Università di Foggia

PhD Course

"Experimental and Regenerative Medicine"

XXXI Cicle

Doctoral Thesis:

Evaluation of 3-Tesla MR Spectroscopy for assessment of bone marrow fat in patients with chronic kidney disease

Supervisor

Prof. Giuseppe Guglielmi

PhD Student

Dr. Cristina Borelli

INTRODUCTION

Bone marrow is a complex connective tissue consisting of two components, hematopoietic bone marrow and fat bone marrow. From the beginning of intrauterine life, hematopoietic bone marrow transforms itself into fat bone marrow, mainly made of adipocytes and their derivates, following a reversible process known as bone marrow conversion, which primarily depends on bone and age (1).

Several studies have recently highlighted that bone marrow adiposity may play a role in modulating both bone quantity and quality and that abnormalities in bone marrow fat composition could occur in a various spectrum of diseases (2,3). Consequently, quantification of bone marrow fat has recently gained an increasing interest.

Magnetic resonance spectroscopy (MRS) has been proposed as a promising technique for non-invasively quantifying bone marrow components, permitting its integration into clinical practice (4,5).

MRS utilizes the resonance frequency of protons to differentiate lipids from water, assessing the chemical composition of bone marrow in vivo.

In animal studies, the volume of bone marrow fat by biopsy strongly correlates with fat content assessed by MRS (6).

MRS provides highly reproducible fat content values at various skeletal sites, with the lumbar spine being the most studied site (7,8).

Moreover, MRS quantification of vertebral bone marrow fat content by using 3T MR allows to obtain an improvement of spectral resolution and a higher signal-to-noise ratio compared to 1.5 Tesla (9).

So far, researchers have focused on the MRS evaluation of bone marrow modifications in hematological diseases or chronic disorders such as osteoporosis or type 2 diabetes mellitus (10-12).

Few investigations exist regarding the assessment of bone marrow fat content by MRS in patients with renal osteodystrophy (13,14).

Renal osteodystrophy is an abnormal bone histomorphometry associated with chronic kidney disease (CKD) and represents one component of the systemic symptoms of CKD-mineral bone disorder (15-17).

Patients with CKD have a wide spectrum of findings on bone biopsy from high-turnover forms associated with hyperparathyroidism to adynamic bone disorders or osteomalacia related to mineralization defects. Various paracrine and endocrine signals participate in bone remodeling, with parathyroid hormone (PTH) playing a central role. In CKD patients PTH metabolism is altered and secondary hyperparathyroidism is an almost universal complication of advanced stages of disease due to many factors such as hypocalcemia, retention of phosphorus, decreases in the levels of calcitriol, intrinsic alterations within the parathyroid gland and skeletal resistance to the actions of PTH (18).

Sustained high PTH levels in CKD cause a high turnover state in bone, where bone resorption and, in a lesser degree, bone formation are stimulated. The disturbed osteoblast activity results in disorganized bone formation, with an excessive amount of non-mineralized component of bone, leading to skeletal fragility and subsequent increased fracture risk, particularly at the peripheral skeleton (19).

In fact, fractures are two to four times more prevalent in patients with CKD compared to the general population (16,20).

Fracture risk is determined by bone strength, which is affected by bone quality and bone mass.

Bone quality is determined by bone turnover and mineralization (assessed by histomorphometry), as well as by bone microarchitecture and chemical composition.

Bone mass is traditionally measured by bone mineral density using dual energy X-ray absorptiometry (DXA) but in the last years other bone indices such as volumetric bone mineral density (vBMD) using quantitative computed tomography (QCT) have been shown to be more accurate than DXA bone measurements (21,22).

Recent studies have shown that alterations of marrow composition with an increased fat deposition may result in deterioration of bone quality in both CKD and aging (20,23).

Adipocytes and osteoblasts arise from common cellular precursors, mesenchymal stem cells (MSCs), suggesting that the preferential differentiation towards adipocyte lineage, assessed by increased bone marrow fat, may alter osteoblast differentiation from mesenchymal stem cells. Whether the increase in bone marrow fat content correlates with bone loss in CKD patients is still unclear.

OBJECTIVES

During the first year of Phd Course, my study was focused on evaluation of the vertebral bone marrow fat composition in healthy population by using MRS. I evaluated the reproducibility of spectroscopic measurements for assessing vertebral bone marrow fat content at 3T MR.

The successive phase of this diagnostic prospective study consisted of comparing the bone marrow fat content between healthy controls and CKD patients. In this last group of subjects, I correlated spectroscopic measurements of bone marrow fat with volumetric bone mineral density (vBMD) obtained by QCT. During the final phase, I investigated if bone marrow fat composition is associated with severity of renal function impairment based on glomerular filtration rate (GFR) and blood levels of parathyroid hormone (PTH).

MATERIALS AND METHODS

Study population

CKD subjects were recruited consecutively for participation in the study, and the inclusion criteria were age ≥45 years old, estimated GFR ≤ 45 ml/min/1.732 m2 (MDRD formula) in the last 6 months, elevated PTH above the normal range (65 pg/ml) in the past year and QCT examination as part of diagnostic workup.

Exclusion criteria were females who were pregnant or nursing, a prior history of lumbar fracture, general contraindications to MRI/MRS exam (e.g., IUD, pacemaker), history of hematological malignancy, chemotherapy or radiation therapy, subjects unable to give consent.

The healthy control participants were asymptomatic subjects, had no history of spinal or other disease and had not received any drug that could have altered their bone marrow. They were matched for race, gender, and age ±10 years to CKD patients.

The study was approved by the Ethics Committee Review Board at our institution.

Written informed consent was obtained from all participants.

Study procedures

To evaluate the reproducibility of the MRS technique, 8 subjects were scanned twice at lumbar vertebrae on the same day with repositioning between the two scans. The repositioning of the subjects was done by the same technologist. After evaluation of intra-patient variability at each vertebral level, L1, L2 and L3 were selected for MRS analysis.

In CKD subjects, QCT and MR examinations of the lumbar spine were performed on the same day. For each patient, a blood sample for PTH measurement was collected at the time of MRS scan. GFR values were taken from the last clinically available assessment, within the last 6 months. CKD patients were stratified in three groups according to GFR levels in order to examine the influence of increasing impairment of renal function on bone marrow fat content. The group 1 included patients with moderate chronic renal insufficiency (GFR from 45 to 30 ml/min), the group 2 patients with severe chronic renal insufficiency (GFR from 29 to 15 ml/min) and the group 3 patients with the most advanced stage of renal failure (GFR <15 ml/min).

QCT examination

Volumetric bone mineral density of the lumbar spine was assessed by QCT, a three-dimensional method which measures trabecular BMD in milligrams per cubic centimeter by indirectly quantifying hydroxyapatite in comparison to a reference phantom.

All examinations were performed by using a 16-row multidetector CT scanner (Aquilion, Toshiba Medical System, Tokyo, Japan). A non-simultaneous calibration system was used (Lumbar Reference Simulator, CIRS, Norfolk, VA).

The scan protocol was standardized and consisted of a tube voltage of 120kVp and a tube corrent of 200mAs. Slice thickness was 3mm.

QCT scans were analyzed on a workstation using the available software.

Elliptical regions of interest (ROIs) were manually placed in the trabecular part of vertebral bodies from L1 to L3, avoiding cortical bone (Fig 1).

For each patient, vBMD values derived from the three lumbar vertebrae were annotated and mean lumbar vBMD was computed by averaging the vBMD measurements.

To assessed the precision error of CT system, vBMD measurements of each subject were repeated three times by the same experienced technician over 6 weeks.

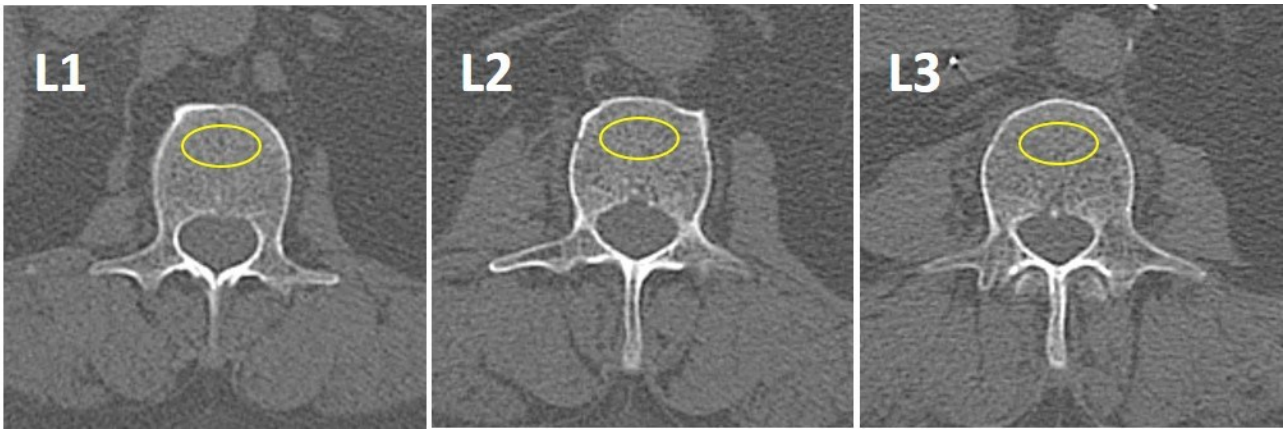

Fig 1 QCT based vBMD measurements of L1, L2 and L3 vertebrae. Measurements were performed in manually placed regions of interest (yellow marked).

MR Spectroscopy

All MR examinations were performed on a 3-T MRI scanner (Philips Ingenia 3.0) using a phased array spine coil.

Subjects were in supine position with knees supported by a form wedge to reduce spine curvature and motion by improving patient comfort.

The imaging protocol included T1-weighted TSE sequence (sagittal plane, repetition time [TR] 450-700 ms, echo time [TE] 9 ms, turbo factor 5, field of view [FOV] 32 cm, slice thickness 3 mm, spacing 0.3 mm, acquisition time 3min and 21sec) and T2-weighted mDIXON TSE sequences (sagittal and coronal planes, TR 2500-4000 ms, TE 80 ms, turbo factor 19, FOV 32 cm, slice thickness 3mm, spacing 0.3mm, acquisition time 3min and 5sec).

These sequences were obtained to guide the optimal positioning of volumes of interest (VOI) within the selected lumbar vertebrae (L1 – L3).

Proton MR spectroscopy was performed using a single voxel spectroscopy technique: voxel size: 15 x 15 x 15 mm^3.

Unless spine fractures, degenerative alterations or focal lesions (eg, hemangiomas) were identified, the spectroscopy measurement box was placed in the center of the vertebral bodies, avoiding the posterior venous plexuses.

The Point Resolved Spectroscopy (PRESS) method was used, and the spectroscopy sequence parameters were: TR 2000 ms, TE 40 ms, spectral bandwidth 2000 Hz, samples 1024, acquisition time 5min and 24 sec).

Neither pre-saturation bands or water suppression was used.

MR image analysis

All spectroscopy data were transferred to a research workstation. Using an in-house developed software, corrections for phase, frequency shift, and baseline distortion were performed on reconstructed spectral data.

Four main peaks were resolved and included: unsaturated lipids [5,35 ppm (olefinic **CH=CH-**)] water [4,7 ppm], residual lipids [2,06 ppm (methylene **-CH=CHCH2 -**)], and

saturated lipids [1,3 ppm (bulk methylene **-(CH2)n-**), 0,9 ppm (terminal methyl **-CH3**)] (Fig.2).

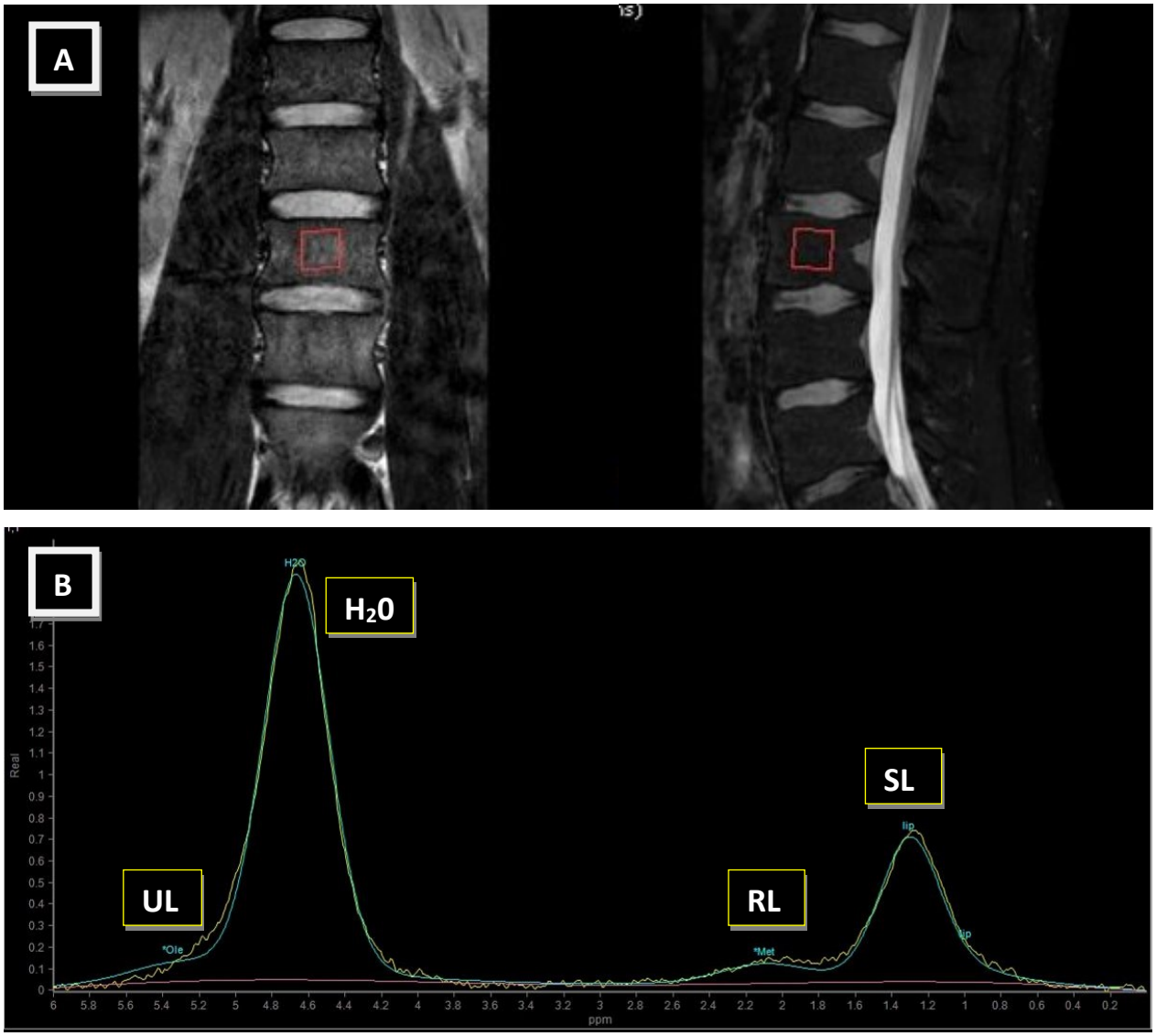

Fig.2 Bone marrow MRS. **A**. Dimension and localization of spectroscopy measurement box. **B.** Representative bone marrow spectrum with individual peaks for saturated lipids (SL), unsaturated lipids (UL), residual lipids (RL) and water (H_2O) after fitting.

The peaks were fitted to obtain their line widths and areas. Subsequently total bone **fat content (FC)** was calculated for each vertebral level (L1, L2 and L3), using the following equation:

FC (%) = total fat/(total fat+water) x 100%

Average fat content (L1-L3) was calculated using all available levels for each participant.

Statistical Analysis

The reproducibility of bone marrow fat measurements was evaluated using coefficients of variation (CV).

Paired t tests were used to compare bone marrow fat content at each lumbar site between CKD patients and healthy controls, matched according to race, gender and age ± 10 years.

The relationship between vBMD and fat content measurements was studied by Pearson's correlation coefficient. Pearson's correlation was also calculated to determine the association between fat content and PTH values.

One-way analysis of variation (ANOVA) was used to identify significant differences in fat content among the three groups of patients formed according to the severity of CKD.

Results were expressed as mean ± standard deviation. All statistical analysis was performed using SPSS (SPSS, Chicago, IL, USA). Statistical significance was defined as $p < 0.05$.

RESULTS

Study population

A total of 42 subjects (34 CKD patients and 8 healthy controls) were enrolled. Four patients of CKD group were excluded due to technical difficulties in MR scans, so a total of 38 subjects were included in the analysis. All CKD patients performed QCT examination.

Among all patients, nine were female. The mean age of patients was 59,6 ± 11,5 years, mean GFR value was 21,5 ±8,8 ml/min and mean PTH value was 149,2 ± 53,1 pg/ml. The mean age of controls was 56,3 ± 12,4 years. The mean body mass index (BMI) of patients was 27,1 ± 5,1 kg/m^2 and the mean BMI of controls was 26,9 ± 4,5 kg/m^2 (p=0.32).

Reproducibility of marrow fat content measurements

Bone marrow spectrum was dominated by water peak (4,7 ppm) and an intense lipid peak (1,3 ppm) deriving from bulk-methylene proton, while olefinic (5,35 ppm), methylenic (2,06 ppm) and terminal methyle protons (0,9 ppm) were more difficult to resolve. The mean bone marrow fat, measured in eight subjects (all healthy controls) was 53,1 ± 8.6% at L1, 56,3 ± 7% at L2 and 57,1 ± 7,3% at L3.

MRS showed a high reproducibility at all vertebral levels, with CV values ranging from 2.1 to 4.6% (Table 1).

Table.1. Marrow fat % at different vertebral levels and coefficients of variation (n=8).

Vertebral level	Mean ± SD (%)	CV (%)
L1	53,1 ±8,6	2.1
L2	56,3 ± 7	4.6
L3	57,1 ± 7,3	4.3

Differences in marrow fat content between CKD patients and controls

At each lumbar spine site, the mean marrow fat content was significantly elevated in patients compared to controls (Table 2). The mean fat content at L1 was 70,5 ± 8,3% in subjects with CKD versus 53,1 ±8,6 % in controls (p<0,001). At L2, the bone marrow fat was 71,5 ± 11.9 % in CKD versus 56,3 ± 7% in controls (p<0,001). Similarly, at L3, the bone marrow fat was 72,4 ± 9,5 % versus 57,1 ± 7,3 % (p=0,02).

Table.2. Marrow fat % at L1, L2 and L3 in CKD patients and controls

Vertebral level	**CKD Patients (n=30)**	**Controls (n=8)**	***p*-value**
L1	70,5 ± 8,3	53,1 ± 8,6	<0,001
L2	71,5 ± 11,9	56,3 ± 7	<0,001
L3	72,4 ± 9,5	57,1 ± 7,3	0,02
All levels (L1-L3)	71,4 ± 8,7	55,5 ± 7,6	<0,001

Data are expressed as means ± SD (%).

Correlation between bone marrow fat content and vBMD

The average values of vBMD, measured in all CKD patients, were 104,1 ± 32.6 mg/cm^3 at L1, 97,1 ± 26,4 mg/cm^3 at L2 and 93,0 ± 34,7 mg/cm^3 at L3. The mean value of vBMD at all lumbar sites was 98,1 ± 27,9 mg/cm^3. The precision error of vBMD measurements by QCT, expressed as CV, was 1,8%.

Mean fat content showed a strong negative correlation with vBMD (r = -0,71; p<0,001) as represented on Fig. 3.

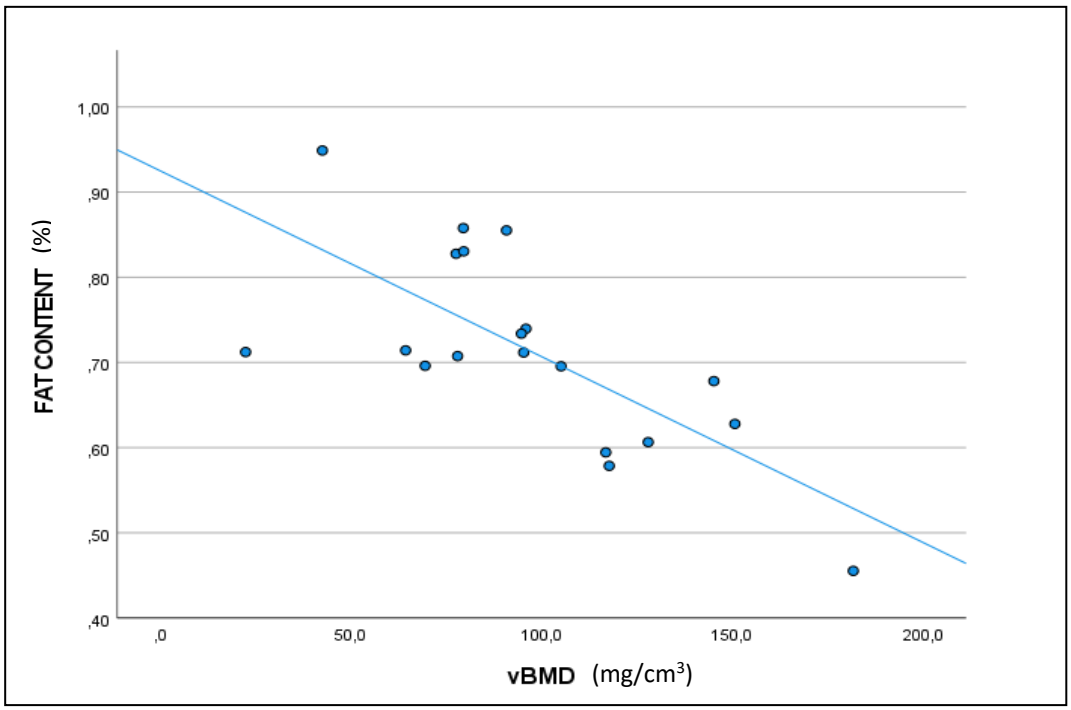

Fig.3. Scatter plot showing the correlation between bone marrow fat and lumbar vBMD in CKD patients. There was a strong negative correlation between the two variables, as better represented by the trend line ($r = -0,71$; $p<0,001$).

Bone marrow fat association with CKD severity and PTH values

In CKD patients divided into three groups according to severity of renal function measured by GFR, the mean fat content was compared among the group 1, 2 and 3. As shown in Fig.4, bone marrow fat increased significantly with the decline of GFR ($p = 0,002$). There was no significant correlation between the mean marrow fat and PTH values ($p>0,05$).

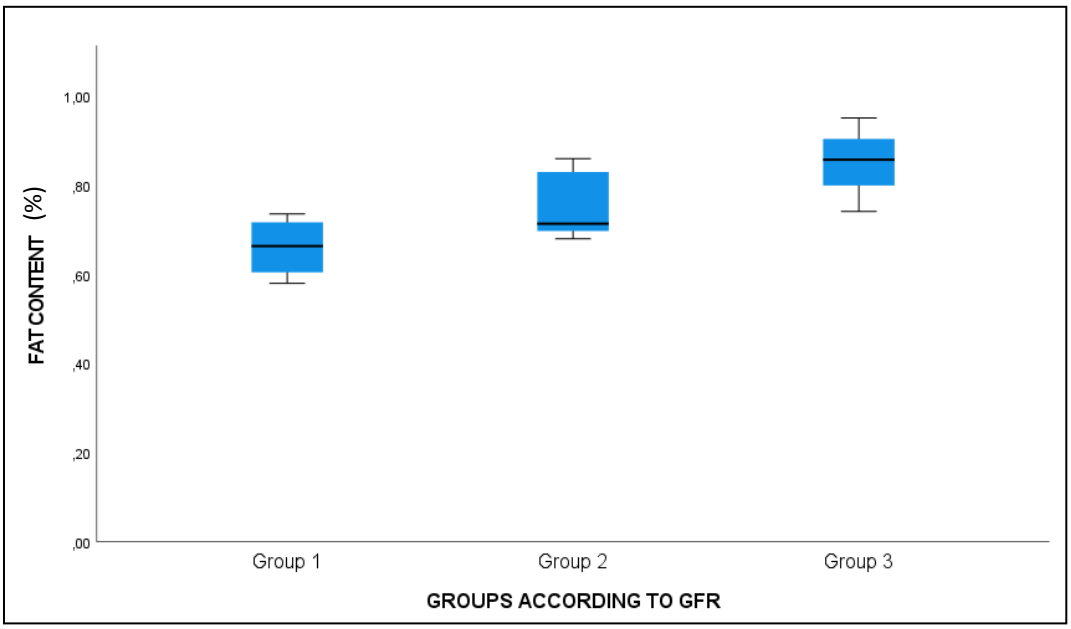

Fig.4. Boxplot analysis of fat content in three CKD groups (group 1 with GFR values from 45 to 30 ml/min, group 2 with GFR values from 29 to 15 ml/min, group 3 with GFR values inferior to 15 ml/min). Bone marrow fat significantly increased from group 1 to group 3, with the worsening of renal function (p=0,002).

DISCUSSION

In this study, we used MRS at 3T for evaluating bone marrow fat at lumbar spine. The reproducibility of bone marrow fat measurements by MRS was optimal at all lumbar vertebral levels. Recent studies have documented the reliability of MRS in marrow lipids assessment and the advantages of acquiring spectral data with a high field strength (9,24-26). In particular, 3T MR technique has a high spectral resolution that helps to better detect individual peaks with low signal intensity. In bone marrow fat assessment, the use of a high

field MR imaging allows accurately measuring unsaturated lipids (5,31ppm), partially overlapped with the water peak, and residual lipids (2,06 ppm), that have a weak intensity.

Given the increasingly relevance of marrow fat composition as indicator of bone weakening, we believe that the use of MRS for marrow fat assessment would be included in clinical setting of patients with bone disorders, as extension of routine MR examination of lumbar spine. Despite a relatively longer scan time of spectroscopy technique, post processing analysis is feasible in clinical practice and coils and patient position do not differ from diagnostic MR imaging.

In our research, we focused on bone marrow fat composition quantified by MRS in patients with chronic kidney disease and compared them with healthy subjects. Our results demonstrated that MRS fat content measurements were significantly elevated in patients with chronic renal impairment. So far, few studies have investigated bone marrow composition by MR imaging in patient with CKD (13,14). Moorthi et al found that marrow fat content by MRS was significantly higher in CKD patients compared to controls (14). Our data are consistent with these findings.

The novel finding of our study was that bone marrow fat and vBMD were inversely related in patients with CKD. In this population, we found elevated values of fat content and low levels of bone density at lumbar spine, with a significant negative correlation between the two parameters, both measured at lumbar spine respectively by MRS and QCT. We chose QCT instead of DXA because this three-dimensional technique allows obtaining volumetric BMD measurements, that are more consistent with those of MR spectroscopy than bi-dimensional BMD measurements of DXA.

In addition we explored the possible connection between marrow fat content and severity of chronic renal failure. To our knowledge, this investigation has not been performed so far.

In aging population, MRS measurements of bone marrow fat correlate with osteopenia and osteoporosis assessed on the basis of BMD and vertebral fractures, as demonstrated in several reports (4,22,23).

The similarities among these studies and our results suggest that in both CKD and aging there is an increased marrow fat deposition, whose cause is still unknown.

However, the evidence of a reciprocal relationship between marrow fat and bone mineral density supports the observation that bone weakening depends not only on trabecular bone loss but also on increased adipogenesis. In fact, excessive amounts of marrow fat determine the reduction of bone mass, and consequently of bone strength. In addition, a disproportionate adipogenesis may inhibit normal osteoblast activity, resulting in bone loss and alteration of bone quality. These mechanisms could be responsable for the higher fracture rates in CKD patients compared to general population (16,20).

Bone abnormalities affect almost all patients with CKD requiring dialysis and the majority of patients with CKD stages 3–5 (27). So we hypothesized an increased marrow fat content in patients with late stages of chronic renal insufficiency. In effect, our results demonstrated that bone marrow fat increased significantly with the decline of GFR, suggesting that the normal mineral and endocrine functions disrupted in CKD patients play an important role in modulation of marrow adipogenesis.

We also investigated the relationship between marrow fat content and blood levels of PTH, that is considered a valuable biomarker of bone remodeling in CKD. Our results did not show a significant association between these two parameters. A small portion of CKD patients received treatment for secondary hyperparathyroidism and the effects of drugs may have influenced blood levels of PTH. In the future, we will correlate bone marrow fat with biochemical markers of bone turnover in patients with chronic renal insufficiency, at

baseline and after specific treatment to correct secondary hyperparathyroidism. These observations may help to understand if bone marrow fat composition is a “reversible” condition that changes with clinical improvement of mineral disorder related to CKD.

The current study has some limitations. The population size is relatively small, in particular the healthy controls, and measurements of vBMD by QCT were no available in healthy subjects. It should be emphasized that several studies have already examined association between marrow fat and bone density in healthy population (9,10,23). However, further investigations are needed to recruit a larger sample of subjects for comparing their bone composition with those of CKD patients.

In our research, we did not have histological data derived from bone biopsy to confirm MRS marrow fat measurements in CKD patients. Nevertheless, a recent study of Cohen et al. performed in osteoporotic and control subjects demonstrated a correlation between marrow fat content in trans-iliac crest biopsy and lumbar spine marrow fat content by MRS (28). Although related to a different study population, these results contribute to better validate MRS in bone marrow fat assessment.

Lastly, in a small number of our study population eventual drug therapies affecting bone and mineral metabolism may have had effects on blood PTH values measured in those patients, influencing our results in terms of correlation with marrow fat content. Regardless of that, there is a need to understand if CKD-induced disturbances of PTH metabolism may have a role in changes of bone marrow composition.

Conclusions

This study demonstrates that a decrease in bone density within lumbar vertebrae is associated with a significant increase of bone marrow fat content in CKD patients.

Moreover, bone marrow fat modifications correlate with the severity of renal function impairment.

On the basis of our results, we believe that 3T MRS quantification of bone marrow fat is reliable and highly reproducible, indicating its possible role as qualitative and quantitative imaging biomarker for bone marrow changes in CKD patients.

REFERENCES

1. Gurevitch O, Slavin S, Feldman AG. Conversion of red bone marrow into yellow - cause and mechanisms. *Med Hypotheses* 2007;69:531–536.

2. Krings A, Rahman S, Huang S, et al. Bone marrow fat has brown adipose tissue characteristics, which are attenuated with aging and diabetes. *Bone* 2012;50:546–552.

3. Rosen CJ, Klibanski A. Bone, fat, and body composition: evolving concepts in the pathogenesis of osteoporosis. *Am J Med* 2009;122:409–414.

4. Griffith JF, Yeung DK, Antonio GE et al. Vertebral bone mineral density, marrow perfusion, and fat content in healthy men and men with osteoporosis: dynamic contrast-enhanced MR imaging and MR spectroscopy. *Radiology* 2005;236:945–951.

5. Machann J, Stefan N, Schick F. (1) H MR spectroscopy of skeletal muscle, liver and bone marrow. *Eur J Radiol*. 2008;67:275-84.

6. Pichardo JC, Milner RJ, Bolch WE. MRI measurement of bone marrow cellularity for radiation dosimetry. *J Nucl Med* 2011;52:1482–1489.

7. Yeung DK, Lam SL, Griffith JF et al. Analysis of bone marrow fatty acid composition using high-resolution proton NMR spectroscopy. *Chem Phys Lipids* 2008;151:103–109.

8. Griffith JF, Yeung DK, Chow SK, et al. Reproducibility of MR perfusion and H spectroscopy of bone marrow. *J Magn Reson Imaging* 2009;29:1438–1442.

9. Liney G, Bernard C, Manton D et al. Age, gender, and skeletal variation in bone marrow composition: a preliminary study at 3.0 Tesla. *J Magn Reson Imaging* 2007;26:787–793.

10. Kugel H, Jung C, Schulte O et al. Age- and sex-specific differences in the 1H-spectrum of vertebral bone marrow. *J Magn Reson Imaging* 2001;13:263–268.

11. Yeung D, Griffith J, Antonio G et al. Osteoporosis is associated with increased marrow fat content and decreased marrow fat unsaturation: a proton MR spectroscopy study. *J Magn Reson Imaging* 2005;22:279–285.

12. Tang GY, Lv ZW, Tang RB et al. Evaluation of MR spectroscopy and diffusion-weighted MRI in detecting bone marrow changes in postmenopausal women with osteoporosis. *Clin Radiol* 2010; 65:377-381.

13. Wehrli FW, Leonard MB, Saha PK et al. Quantitative high-resolution magnetic resonance imaging reveals structural implications of renal osteodystrophy on trabecular and cortical bone. *J Magn Reson Imaging*. 2004 Jul;20:83-9.

14. Moorthi RN, Fadel W, Eckert GJ et al. Bone marrow fat is increased in chronic kidney disease by magnetic resonance spectroscopy. *Osteoporos Int*. 2015;26:1801-7.

15. Moe S, Drueke T, Cunningham J et al. Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2006;69:1945–1953.

16. Piraino B, Chen T, Cooperstein L et al. Fractures and vertebral bone mineral density in patients with renal osteodystrophy. *Clin Nephrol* 1988; 30:57–62.

17. Gerakis A, Hadjidakis D, Kokkinakis E et al. Correlation of bone mineral density with the histological findings of renal osteodystrophy in patients on hemodialysis. *J Nephrol* 2000; 13:437–443.

18. Evenepoel P, Bover J, Ureña Torres P. Parathyroid hormone metabolism and signaling in health and chronic kidney disease. *Kidney Int* 2016;90:1184-1190.

19. Carrillo-López N, Panizo S, Alonso-Montes C et al Direct inhibition of osteoblastic Wnt pathway by fbroblast growth factor 23 contributes to bone loss in chronic kidney disease. *Kidney Int* 2016; 90:77–89.

20. Alem AM, Sherrard DJ, Gillen DL et al. Increased risk of hip fracture among patients with end-stage renal disease. *Kidney Int* 2000;58:396–399.

21. Li N, Li XM, Xu L, et al. Comparison of QCT and DXA: Osteoporosis detection rates in postmenopausal women. *Int J Endocrinol* 2013;2013: 895474.

22. Mao SS, Li D, Syed YS, et al. Thoracic quantitative computed tomography (QCT) can sensitively monitor bone mineral metabolism: comparison of thoracic QCT vs. Lumbar QCT and dual-energy x-ray absorptiometry in detection of age-relative change in bone mineral density. *Acad Radiol* 2017;24:1582–1587.

23. Shen W, Chen J, Punyanitya M et al. MRI-measured bone marrow adipose tissue is inversely related to DXA-measured bone mineral in Caucasian women. *Osteoporos Int* 2007;18:641–647.

24. Pansini VM, Monnet A, Salleron J et al. Reproducibility of 1H MR spectroscopy of hip bone marrow at 3 Tesla. *J Magn Reson Imaging*. 2012;36:1445-9.

25. Karampinos DC, Ruschke S, Dieckmeyer M et al. Quantitative MRI and spectroscopy of bone marrow. *J Magn Reson Imaging*. 2018;47:332-353.

26. Schmeel FC, Vomweg T, Träber F et al. Proton density fat fraction MRI of vertebral bone marrow: Accuracy, repeatability, and reproducibility among readers, field strengths, and imaging platforms. *J Magn Reson Imaging*. 2019 Dec;50:1762-1772.

27. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Update Work Group. KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD). *Kidney Int Suppl*. 2017; 7: 1–59.

28. 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 Oct;26:2471-8.