74(6):269-274.
38. Bryant RG, Marill K, Blackmore C, Francis C. Magnetic relaxation in blood and blood clots. *Magn Reson Med*. 1990;13(1):133-144.
39. Oja JME, Gillen JS, Kauppinen RA, Kraut M, van Zijl PCM. Determination of oxygen extraction ratios by magnetic resonance imaging. *J Cereb Blood Flow Metab*. 1999;19(12):1289-1295.
40. Golay X, Silvennoinen MJ, Zhou J, et al. Measurement of tissue oxygen extraction ratios from venous blood T2: Increased precision and validation of principle. *Magn Reson Med*. 2001;46(2):282-291.
41. Silvennoinen MJ, Clingman CS, Golay X, Kauppinen RA, van Zijl PCM. Comparison of the dependence of blood R2 and R on oxygen saturation at 1.5 and 4.7 Tesla. *Magn Reson Med*. 2003;49(1):47-60.
42. Chen JJ, Pike GB. Human whole blood T2 relaxometry at 3 Tesla. *Magn Reson Med*. 2009;61(2):249-254.
43. Qin Q, Grgac K, van Zijl PCM. Determination of whole-brain oxygen extraction fractions by fast measurement of blood T2 in the jugular vein. *Magn Reson Med*. 2011;65(2):471-479.
44. Schick F, Einsele H, Weiß B, Jung W-I, Lutz O, Claussen CD. Characterization of bone marrow after transplantation by means of magnetic resonance. *Ann Hematol*. 1995;70(1):3-13.
45. Arentsen L, Yagi M, Takahashi Y, et al. Validation of marrow fat assessment using noninvasive imaging with histologic examination of human bone samples. *Bone*. 2015;72:118-122.
46. Cohen A, Shen W, Dempster DW, et al. Marrow adiposity assessed on transiliac crest biopsy samples correlates with noninvasive measurement of marrow adiposity by proton magnetic resonance spectroscopy ((1)H-MRS) at the spine but not the femur. *Osteoporos Int*. 2015;26(10):2471-2478.
47. MacEwan IJ, Glembotski NE, D'Lima D, et al. Proton density water fraction as a biomarker of bone marrow cellularity: validation in ex vivo spine specimens. *Magn Reson Imaging*. 2014;32(9):1097-1101.
48. Ballon D, Jakubowski A, Gabrilove J, et al. In vivo measurements of bone marrow cellularity using volume-localized proton NMR spectroscopy. *Magn Reson Med*. 1991;19(1):85-95.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Ruschke S, Syväri J, Dieckmeyer M, et al. Physiological variation of the vertebral bone marrow water T2 relaxation time. *NMR in Biomedicine*. 2021;34:e4439. https://doi.org/10.1002/nbm.4439