Bone Marrow Fat Quantification in calcaneus. Why not?

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TARGET AUDIENCE – Translational researchers interested in noninvasive assessment of bone quality

PURPOSE – The importance of evaluating bone marrow fat (BMF) is due to the correlation between BMF growing in prevalence and osteoporosis. In this contest, single voxel Magnetic Resonance Spectroscopy (MRS) is a robust method which can provide an in vivo estimation of total and specific fats, as previously reported[1-4]. Because fats quantity and their metabolism in bone marrow are skeletal location dependent[5], MRS investigation in different skeletal sites, performed in large population, are desirable to increase the physiological and metabolic understanding of osteoporosis, and to find new osteoporosis markers bone mineral density (BMD)-independent. The investigation of calcaneus bone marrow might offer the chance of using dedicated (and, as a consequence low cost) scanners reducing the risk of subjects' claustrophobic reactions, which remains a major source of drop-outs in Magnetic Resonance (MR) examinations. Therefore, in this work the reliability and the potential of MRS in calcaneus is discussed. **METHODS –** *Subjects*. Sixty-two Caucasian postmenopausal women (mean ± SD age: 62.1±5.8 years) were recruited to investigate calcaneus BMF. Subjects were excluded in the case of clinical evidence for metabolic bone disease or metastasis, previous history of lumbar spinal surgery or irradiation and current use of steroids or hormone replacement therapy. Each volunteer underwent BMD evaluation by quantitative computed tomography (QCT) in the lumbar region (L2-L4). The study was approved by the Local Ethics Committee. Written informed consent was collected from each subject before BMD and MR examination. According to current indications[6], subjects were divided in three groups with the following cut-off values: i) healthy individuals (H, T-score ≥ -1.8); ii) subjects with osteopenia (OPE, T-score between -1.8 and -3.3); iii) patients with osteoporosis (OPO, T-score ≤-3.3). *Experiments*. Single voxel MRS was performed by using PRESS sequence at 3T with a circularly polarized volume head-coil for radiofrequency transmission and reception. A homemade positioning device was used to immobilize the right foot during MR acquisition. Axial and coronal T2-weighted images of foot were acquired for positioning a 15×15×15mm^3 voxel in the center of calcaneus. The acquisition parameters used for MRS were: TR/TE=4000/22ms, signal average NS=32, spectral bandwidth 2000 Hz and 1024 data points. Prior to all acquisitions, the static field homogeneity was optimized in the volume of interest using an automated, second-order shimming routine. No conventional fat or water suppression acquisition or longer TEs methods were used in order to avoid underestimation of some resonances due to peaks overlapping (especially the resonances between 4 and 5.3 ppm) and to avoid additional acquisitions required to evaluate transversal relaxation times for T_2 correction of the spectral lines. Moreover, T_2 * was obtained with a FLASH sequence (TR/TE=1500/5-7-10-20 ms; flip angle, 30°; FOV=192x192 mm2; matrix, 128x128; BW=260 Hz/pixel; NS=1). *Method.* Row data of spectra acquired from each subject were exported and analyzed on an offline computer using the LcModel software (SPTYPE 6). Zero and first order phase corrections were performed. Nine proton resonances could be fitted using LcModel (Table 2): CH_3 (labeled L09, at ~ 0.90 ppm), bulk CH_2 (L13 at~ 1.3 ppm), the CH_2 α- (L23 at~ 2.3 ppm) and β- (L16 at~ 1.59 ppm) to the carbonyl, allylic CH_2 α to a double bound (L21 at~ 2.03 ppm), water (W at at~ 4.7 ppm), the glycerol CH_2, the glycerol CH (L41 and L43 at~ 4.10-4.35 ppm) and olefinic double bond protons (L52 +L53 at~ 5.20 ppm). In this analysis data with an %SD equal or higher than 10% were discarded. Marrow fat content percentage (FC%) was calculated according to the following relation: $FC\% = I_{fat} / \left(\sum_i I^i_{fat} + I_{water}\right) * 100$, where $\sum_i I^i_{fat}$ is the sum of the area amplitudes of the resonances: L09, L13, L16, L21, L23, L28, L43, L41, L52+L53 and I_{Water} is the area amplitude of water resonance. Total lipid quantification was performed by using: $TL\% = \sum_i I^i_{fat} / \left(\sum_i I^i_{fat} + I_{water}\right) * 100$. FC% for each lipid resonance TL, L13/L52+L53, L13/L41, L13/L43, were computed as mean ± SD. In order to evaluate the robustness of each resonance quantification a reproducibility analysis was performed. Three healthy subjects from the overall study cohort were re-scanned on two separate sessions using identical scanning protocols (median 36 days apart) comprised of single voxel PRESS sequence. To determine the short term reproducibility, three acquisitions were obtained for each volunteer over a period of 90 min with repositioning the subject in the scanner. We calculated the coefficient of variation CV_s of each peak as the ratio of the SD and the mean of each resonance. The long term reproducibility, CV_l, was evaluated by computing the variance of the mean of the two separate scanning session for each individual. *Statistic.* Homogeneity of variances between groups (H, OPE, OPO) was tested by using Levene's test. Pairwise comparisons between groups were made using a Welch ANOVA. Games-Howell corrections were performed to correct for multiple testing. **RESULTS** -The CV was excellent (ranging from 0.09% and 2.27%) for L13, L16+L13, L16+L09+L13 and TL, but for olefinic and glycerol resonances CV was less optimal (see Table 1). According to previous studies[7,8], there was no significant correlation between TL and T-score. L13/L52+L53 significantly discriminated between H and OPE groups, while both L13/L41 and L13/L43 significantly discriminated between OPE and OPO subjects (Table 2). Moreover, significant negative correlation was found between L13 and TL (r=-3.67, P=0.001), and between L53+L52 and L13 (r=-0.48, P=0.001), together with a significant negative correlation between glycerol resonances and T-score. **DISCUSSION** - Excellent reproducibility values were found for TL, L13, L16+L09+L13 and L16+L13 in the calcaneus, equal or better than those found in vertebral and femoral skeletal sites[9]. As a consequence low spectral resolution in calcaneus bone marrow spectra (due to the presence of magnetic susceptibility differences between trabecular bony structures and bone marrow) seems did not affect fats resonance reproducibility in highest trabecular bone density skeletal sites, such as calcaneus. Moreover, no significant correlation was found between TL% and T2*. Consistently with correlations results, L13/L41 and L13/L43 were identified as potential biomarkers to discriminate between OPE and OPO, being significantly lower in OPO compared to OPE group. Although the reproducibility of glycerol resonance was less optimal as compared to that of L13 (see Table 1), the percent differences in L13/L43 value between OPE and OPO groups are much higher than the CV of glycerol. Indeed for L13/L43 it was (99.49-61.41)/99.49=38.28%, while for L13/L41 it was (99.25-61.23)/99.25=38.31% between OPE and OPO groups. This result is consistent with an increased mobilization of fatty acid that occur with a release of free glycerol in oxidative fatty acids processes. Moreover, L13/L52+L53 discriminated between H and OPE, being significantly lower in H compared to OPE subjects. This result is in agreement with previous observations about the higher level of unsaturated fat (identified by olefinic double bond protons) found in H compared to low BMD subjects[3,9]. Although the CV_l of L52+L53 is about 10.4%, the percent differences in L13/L52+L53 value between OPE and H groups equal to (10.33-5.65)/10.33=45.3% is much higher than the CV of olefinic double bond protons. **CONCLUSION –**MRS in calcaneus can provide reliability fats estimation and L13/L43, L13/L41, L13/L52+L53 may be reliable markers of osteoporosis. Since BMF in calcaneus is characterized by a different metabolism compared to that of vertebral or femoral sites, MRS in calcaneus performed in large population, may increase our pathophysiological understanding of osteoporosis from a metabolic point of view.

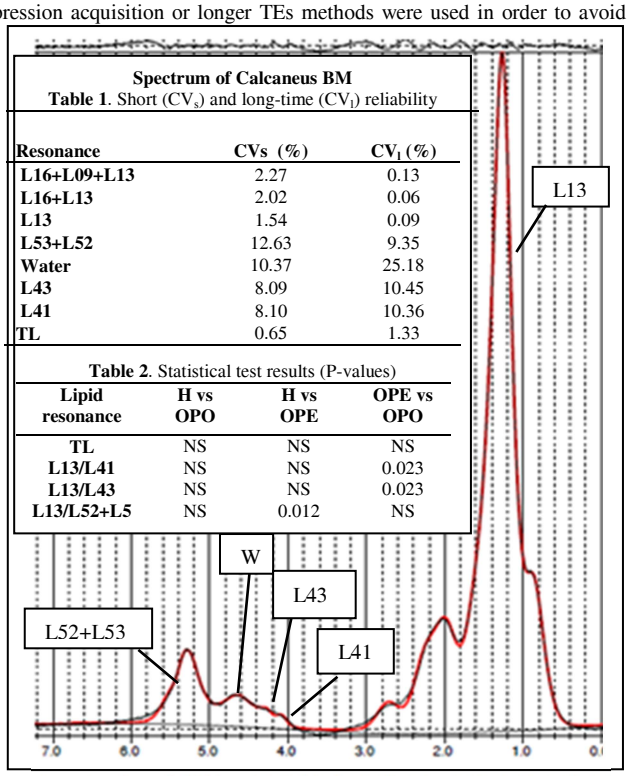
Spectrum of Calcaneus BM

Table 1. Short (CV_s) and long-time (CV_l) reliability

Resonance	CVs (%)	CV_l (%)
L16+L09+L13	2.27	0.13
L16+L13	2.02	0.06
L13	1.54	0.09
L53+L52	12.63	9.35
Water	10.37	25.18
L43	8.09	10.45
L41	8.10	10.36
TL	0.65	1.33

Table 2. Statistical test results (P-values)

Lipid resonance	H vs OPO	H vs OPE	OPE vs OPO
TL	NS	NS	NS
L13/L41	NS	NS	0.023
L13/L43	NS	NS	0.023
L13/L52+L5	NS	0.012	NS



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