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Assessment of alveolar bone marrow fat content using 15T MRI

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Abstract

Objectives—Bone marrow fat is inversely correlated with bone mineral density. The aim of this study is to present a method to quantify alveolar bone marrow fat content using a 15T MRI scanner.

Study Design—A 15T MRI scanner with a 13-mm ID loop-gap radiofrequency coil was used to scan seven 3 mm diameter alveolar bone biopsy specimens. A 3D gradient-echo (GRE) T1-weighted pulse sequence was chosen to obtain images. All images were obtained with a voxel size ($58 \mu\text{m}^3$) sufficient to resolve trabecular spaces. Automated volume of the bone marrow fat content and derived bone volume fraction (BV/TV) were calculated. Results were compared with actual BV/TV obtained from micro-CT scans.

Results—Mean fat tissue volume was $20.1 \pm 11\%$. There was a significantly strong inverse correlation between fat tissue volume and BV/TV ($r = -0.68$, $p = 0.045$). Furthermore, there was a strong agreement between BV/TV derived from MRI and obtained with Micro-CT (ICC = 0.92, $p = 0.001$).

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Conflict of Interests

The authors declare that they have no conflict of interest.

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Conclusion—Bone marrow fat of small alveolar bone biopsy specimens can be quantified with sufficient spatial resolution using an ultra-high-field MRI scanner and a T1-weighted pulse sequence.

Keywords

Yellow bone marrow; MRI; micro-CT

Introduction

Morphological conditions of the alveolar bone may have an impact on outcomes of different dental treatment modalities such as implant dentistry and periodontology.^{1, 2} Alveolar bone density has been quantified *in vivo* with computed tomography methods^{1, 3} and *ex vivo* with histomorphometry⁴ and micro-CT.³ Alveolar bone density also plays an important role in dental implant osseointegration where an inverse relationship has been determined between the contact extension between implant body and bone marrow fat.⁵ Bone mineral density (BMD) and bone volume fraction (defined as bone volume over total volume, BV/TV) generally vary inversely with yellow bone marrow fat (BMF) content,⁶ which may therefore also have an impact on dental treatments. Furthermore, BMF quantification is also important in radiotherapy to prevent and detect marrow composition changes that could lead to hematologic toxicity.^{7, 8}

BMF is commonly measured with magnetic resonance imaging (MRI).⁹ In MRI, images are formed from radiofrequency signals that are generated from the spins of the nuclei (¹H) of hydrogen atoms. In bones, hydrogen atoms can be found mainly in the water and protein contained in the bone matrix, and in the water and fat in marrow. However, trabecular bone measurements with MRI at conventional magnetic field strengths are challenging due to limited signal-to-noise ratio (SNR) resulting in low spatial resolution that is often insufficient to visualize individual marrow spaces.¹⁰ Therefore, to obtain adequate SNR, small bone specimens require the use of small high-performance radiofrequency (RF) coils as well as high magnetic fields. Little is known of the benefits of using an ultrahigh field (15T) MRI scanner with custom-built RF coils to analyze the small (3 mm diameter) bone specimens commonly used in studies on alveolar bone and dental implants.¹

Thus, the aim of this study was to present a method to assess and quantify the bone marrow fat content from small alveolar bone samples using a 15T MRI scanner with a custom-built RF coil.

Materials and Methods

Subjects

This study was approved by the ethics committee of the University of São Paulo (protocol number: 104/11). An informed consent form was signed by all patients willing to participate in this study. The guidelines of the Helsinki Declaration were followed in this investigation.

Inclusion and exclusion criteria

This study was conducted on cylindrical bone samples from partially edentulous patients attending a private dental clinic with a research partnership with the University of São Paulo. One bone specimen per patient was assessed. All patients had been referred for implant placement (maximum of one implant per dental arch, regardless of the number of missing teeth) between October 2014 and March 2015. Only mandibular implant sites more than 5 mm in width were included.

Patients with metabolic disorders, such as vitamin D deficiency and diabetes, or with insufficient alveolar bone volume for implant placement were excluded from the study. History of ridge grafting was also considered an exclusion criterion.

Bone sample preparation

All bone specimens had been retrieved at time of implant placement by using a trephine bur (3mm internal diameter) to prepare the implant sites. In all cases, implants were successfully placed, restored, and followed up for a minimum of one year. No postoperative complications were observed during this period.

All specimens had the same dimensions (3 mm diameter and 6 mm length) and were fixed in 10% buffered formalin, in accordance with previously described methodologies for trabecular bone assessment.^{11, 12} Prior to the scans, the surfaces of all specimens were patted dry to minimize contamination of the total proton signal by the formalin proton signal. The specimens were then transferred to 13 mm glass NMR tubes containing liquid fluorocarbon (Fomblin perfluoropolyether, Ausimont, Thorofare, NJ, USA). This fluid does not contain hydrogen atoms, and eliminates the intense proton signal from the fixation medium, mitigates susceptibility difference artifacts at tissue-air interfaces, and slows sample dehydration.

RF Coil

A custom-built RF probe based on a loop-gap-resonator design,¹³ with a 13-mm ID (Figure 1) was used for all measurements at room temperature. To build the RF coil, coaxial cylinders made of plastic (outer cylinder) and quartz (inner cylinder) were used as formers for the coupled resonators. Both cylinders were covered with self-adhesive copper tape (Scotch Brand Foil Tapes, 3M, Inc., Minneapolis, MN, USA) split into two parts by 5 and 2 mm gaps for the outer and inner cylinders respectively. The gap widths were chosen to adjust the resonance frequency of the resonator to that of the 619 MHz Larmor frequency of the scanner. Adhesive Teflon tape was used to secure the copper tape to allow smooth rotation of the two cylinders and increase the dielectric constant of the inter-cylinder space. The gap in the inner cylinder was bridged by a 24 mm long × 8 mm wide copper tape patch to improve the RF magnetic field homogeneity.

MRI scans

All scans were performed on a 15T 130 mm horizontal bore magnet (Agilent, Yarnton, Oxford, UK) equipped with a 60 mm ID gradient insert (Resonance Research, Inc., Billerica, MA, USA) with 2370 mT/m maximum gradient, interfaced to a Siemens console

(Siemens Medical Systems, Erlangen, Germany). Prior to the imaging procedure, the presence of a weak water signal peak (observed at a chemical shift of 4.7 ppm), presumed to be predominantly from residual formalin, and a strong fat signal peak (at 1.3 ppm) was confirmed in all cases by means of a spectroscopy assessment that is normally performed by the 15T scanner during its frequency tuning procedure. This confirmed that the signal from these specimens was predominantly from marrow fat. All scans were acquired with a field of view of $7.5 \times 7.5 \times 7.5$ mm and a matrix of $128 \times 128 \times 128$ pixels, yielding voxel dimensions of $59 \mu\text{m}^3$ (0.20 pL).

RF pulse sequence to detect fat tissue

A 3D gradient-echo (GRE) pulse sequence was used in all scans with a 255 Hz receiver bandwidth, 25° flip angle, 16 averages, 50 ms repetition time (TR) and 3.3 ms echo time (TE), resulting in a total acquisition time of 32:07 min:sec per scan.

Fat protons occur overwhelmingly in the methylene (CH_2) groups in the hydrophobic chains of lipid molecules, and generally exhibit a much shorter spin-lattice relaxation time (T_1) than do water molecules in pure water or in marrow. Since the T_1 of the water in formalin is similar to that of pure water, the short TR used in the pulse sequence tends to suppress signals from water in residual formalin as well as from erythropoietic (red) marrow, while retaining the signals from fatty (yellow) marrow.^{9, 14, 15}

Bone marrow fat content measurement

All analyses were performed on digital imaging and communications in medicine (DICOM) files using an open-source DICOM viewer (OsiriX v. 6.0; Pixmeo, Geneva, Switzerland). A region of interest (ROI) enclosing the total estimated volume with fat tissue was generated using a previously described methodology.¹⁴ The threshold was defined according to the histogram of the signal intensities (12-bit pixel values). An optimized lower threshold value of 2000 was estimated and set for all measurements. Accordingly, all pixels with a value greater than 2000 were classified as fat tissue and included in the auto-generated ROI (Figure 2). The 3D ROI measurement tool of the software was then used to calculate the volume of the ROI in cubic millimeters. Since this method for measuring an automated ROI is highly reproducible,¹⁶ a single observer with expertise in radiology (PhD in oral radiology and postdoctoral fellow trained in radiology and nuclear magnetic resonance science) performed all volume calculations.

Derived bone volume fraction (BV/TV) calculation

The MR images obtained were also used to calculate BV/TV, as previously described.¹⁰ Briefly, binary images were created with the assumption that dark pixels correspond to bone tissue. For this purpose, water and fat proportions were not taken into consideration, since the water content is extremely low, and the main objective was to calculate the percentage of the volume that corresponds to bone tissue.

Micro-CT analysis

A micro-CT scanner (SkyScan1172®; SkyScan; Kontich, Belgium) was used to analyze the bone specimens with the source running at 100 kV and 100 micro-amperes, a voxel size of

6.0 μm , with a 0.5 mm Al filter. Time exposure per frame was 450 ms. The Nrecon® (Sky-Scan, Kontich, Belgium) software was used to reconstruct the resulting x-ray images, which were further analyzed for BV/TV. All micro-CT analyses were performed by an examiner with expertise in bone morphometry (PhD student and master in biomaterials).

Statistical Analysis

Since strong correlations were expected, the sample size used was determined to detect a minimum correlation of 0.8, to give the study a power of 80% at a significance level of 5%. Normality of all variables was assessed using the Shapiro-Wilk test. Pearson's test was used to assess the correlation between the total fat volume and BV/TV, while agreement between BV/TV from MRI and micro-CT was calculated with the intraclass correlation coefficient (ICC). All statistical analyses were performed at a level of significance of 5%, using the IBM SPSS Statistics 17 software (SPSS, Inc, Chicago, IL).

Results

Seven subjects – totaling seven bone specimens (one per subject) – were included in this study (Table 1). All patients were female and presented a mean age of 53.7 ± 4.4 years. Mean fat tissue volume was $20.1 \pm 11.3\%$. There was a significantly strong inverse correlation between fat tissue volume and BV/TV obtained with micro-CT ($r=-0.68$ and $p=0.045$, Figure 3). Furthermore, there was also a strong agreement between BV/TV derived from MRI and that obtained with micro-CT ($\text{ICC}=0.92$, $p=0.001$).

Discussion

Bone marrow fat quantification with MRI has been described as a promising diagnostic method, despite challenges in obtaining high-resolution images.^{11, 14, 15, 17} The present findings indicate that quantification of BMF content in small specimens is feasible using a T_1 -weighted gradient echo pulse sequence and a custom made RF coil in a 15T MRI scanner. The high SNR provided by the strong magnetic field and large filling factor of the coil led to high spatial resolution with voxel size of $59 \mu\text{m}^3$ and consequently improved image quality. The improved resolution allowed greater contrast between pixels and facilitated the subsequent automated volume measurements (segmentation) of the fat content. The automatic segmentation technique used here has been shown to compute fat tissue volumes accurately on T_1 -weighted images.¹⁸ In particular, the reproducibility of the measurements was not affected by the choice of segmentation algorithm.

To our knowledge, this is the first application of ultra-high-field MRI in bone morphometry for dental research, supporting previous medical studies.^{9, 12, 14, 15, 17, 19} BMF quantification has been shown to yield clinically relevant data in the detection of bone marrow alterations associated with radiotherapy.^{7, 8} In accordance with our findings, clinical applications of T_1 -weighted images have also been described to detect the increase of BMF volume resulting from radiation therapy.^{20–22}

Alveolar bone research frequently involves the use of small diameter jawbone specimens obtained with a trephine. Imaging and structural findings of alveolar bone have been

correlated with bone grafts and dental implant outcomes,^{1, 2} and systemic bone findings.²³ As demonstrated in this study, fat content assessment by MRI can be achieved with pulse sequences that emphasize differences in T_1 between water and fat.^{6, 9, 14, 15}

Importantly, the inverse relationship between morphometric BV/TV and BMF enables indirect assessment of actual bone volume fraction. A recent animal study on dental implants suggested that implant osseointegration is enhanced in the presence of blood clots and indirectly proportional to the extension of the contact between the implant body and BMF.⁵ Therefore, quantifying the fat content in jaws may indicate sites that are more favorable for dental implant installation. Although the present MRI methodology is indicated for BMF assessment, other MRI protocols could be used for dental implant surgical planning.^{24–26} Further clinical studies would be recommended to address possible clinical advantages of incorporating the fat quantification method described in this proof-of-concept study.

This investigation has several limitations. First, since only bone specimens were scanned, and anticipating the eventual use of this technique *in vivo*, the effect of motion artifacts in live human subjects could not be ascertained. Nevertheless, similar methodologies have been adapted to clinical scanners to measure BMF *in vivo* with optimized pulse sequences, and our methodology could be similarly adapted as well.^{6, 9, 14, 15} An additional limitation is that only samples from female patients were analyzed. Further studies with larger sample sizes on clinical scanners testing different pulse sequence parameters would be recommended to address the *in vivo* feasibility of the methodology presented herein.

In this study, water and fat content were not unambiguously and quantitatively assessed by a spectroscopic measurement such as quantitative water and fat imaging using Dixon or similar MRI pulse sequences.²⁷ The use of the chemical shift difference between water and fat in MR spectroscopy or spectroscopic imaging in trabecular bone can be challenging at very high field due to the significant line broadening caused by the increased magnetic susceptibility effect in trabecular bone.¹⁰ This effect hinders a clean separation of water and fat content, adds to the duration of the measurement, and reduces the signal-to-noise ratio when images must be combined by addition or subtraction. In contrast, the use of T_1 weighting is a well-established method and relatively quick to perform.^{6, 9, 14, 15} Furthermore, because it is generally established that the marrow of the mandible is predominantly yellow, especially in adult patients who are likely to be receiving dental implants,¹⁵ it is reasonable to assume that the MR signal in the specimens examined in this study comes predominantly from fat, which further supports the clinical relevance of this study.

Conclusion

Within the limitations of this study, our findings suggest that BMF of small alveolar bone samples can be segmented with satisfactory contrast resolution using an ultra-high-field MRI scanner with a customized coil of small diameter and a T_1 -weighted pulse sequence. To our knowledge, this is the first demonstration of alveolar bone morphometry using MRI to estimate BV/TV, validated by means of micro-CT morphometric analyses.

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Statement of sources of funding for the study

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Statement of clinical relevance

This is the first study on MRI for alveolar bone morphometry. According to the evidence discussed herein, with sufficient development our methodology may be adapted to clinical MRI scanners. This would allow for assessing conditions of alveolar bone in patients without the need of ionizing radiation.

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Figure 1.

Photographs of the RF coil design (a), (c) along with exploded view illustrations (b),(d). Relative rotation of the concentric resonators provides tuning of the coil frequency. An additional piece of copper foil (dotted rectangle), insulated from both cylinders and rotating with the inner cylinder, was used to bridge the gap on the inner resonator and improve the RF homogeneity. Adjusting the height of the loop above the coil with the gear and rack mechanism (c) allowed fine tuning of the resonance frequency of the coil while inside the scanner. Coupling to the scanner was achieved using the transmit/receive (TR/RX) loop shown.

Figure 2.

Automated segmentation of the bone marrow fat content in a small (3mm in diameter) bone specimen. A) Original T1-weighted longitudinal image of a single slice. B) Automated segmentation of the BMF area of the same slice.

Figure 3.

Correlation between 15T MRI and Micro-CT. A) Micro-CT reconstruction of a bone sample, showing areas with absence of bone tissue (arrows). B) 15T MRI of the same bone sample, showing that the areas without bone tissue generally had a large fat content (arrows).

Table I

Results for BMF measurements of each bone sample.

Bone Sample	Patient's Age	15T MRI		Micro-CT
		Fat tissue volume (%) [*]	Derived BV/TV (%) ^{**}	BV/TV (%)
1	55	16.30	76.30	66.39
2	51	4.86	96.11	94.60
3	61	39.34	75.14	73.10
4	47	20.55	75.67	78.97
5	56	14.90	86.20	84.68
6	52	30.04	65.18	63.22
7	54	15.39	83.22	85.73

Abbreviations: BMF, bone marrow fat; 15T MRI, 15 Tesla Magnetic Resonance Imaging; Micro-CT, micro-computerized tomography; BV/TV, bone volume fraction.

^{*} Results are significantly inversely correlated with Micro-CT BV/TV, according to Pearson's test ($r=-0.684$, $p=0.045$)

^{**} Results are in strong agreement with Micro-CT BV/TV, according to the Intraclass Correlation Coefficient (ICC=0.920, $p=0.001$)