Rapid Determination of Vertebral Fat Fraction Over a Large Range of Vertebral Bodies
Abstract

Introduction

Vertebral body Fat Fraction (FF) has been found to vary between lumbar vertebrae using Magnetic Resonance Spectroscopy (MRS). We aim to more quickly assess a larger number of adjacent vertebrae using a single LINEAR In:Out sequence.

Methods

Five males had DEXA and 1.5 Tesla MR scans performed. MRS was performed at L3, and sagittal LINEAR measurements from T10 to S2. For the LINEAR measurements, two independent observers followed a set reading protocol.

Results

For FF measurements there was limited intra-observer variation, with observers being on average within 3.4% of the pooled mean value. Similarly, there was good interobserver agreement with an average variation of 2.1%. All men showed a lowering FF between L5-S1 of between 1.6–7%. Otherwise, there was a trend for increasing FF moving inferiorly from T10 to L5. This averaged 2.7%/vertebra (range 1.1-3.8%), and appeared to be independent of MRS measured FF at the L3 level. Excluding one MRS FF outlier, there was good correlation between MRS FF at L3 and BMD using DEXA (R²=0.57).
Conclusion

LINEAR measurements are largely reproducible between observers following a set protocol. There appears to be a gradient in FF moving from T10 to L5. This variation may better describe overall marrow function than a single vertebral reading.

Key Words:

Magnetic Resonance Imaging
Magnetic Resonance Spectroscopy
Bone Marrow
Fat Fraction
Fat Imaging
Osteoporosis
Introduction

Bone marrow contains hematopoietic stem cells, generating circulating blood and osteoclasts as well as mesenchymal stem cells which can mature into osteoblasts and adipocytes/fat. The ratio of these respective red and yellow cells is not constant and changes with age, gender and anatomical location. The fat fraction (FF) is ratio of fat-to-water-plus-fat and has been determined by magnetic resonance spectroscopy (MRS) and imaging (MRI). Marrow Fraction is the inverse of FF (ie 100% - FF), and is thought to correlate with marrow function.

The FF is known to vary according to anatomical site, as has been demonstrated in work with 18F-fluoro-L-deoxythymidine Positron Emission Tomography (FLT-PET) imaging. This group reported high FF in cervical spine, lowest FF in the thoracic spine and lumbar spine decreasing again in the sacrum. However, PET/CT studies are not suitable for repeated measures or acquiring normative data due to the radiation exposure. Early experience measuring FF via MRS at two separate lumbar vertebrae showed increasing FF for the more inferior vertebra. This observation is consistent with a later report assessing the four vertebrae from L1 to L4, showing the same trend, even between neighbouring vertebrae. It is therefore plausible that a point estimate of FF in a single vertebra doesn't completely describe marrow function. A more comprehensive measurement of not only FF but also change in FF according to anatomical site and time may provide a more complete description.

Preliminary work suggests there is a correlation between the Fat Fraction (FF) estimated by Magnetic Resonance Spectroscopy (MRS) in Lumbar vertebrae and
the accepted standard of Bone Mineral Density (BMD) measured by DEXA \(^7\). However, there is a wide degree of overlap between subjects classified as normal, osteopaenic and osteoporotic on DEXA and their corresponding FF values. Some of this variability would be explained by clinical factors such as age and gender \(^4\), which are routinely incorporated into fracture risk nomograms such as FRAX \(^8\). However, due to the complex physiology of bone beyond what can be quantified through DEXA imaging and clinical parameters, it is plausible that some of this variability may be due to other factors which MRI would be well placed to assess.

The LINEAR In:Out Phase image sequences produce separate water and fat only images, and have previously been shown to correlate very strongly with MRS derived FF measurements in bone \(^3\), and liver \(^9\). The advantage of imaging approaches applied to a sagittal section of vertebrae is that the FF can be estimated for a larger number of structures in a much shorter time than performing MRS in multiple Regions of Interest (ROI). We aim to use the LINEAR In:Out sequence in a group of older male patients entering onto a clinical trial investigating the effects of androgen deprivation on marrow function.

Evaluation of normal and pathological changes in bone marrow FF preferably requires non-invasive methods that do not require x-rays or radiotracers. The variation of FF along the vertebral column suggests the requirement of assessing a large a range of the spine as possible. MRI methods including In:Out phase imaging as investigated in this study show great promise in this regard.
Methods

Participants

Eligible patients were men with localized prostate cancer who were to receive androgen deprivation therapy (ADT) and prostate radiotherapy. None had a history of lower back problems. Screening thoracolumbar X-Rays were performed to exclude oestoporotic compression fractions, advanced degenerative changes or any other gross abnormalities. A whole body 99m-Tecnicium bone scan was performed and needed to be negative for the presence of metastatic bony disease. Signed informed consent was obtained from five participants. This project received Human research ethics committee approval.

Imaging details

All MR examinations were performed on a GE 1.5 Tesla Signa Excite system using a lumbar phased-array coil. Patients were positioned using a knee rest to minimise lumbar lordosis. Initial scout images were followed by the dedicated In-phase and Out-of-phase imaging. Specifically this was a T2-weighted iterative decomposition of water and fat with echo asymmetric and least squares estimation (IDEAL)-FSE sequence (TE/TR = 60 ms / 3000 ms). This produced separate water-only and fat-only images. Twenty sagittal images of the spine from T10 to the mid-Sacrum were obtained using this approach for each patient, with a slice thickness of 3 mm.
DEXA scanning was performed on all individuals with individual readings from L2, L3 and L4 vertebral bodies. Z- and T-Scores were also calculated based on Australian data.

Data Extraction

Offline two independent observers (JM and WW) extracted data from the sagittal Fat T2 LINEAR images using proprietary software (Voyager Telerad Picture Archive and Communication System, Intellrad Solutions Pty Ltd, Melbourne, Australia). A set protocol was followed with multiple observations. The observers were instructed to begin at the sagittal slice 5 mm from cortical bone and to make freehand ROIs to provide measurements of vertebral fat content for all vertebral bodies within the field of view. To limit peripheral artefact, the most superior and inferior vertebrae were not assessed. Observers were instructed to exclude bony cortex, or any anatomical abnormalities observed on the T2 images. They would then proceed from right to left on serial sagittal slices repeating the measurements five times overall.

Magnetic Resonance Spectroscopy

MR spectroscopy (MRS) was performed at the L3 level on all five patients. The software package SAGE (GE Medical Systems) was used to extract the areas under the peaks for separate fat and water peaks ($A_f$ and $A_w$ respectively). Fat fraction (FF) was calculated as $A_f / (A_w + A_f)$. As this has very high correlation with FF using the LINEAR approach ($r^2$ values of 0.85-0.9 $^{3,10}$), this figure was used to calculate a measurement for pure fat on the Fat T2 LINEAR images by dividing the measure
from the Fat LINEAR image at L3 by the FF figure from the MRS for the same vertebral body. The FF for all other vertebrae were then calculated by dividing the Fat:LINEAR measure for that vertebra by the pure fat measure.

Data Analysis

Descriptive plots were constructed to assess for trends in observer variation as well as FF across adjacent vertebral bodies. Linear regression was used to model the changes observed in FF within each participant.
Results

Patient Characteristics

Five men with locally advanced prostate cancer consented to this study. All were aged between 70 and 75 years. None was being treated with bisphosphonate therapy, corticosteroids or ADT, and none had a history of previous low trauma fracture or osteoporosis. Figure 1 shows an example of a false colour LINEAR Fat MRI sagittal image.

Reproducibility of Marrow Fat Fraction Measures

To ensure stability of the FF measures, tests were done of intra- and inter-observer variation. Between the two observers, 94 vertebral body Fat measurements were obtained, with a median of 5 observations per vertebral body. A total of 464 measurements were recorded. For each observation, the percentage deviation from the mean for that vertebra and observer was calculated. Figure 2 shows the percentage deviation of a single observation from the mean for a particular vertebral level and observer. The overall average intra-observer variation was 3.4%, and in greater than 95% of instances, observations were within 9% of the mean. No one patient’s vertebra had two separate measurements greater than 9% from the mean, nor was any individual deviation greater than 18%. It was therefore concluded that there would be little effect from outliers on the data, and that the mean rather than the median would be a robust measure of data location.
Similarly, inter-observer variation was compared across 45 different vertebral levels between the two observers. The inter-observer percentage deviation was calculated as the absolute value for Observer A – Observer B divided by the average of the two. Of the total of 45 observations, the overall average inter-observer variation was 2.1%, with 21 of the vertebrae within 1% between the two observers, 33 within 3% and 39 within 5%. The maximal variation was 8.1%. This suggests that the reading protocol lead to consistent results within and between observers, and justified using a pooled mean value in the subsequent analyses.

**Fat Fraction**

Figure 3 shows FF at each vertebral level for each of the five patients. Note that all show a gradual trend of increasing FF moving from the most rostral towards the most caudal vertebral body. Table 1 quantifies the average differences in FF between adjacent vertebrae. Note the anomaly in the trend that all patients show a reduced FF in S1 compared with L5. This ‘L5-S1 Dip’ is noted in all five patients with figures of -4.9%, -6.1%, -2.4%, -7.0% and -1.6% respectively. Due to one patient being shorter than the others, it was possible for a larger range of twelve vertebral bodies to be measured, and his FF per vertebrae is shown in Figure 4. The trend of increasing FF moving inferiorly appears even more pronounced, with a 50.9% difference in FF seen between T10 (29.1%) and S4 (80.0%) seen.

**Fat Fraction Gradient**
The gradient in FF was fitted to various models. Given the possibility of other factors affecting FF measurements at S1, the models were trialled from T11 to L5. For all five patients, a linear regression model proved a very good fit of the data, with $R^2$ values of between 0.81 and 0.96 (see Table 2). More complex modelling offered only marginal improvements in the $R^2$ over the linear model, and was usually inconsistent (eg quadratic model was convex for some patients, and concave for others). The slope of the regression line varied from 1.1%/vertebra to 3.8%/vertebra (see Table 2). Note how patients with similar L3 MRS FF readings can have different FF gradients. Patients 1, 4 and 5 have L3 MRS FF of 34.8±1%, yet despite this similarity the FF gradient varies by a factor of 3.5 across the full range of observed FF gradient values for these 3 patients. This suggests that a FF reading at a single vertebral level does not completely describe the functional marrow distribution.

**DEXA versus MRS FF**

The T-scores for the Lumbar vertebra were -2.1, -0.2, 2.3, -0.2 and 0.65, suggesting that 4 of the participants had normal BMD and one was osteopaenic. The isolated DEXA BMD reading at L3 was compared with the MRS FF reading at the same vertebral level. Only a weak negative correlation was noted between the two readings ($R^2=0.17$). One participant had a high MRS FF which was an outlier compared with the other four. He was not the osteopaenic individual. If the man with an abnormally high MRS FF was excluded, the negative correlation linear fit was much better ($R^2=0.57$).
Discussion

The use of LINEAR In:Out phase imaging, with ROI analysis, proved to be a rapid and reliable method for determination of vertebral FF. This method enables a large series of vertebral bodies to be measured following a single rapid acquisition. This contrasts with spectroscopy, which normally evaluates a single vertebra per acquisition. There was minimal inter- and intra-observer variation for these measurements for independent observers following our set protocol. The most striking observation was that the more inferior the vertebral location, the more likely the FF would steadily increase. This FF gradient appears to be largely independent of an isolated measure of FF using MRS at a single vertebral body level. This result has not been reported using the LINEAR approach, but is in agreement with reports in the literature using MRS to estimated FF.\(^2,3\)

An early report touches on the possibility of a marrow gradient noting a trend toward increasing FF for more inferior vertebral bodies measured with MRS.\(^3\) In this study the mean FF value at L1 was 40.5%, and at L5 it was 51.3%, albeit with wide ranges due partially to only ten patients being examined in this manner. This trend was not consistent, and may have been overwhelmed by the stronger relationships noted with both age and gender.\(^4\) Even with these caveats, it is worth noting that the corresponding figures from our series were broadly similar at 37.7% and 46.7%.

Another previous report again using MRS to quantify FF focussed on post-menopausal women, which would be expected to reduce the impact of age and gender on the results.\(^2\) Vertebral levels from L1 to L4 were all measured individually.
for 40 women, some of whom were known to have low BMD. The FF gradient increased by an average of 2.2%/vertebra moving inferiorly in the patients with low BMD, although no strong evidence of a gradient was seen in the healthy controls. The average figure in our series was 2.7%/vertebra, although only one of our five patients had a low BMD reading. A subsequent report from the same group looking at diabetic women, also noted a trend towards reducing FF from L1 to L3.

It would appear that our results using the LINEAR approach are consistent with earlier observations reported using MRS. While the two approaches correlate well with each other, the latter is superior in its ability to examine more vertebral bodies simultaneously in a shorter time. Given the observed gradient in FF, it would seem that future investigators will either need to examine multiple vertebral bodies with MRS, or use an alternative approach such as LINEAR.

There have been several reports suggesting a correlation between FF measured at a single vertebral body level and BMD measured by DEXA imaging. However, there is extensive overlap between normal, osteopaenic and osteoporotic individuals. Part of the reason for this may be anatomical variations such as osteophytes which can interfere with DEXA readings. Although this might be circumvented with the use of qualitative CT, the increased radiation dose and lack of widely validated population data for this modality may curtail its widespread use. Our hypothesis is that the complex functional anatomy, physiology and biology of bone is poorly captured using a single parameter such as Fat Fraction at a single vertebral level. Additional factors such as the FF gradient may be helpful in separated people...
into distinct BMD categories, and as such this is an area that we are continuing to investigate.

A second finding from our study was that there is a consistent reduction in FF moving from L5 to S1. This contrasts with a result published using FLT-PET, although that study did not resolve to the same degree of anatomical precision as in the current report. This may be due to other subtle degenerative pathology at this level such as spondylosis relating to the unique mechanics of the L5-S1 joint compared with the more superior thoracic and lumbar articulations. Further work will be required to clarify both the consistency of the reduction, as well as trying to gain a greater understanding of the underlying causes. If the FF does not change consistently and at an equal rate along the spine, individual measures by spectroscopy may miss changes.

Loss of BMD is a common problem for men managed with ADT for prostate cancer. This results in a higher rate of fractures for such men. Previously, ADT was only used for men being managed palliatively for metastatic disease and hence with relatively low life expectancy. Two key developments now make the long term toxicity of ADT more pertinent. One is the evidence of efficacy of adjuvant ADT in the curative setting, meaning many men expecting to be cured of their prostate cancer will survive long enough to potentially experience the chronic effects of ADT exposure. The second is the increasing number of effective systemic therapies, extending the life of men in the metastatic setting. Abiraterone Acetate in particular requires long term exposure to not only ADT, but also low dose prednisone, which would be expected to further accelerate loss of BMD.
Although our small study is in a relatively homogeneous patient population, we have demonstrated the potential to measure large regions of the spine revealing some consistent findings. Following from the results from this study, we have initiated a larger prospective trial to investigate the capacity of lumbar spine MRI to predict which men are at higher risk of accelerated loss of BMD while on ADT as treatment for their prostate cancer. Several randomized studies have shown improvements in BMD for unselected men on ADT treated with bisphosphonates, RANKL inhibition or selective oestrogen reuptake modulators\textsuperscript{24}. Although there are numerous guidelines recommending pharmacological intervention for such men mainly on the basis of their T-score on DEXA imaging, given potential toxicities like osteonecrosis of the jaw as well as the expense of such agents there is scope to further target therapy to men most likely to benefit\textsuperscript{24-26}.

Our current study focuses on men with prostate cancer being managed with curative intent with an 18 month course of ADT and pelvic radiotherapy. Due to improved signal to noise ratio and shorter image acquisition time to reduce motion artefact, we will use a 3 Tesla system for this successor study\textsuperscript{11}. We aim to investigate whether multiparametric MRI of the lumbar spine at baseline including both In:Out phase as well as diffusion weighted imaging might contribute to a model combined with clinical and DEXA findings to identify a subgroup of patients at risk of accelerated loss of BMD.
Conclusion

Rapid acquisition of a large range of vertebral bodies with accurate determination of FF with ROI was demonstrated. We have observed the existence of a gradient in Fat Fraction from T10 to S2. There is also a consistent dip in Fat Fraction between L5 and S1, which may be due to the different anatomy and degenerative changes at this level. These findings will be explored in a larger prospective study attempting to use such extra information available on MRI to determine which men are at risk of more rapid loss of BMD while on ADT.

Acknowledgements

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References


**Tables**

**Table 1**: Difference in Fat Fraction between adjacent vertebrae. Note the steady increase at all levels except for L5-S1.

<table>
<thead>
<tr>
<th>Vertebra</th>
<th>Fat Fraction Mean (Range)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T11</td>
<td>29.4% (19.7 – 36.9)</td>
<td></td>
</tr>
<tr>
<td>T12</td>
<td>33.9% (25.1 – 44.5)</td>
<td>4.6%</td>
</tr>
<tr>
<td>L1</td>
<td>37.6% (29.6 – 54.4)</td>
<td>3.7%</td>
</tr>
<tr>
<td>L2</td>
<td>38.6% (32.7 – 54.2)</td>
<td>1.0%</td>
</tr>
<tr>
<td>L3</td>
<td>40.0% (33.8 – 55.1)</td>
<td>1.4%</td>
</tr>
<tr>
<td>L4</td>
<td>44.3% (36.3 – 58.7)</td>
<td>4.3%</td>
</tr>
<tr>
<td>L5</td>
<td>46.6% (37.8 – 60.8)</td>
<td>2.3%</td>
</tr>
<tr>
<td>S1</td>
<td>42.2% (31.5 – 58.5)</td>
<td>-4.4%</td>
</tr>
<tr>
<td>S2</td>
<td>47.9% (33.3 – 66.7)</td>
<td>5.7%</td>
</tr>
</tbody>
</table>
Table 2

L3 MRS FF, with linear regression slope (an indicator of gradient in FF across the adjacent vertebral bodies) and $R^2$ values.

<table>
<thead>
<tr>
<th>Patient</th>
<th>MRS FF at L3</th>
<th>FF Slope (FF%/vertebra)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35.8</td>
<td>2.23</td>
<td>0.94</td>
</tr>
<tr>
<td>2</td>
<td>41.2</td>
<td>2.74</td>
<td>0.82</td>
</tr>
<tr>
<td>3</td>
<td>55.1</td>
<td>3.84</td>
<td>0.83</td>
</tr>
<tr>
<td>4</td>
<td>34.2</td>
<td>1.10</td>
<td>0.81</td>
</tr>
<tr>
<td>5</td>
<td>33.8</td>
<td>3.44</td>
<td>0.96</td>
</tr>
</tbody>
</table>
Figure Legends:

**Figure 1**: Colour enhanced Fat T2 IDEAL sagittal image from a patient demonstrating vertebrae from T9 to S2. Note how the more rostral vertebrae are generally more green coloured than the redder caudal vertebra suggesting increasing fat fraction moving rostrally.

**Figure 2**: Line graphs showing low intraobserver variation in Fat measurements.

**Figure 3**: Fat fraction from T11 to S2 for the five patients measured. Most patients demonstrate a trend of increasing fat fraction moving rostrally.

**Figure 4**: The patient who due to his smaller size was able to have FF estimated for 12 adjacent vertebral bodies showing a range of values of over 50% across the field of view.