

1 Quantifying bone structure, microarchitecture and pathophysiology with
2 magnetic resonance.

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14

15 **I. Introduction**

16 Diseases affecting bone are the second most common cause of disability and are predicted to
17 increase in prevalence in an aging population[1]. Imaging plays an increasingly important role in
18 diagnosis, assessment of treatment response and follow up of diseases affecting bone, and provides
19 a valuable alternative to invasive biopsy. Modern ‘physiological’ imaging techniques provide not only
20 anatomical but also functional information, giving us valuable insights into the bone

21 microenvironment in both health and disease. As imaging delves deeper into tissue physiology, it is
22 increasingly important that radiologists are aware of the effect of tissue pathology on the images
23 they interpret. MRI has the versatility to image these aspects of bone pathology and lends itself to
24 quantitative analysis. Furthermore it can image bone in detail or give an overview of skeletal
25 involvement in disease.

26 Although in clinical practice most images are analysed qualitatively by radiologists, there has been a
27 trend towards using quantitative imaging methods which provide objective physical measurements
28 from tissue such as diffusivity, perfusion or tracer uptake. To quantify is to measure, and
29 quantification within the science of magnetic resonance arose from the ability to measure the NMR
30 properties of biological tissue. This led to attempts to characterise the nature of the tissue using
31 these parameters [2]. Quantitative MRI therefore uses measurable MR parameters to describe
32 tissue, rather than forming an image from non-quantitative values.

33 For a parameter to be clinically useful, it has to reflect a biologically significant process, such as
34 change in a meaningful manner with the exacerbation or resolution of a disease process. There are a
35 number of advantages to truly quantifying MR measurements. It is easier to test for reproducibility,
36 sensitivity and specificity of the measurement. The data are easier to model and assess
37 mathematically, and have the potential for machine learning and population studies. There is also
38 the potential for automation of assessment in a manner not amenable to qualitative data.

39 In this work, we provide a brief overview of bone physiology and pathophysiology, before
40 considering how magnetic resonance (MR) techniques can be used to 'probe' these physiological
41 and pathophysiological processes.

42 II. Bone Physiology and Pathology

43 1. What is Bone?

44 Although the skeleton is sometimes viewed as a simple structural support for the body, it is
45 increasingly clear that bone is in fact an active, dynamic organ, which plays a central role in the
46 coordination of metabolic, endocrine and haematological processes. Bone is integral to the
47 homeostasis of minerals such as calcium and phosphate, serves as a reservoir of growth factors and
48 is the cradle of haematopoiesis[3].

49 The skeleton is composed of around 80% cortical bone and 20% trabecular bone [4]. Cortical bone is
50 dense and composed of a branching network of cylindrical osteons called Haversian systems.

51 Trabecular bone consists of osteons called packets arranged in a honeycomb pattern. The non-
52 mineralised component of bone is called bone marrow and consists of adipocytes (yellow marrow)
53 and haematopoietic cells (red marrow). The outer cortical surface of bone is covered in periosteum,
54 except at joints, and the inner surface is covered by endosteum. Periosteum is a fibrous connective
55 tissue whereas the endosteum is a membranous structure; both contain blood vessels, osteoblasts
56 and osteoclasts.

57 The major cellular constituents of bone are osteoclasts, osteoblasts and osteocytes, which are
58 surrounded by mineralised extracellular matrix. Osteoblasts synthesise bone matrix and regulate
59 mineralisation by releasing vesicles that contain calcium and phosphate. The mineralised matrix of
60 bone consists of collagenous proteins (mainly type I collagen) and bone mineral, which is mainly
61 hydroxyapatite (4). Osteoblasts, which are surrounded by and buried within this matrix, then
62 differentiate into osteocytes. A biochemical network forms connecting bone surface lining cells and
63 osteocytes. Their main function is to transduce mechanical stress into a biological response by
64 signalling to the network of osteocytes and osteoblasts. Osteoclasts play a central role in bone
65 remodelling and are the only cell capable of resorbing bone.

66 Bone is a dynamic structure, which undergoes growth, modelling and remodelling during life under
67 influences from mechanical forces, metabolic factors and hormonal action. Bone remodelling is a
68 continuous process where units of old bone are removed and replaced by new proteinaceous
69 matrix, which is then mineralised. [4]. Regulation of osteoclast mediated bone resorption is under
70 the influence of parathyroid hormone, vitamin D and calcitonin. Mineralisation of the matrix is
71 regulated by osteoblasts and this modulates serum levels of calcium and phosphate under the
72 influence of vitamin D. After a cycle of remodelling, 50 to 70% of osteoblasts undergo apoptosis and
73 the others become osteocytes and bone lining cells[4]. Abnormal modelling can be activated in
74 disease states such as multiple myeloma where osteoclasts are activated by bone lining cells
75 expressing tartrate-resistant acid phosphatase due to an abnormal microenvironment created by
76 plasma cell infiltration [4].

77 One of the most important functions of bone is haematopoiesis. The haematopoietic system is
78 responsible for producing more than 100 billion mature blood cells a day [3]. Haematopoietic stem
79 cells reside in the endosteum termed 'the haematopoietic niche' and have a rich vascular supply.
80 The interactions between bone microenvironment and haematopoiesis are complex but its
81 understanding is increasing rapidly. In particular, the bone microenvironment has been shown to
82 play an important role in the pathogenesis of many diseases. For instance in leukaemia, bone
83 marrow infiltration can suppress and stimulate osteoblasts [5]. Metastatic cancer cells have been
84 shown to compete with haematopoietic cells for resources [5]. Hormones also influence the
85 haematopoietic microenvironment. Both parathyroid hormone and oestrogen have been shown to
86 have a role in modulation of the haematopoietic stem cells [3].

87

88 2. Bone Pathology

89 A useful way of classifying bone pathology is by micro-architectural changes, which radiologists can
90 infer from imaging. New imaging techniques can detect abnormalities in density, quality, porosity,
91 cellularity, the presence of fibrosis and fat content.

92 i. Change in cellularity

93 Bone cellularity is increased in pathological processes such as malignancy, infection and
94 inflammation. These pathological processes can be further classified by which compartment they
95 affect. For instance, primary and secondary bone tumour, infection and inflammation cause a
96 change in cellular density and alter the size of the extracellular space. On the other hand, abnormal
97 mineralisation or fibrosis in the extracellular space can cause increased packing of cells. The
98 microenvironment of bone changes early and rapidly in aggressive processes. Rapid increases in
99 bone cellularity cause a loss of fat, destruction of bone trabeculae and formation of new blood
100 vessels, which can be quantified by MR techniques.

101

102 ii. Change of Vascularity

103 Bone is highly vascular and changes in vascularity can be a useful indicator of disease. Perfusion of
104 bone is increased in inflammation and neoplasia. Reduced perfusion is seen in patients with
105 peripheral vascular disease and with red cell abnormalities such as sickle cell anaemia.

106 The effect of reduced perfusion of bone can be seen as fairly characteristic lesions on MR. The
107 earliest imaging sign of bone infarction is bone oedema, which represents cytotoxic oedema. In the
108 chronic phase, fibrosis of the marrow and sclerosis of bone is seen.

109 Increased perfusion to bone can occur in various pathological states. Perfusion of tissues is complex
110 and involves various compartments. One of the simplest models explaining tissue perfusion uses two
111 compartments: blood plasma and the interstitial space[6]. For a given cardiac output, increased

112 tissue perfusion can be due to increased permeability of existing vessels or an increase in the
113 number of blood vessels supplying tissue. Both increased permeability and neo-angiogenesis exist in
114 inflammation and neoplasia; and can be detected by MR techniques.

115 iii. Change of bone remodelling

116

117 Many disease processes affecting bone lead to bone fragility characterised by a decrease in bone
118 mass and quality. Bone quality depends on several factors such as bone mineralisation, remodelling
119 rate, number of micro-fractures and microarchitecture [7]. Bone loss takes place due to remodelling
120 imbalances in the activity of osteoclasts and osteoblasts. Several factors can perturb this balance
121 from changes in hormone concentration in osteoporosis to inflammatory cytokines in rheumatoid
122 disease[8].

123 When this balance is tipped in the favour of bone loss in osteoporosis, there is a reduction in bone
124 mass with thinning of trabeculae and increased porosity of cortical bone. Although the thinning of
125 the trabecular network is well recognised, cortical porosity has been less well studied due to the
126 challenges in its imaging. Traditional approaches have measured cortical bone thickness, which does
127 not fully characterise its quality. In fact the degree of porosity is considered the main microstructural
128 feature of the cortex [9]. Porosity may seem like a property that leads to an inherent mechanical
129 weakness of bone but it serves an important purpose. The vascular channels are required to sustain
130 and nourish the syncytium of interconnected osteocytes, whereas the nanopores play an important
131 role in mechanosensation [10]. Although the mechanical cost of porosity is small in healthy bone, in
132 pathological states, such as chronic kidney disease, disuse and parathyroid treatment, increased
133 porosity leads to bone fragility [9]. Geographical increases in porosity due to inefficient
134 redistribution of bone mass is associated with increase rates of fracture in patients with diabetic
135 patients [11].

136 III. Imaging Methods

137 i. Diffusion weighted imaging

138 Diffusion weighted imaging assesses the Brownian motion of water in its microscopic environment.
139 The signal detected reflects the movement of water at a micrometre scale beyond the usual
140 millimetre resolution of MRI. The diffusion-weighted image however is affected by other parameters
141 other than diffusion such as tissue perfusion and T1 and T2 relaxation times. The DWI image is
142 constructed by applying diffusion sensitising gradients to a T2 weighted image. The degree of
143 diffusion sensitisation is defined by the 'b value'; with lower b values providing perfusion weighting
144 and higher b values providing diffusion weighting [12]. However as T1 and T2 relaxation times of
145 tissue vary under different physiological and pathological conditions, diffusion weighted imaging can
146 be difficult to interpret. Apparent diffusion coefficient calculations can quantify the diffusion effect
147 by using two or more acquisitions at different b values[13]. The ADC value is only truly accurate if
148 water diffusion behaves freely but in tissue it remains useful as it gives a summary of the diffusion
149 characteristics at a voxel level. Diffusion is restricted when molecules encounter boundaries which
150 prevent free movement and in human tissue the main boundaries are cell membranes [14]. The
151 variation of ADC in physiological or pathological conditions is thought to be due to the effect of
152 processes largely affecting the extracellular space. The contraction of the extracellular space from
153 cell proliferation or swelling causes restricted diffusion as indicated by a decrease in ADC. With
154 improving technology, higher b values can be achieved and, with more complex analyses, may reveal
155 intracellular space and membrane interactions [15].

156 ADC values of bone correlate with bone marrow cellularity and micro vessel density in the
157 extracellular space and this has been shown to be useful in neoplastic conditions. For instance, ADC
158 can increase in osteosarcoma following chemotherapy, indicating tumour response even when no
159 reduction in tumour size has occurred [Figure 1] [16]. In multiple myeloma, whole body DWI has
160 been shown to be a highly sensitive technique for quantifying disease burden [17] and can detect

161 early treatment response, relapse and progression even when not captured by serum and urine
162 analysis [Figure 2] [18]. With successful treatment, the volume of cell infiltration decreases and
163 there is less restriction to free water movement, leading to an increase in ADC values [19]. The use
164 of several b-values allows differentiation between perfusion and diffusion effects on signal in bone
165 marrow. Both rapid micro perfusion, which causes a fast initial decay due to abnormal blood vessel
166 proliferation, and slower signal decay related to diffusion in the interstitial space can be evaluated
167 by the intra-voxel incoherent motion model in myeloma [20]. The ability to measure difference in
168 metrics allows for a quantitative assessment of disease burden, which can be monitored on follow
169 up studies.

170 DWI is effective in early diagnosis of sacroiliitis and monitoring treatment response in patients with
171 seronegative spondyloarthropathies [21,22]. ADC values are significantly higher in patients even in
172 the early stages of ankylosing spondylitis compared to normal controls [23]. In enthesitis related
173 arthritis DWI measurements reflect the response to anti-TNF therapies and are more objective than
174 visual scoring [22]. Computation tools have been developed to quantify bone ADC values which are
175 comparable to conventional STIR sequences [21]. DWI has been shown to be useful in the
176 assessment of hip ischaemia in patients with Legg-Calve-Perthes disease [24], and in particular,
177 median ADC ratios have reported as a reproducible means of assessing hip ischaemia.

178 ii. Dynamic Contrast Enhancement

179 DCE-MRI is based on rapid acquisition of images after contrast injection allowing quantification of
180 tissue perfusion and kinetics. The basis of DCE MRI is the rapid acquisition of a series of T1-weighted
181 images before and after infusion of a T1-shortening, diffusible contrast medium [6]. These can
182 provide a detailed time-intensity curve which can then be used to estimate the concentration of
183 contrast medium in the region of interest [25].

184 DCE-MRI is useful in assessing microcirculation of bone marrow infiltrated by tumour. Tumour
185 angiogenesis in myeloma leads to increased uptake of contrast and this subsequently decreases with

186 effective therapy [26]. In myeloma, DCE-MRI has been shown to be useful in distinguishing hyper-
187 cellular haematopoietic marrow from neoplastic marrow. Perfusion changes can occur early in
188 treatment response as has been shown in osteosarcoma correlating with histological necrosis [27].
189 DCE MRI lends itself to quantitative analysis. Semi-quantitative analysis is based on the time to
190 intensity graph, which can be used to calculate various metrics such as time to peak and area under
191 the curve. The early phase of enhancement reflects tissue micro-vascularisation and the later phases
192 of washout reflect capillary permeability and interstitial space enhancement [28]. Quantitative
193 perfusion analysis uses pharmacokinetic models to explain contrast exchange between the
194 intravascular and extravascular space. There are three principle parameters: the transfer constant
195 K_{trans} , the extravascular extracellular space fractional volume (V_c) and K_{cp} (backflow transfer constant)
196 [26]. In highly permeable scenarios, the transfer constant is equal to blood plasma flow per unit
197 volume of tissue and in low permeability it depends on the permeability between blood plasma and
198 the extravascular extracellular space. Characteristic perfusion patterns can aid the diagnosis of
199 osteoid osteomas, osteblastomas, and giant cell tumours of bone [29].

200

201 iii. Chemical Shift-Encoded Imaging

202 Chemical shift-encoded imaging (CSI) was first described by WT Dixon, using a simple modification of
203 a spin echo sequence to acquire 'fat-water in phase' and 'fat-water opposed phase' images,
204 facilitating the generation of water-only and fat-only images [30]. Although there were a number of
205 technical limitations with the original technique, this technology has now developed to the point
206 where fast, accurate and quantitative CSI is relatively easy to implement on most clinical scanners.
207 Modern CSI typically uses multi-echo spoiled gradient echo (SPGR) sequences, with data acquisition
208 at multiple echo times (usually between 3 and 8). There are a variety of analytic tools available that
209 can generate fat fraction maps, for example 'Iterative Decomposition with Echo Asymmetry and

210 Least squares (IDEAL)' [31]. Each pixel has a value of between 0 (pure water), and 1 (pure fat). In
211 normal bone marrow, most pixels have a value around 0.5, indicating approximately equal signal
212 contributions from water and fat.

213 CSI is particularly useful for disorders, which affect the bone marrow, where pathological processes
214 tend to cause either an increase or a decrease in fat content. For example, a number of authors have
215 demonstrated a reciprocal relationship between marrow fat and bone mineral density in patients
216 with osteoporosis, leading to investigation of FF as a biomarker in osteoporosis [32–35]. Similarly, in
217 obese patients, marrow fat has an adverse effect on bone microarchitecture [36]. Interestingly,
218 patients with anorexia nervosa undergo an increase in marrow fat content despite losses in overall
219 body fat, possibly because marrow adipose tissue undergoes a homeostatic change designed to
220 increase appetite and insulin sensitivity [37,38].

221 In patients with metastatic cancer, tumour cells infiltrating the marrow effectively 'displace' the
222 normal fatty marrow and therefore cause a reduction in FF. For example, symptomatic multiple
223 myeloma patients have significantly lower FF measurements than those with symptomatic disease
224 [39]; FF measurements can potentially also be used to stratify patients according to their depth of
225 response to treatment [Figure 3] [40].

226 An interesting recent development is the use of CSI to quantify inflammation in patients with
227 spondyloarthritis. Areas of 'active' juxta-articular inflammation (bone marrow oedema) cause a
228 reduction in FF, whereas chronically inflamed sites (fat metaplasia) undergo an increase in FF [Figure
229 4] [41]. FF measurements could therefore be useful as a marker of inflammatory disease severity
230 and activity. A key advantage of CSI in this setting is that disease severity can be assessed directly
231 from the image, removing the need for subjective interpretation by a radiologist.

232

233 iv. Ultra short TE and Zero-TE

234 The MR signal intensity of a voxel containing tissue is dependent on many factors including the mean
235 transverse relaxation time (T2 and T2*) of the tissue being examined in a particular voxel. Tissues
236 are heterogeneous and are composed of a spectrum of transverse relaxation times. Bone, especially
237 cortical bone, contains a high fraction of components with ultrashort transverse relaxation times,
238 which are in the order of 0.39-0.5 msec. However ultrashort time to echo (UTE) sequences including
239 zero TE using short minimum echo times below 1 msec are now able to interrogate the
240 microarchitecture of bone. One of the main challenges in cortical bone imaging is the contamination
241 of signal from muscle and fat, which is being addressed by novel subtraction techniques [42] These
242 techniques have produced promising quantitative cortical bone maps [Figure 5]. Zero-TE sequences
243 differ from other ultrashort TE sequences because the readout gradient is applied prior to excitation.
244 It has some advantages over other UTE sequences including reduced eddy current effects and
245 minimal acoustic noise due to the elimination of rapidly switching gradients in between TRs.

246 UTE sequences have been used to study cortical porosity by characterising bound water versus free
247 water. Porosity is an important determinant of bone quality and strength[43]. A study has shown
248 that indirect measurements of porosity and T2 relaxation times of cortical bone may be correlated
249 with its material property; for instance short T2 relaxation times have been shown to correlated with
250 failure strain in cadaveric femoral bone [44]. Zero TE sequences have been used to study in vivo
251 trabecular bone architecture [45].

252

253

3. Imaging at the Extremes of Scale: From Single Voxel Spectroscopy to Whole

Body Imaging

i. MR Spectroscopy

MR Spectroscopy (MRS) provides information on the molecular composition of tissue and has been used in the brain to characterise lesions but it also shows promise for bone lesions and bone marrow imaging. MRS spectra can be acquired from nuclei, which have non-zero spin such as protons, carbon-13 (^{13}C), sodium (^{23}Na) and phosphorus-31 (^{31}P). In musculoskeletal imaging, proton MRS has been studied the most in the context of tumour and fat characterisation. ^{31}P spectroscopy requires specialised hardware and provide lower spatial resolution [46] but has been used to investigate energy metabolism in normal and diseased states. Sodium MRI images the sodium nuclei of tissues but in musculoskeletal imaging, it remains a research tool primarily in early osteoarthritis looking at hyaline cartilage proteoglycan losses [47].

The most common methods of fat characterisation by proton MRS are single voxel methods such as stimulated echo acquisition mode (STEAM). Single voxel methods are simpler, faster and suffer less from magnetic field inhomogeneity compared to multivoxel techniques [46]. Aggressive bone lesions demonstrate high cell membrane turnover and studies have shown that the metabolite choline, which encompasses free choline, phosphocholine and glycerophosphocholine, is increased in malignant lesions [43]. Early studies on MR spectroscopy of bone lesions used a qualitative assessment of choline content but more recent studies have calculated absolute choline concentrations [48]. There are limitations to this method but there is a movement towards quantitative assessments of the tumour metabolic signature in the literature [43]. MRS therefore shows promise in increasing sensitivity and specificity of MR in detecting malignancy and therefore obviating invasive biopsies.

^1H -MRS has also been used to study the triglyceride chemical composition of bone marrow in vivo [49] and elevated marrow lipid content has been found in patients with osteoporosis and osteopenia

279 [50]. Since lipid peaks in marrow are usually incompletely resolved on ¹H-MRS, the application of
280 prior knowledge in spectral analysis can enable the reliable assessment of overlapping lipid peaks
281 [51]. Provided that signal contributions from individual lipid peaks can be identified and measured,
282 ¹H-MRS can also assess changes in lipid composition that occur in osteoporosis.

283

284 ii. Micro-MRI

285 Micro-MRI provides high resolution imaging of bone allowing the evaluation of both cortical and
286 trabecular properties at a scale of 100-200 micrometres (in plane resolution)[52]. It rivals and
287 performs similarly to high-resolution peripheral quantitative computed tomography (HR-pQCT)
288 without using ionising radiation. Micro-MRI use sequences such as spoiled gradient echo, balanced
289 steady state free precession (b-SSFP) and fast large spin echo (FALSE) to provide exquisite detail of
290 bone [53][54]. Metrics such as bone volume fraction, topology and orientation can be quantified
291 which correlate well with equivalent CT measurements [55].

292 Micro-finite-element analysis can be applied to high resolution data sets to analyse the examined 3D
293 trabecular network and estimate mechanical properties such as stiffness and elastic modulus [56]..
294 Furthermore 3D voxel models can be fed into a micro-finite-element stimulator, which can model
295 the change in parameters in response to intervention and predict the mechanical implications of
296 hormonal treatments such as in osteoporosis [20].

297 iii. Whole Body MRI

298 From detailed imaging of the microarchitecture of bone, WBMRI images abnormalities throughout
299 the skeleton. This approach is useful for systemic conditions affecting bone such as haematological
300 malignancies, bone metastases and rheumatological disorders. Studies comparing WB-MRI and PET-
301 CT on a lesion by lesion basis have shown higher overall diagnostic accuracy for WB-MRI [57].
302 Furthermore whole body data sets using functional sequences such as DWI and DCE can be used to
303 create quantitative maps of disease burden and activity [58]. DWI lends itself to easy delineation of

304 bone lesions with semi-automated segmentation software. This has been used to quantify burden of
305 disease which correlates with established prognostic biomarkers [59]. Furthermore ADC changes in
306 individual lesions and globally in the whole body can be used to determine treatment response in
307 patients with metastatic bone disease and myeloma [60,61].

308 WBMRI has become the gold standard for assessment of multiple myeloma as it is more sensitive for
309 marrow infiltration by plasma cells compared to conventional radiography and CT [Figure 2] [62]. MR
310 imaging patterns of bone marrow involvement have been shown to have prognostic value (diffuse
311 disease has a better outcome than focal lesions) and correlate with 5 year survival rate in patients
312 treated with autologous bone marrow transplantation[63]. WB-MRI outperforms bone scintigraphy
313 in the detecting metastatic bone disease from solid cancers as shown in meta-analyses[64].

314 In the setting of ankylosing spondylitis, WBMRI allows the global assessment of both acute and
315 chronic involvement of the axial and peripheral skeleton. Detecting pre-structural changes are
316 important in diagnosing the condition early allowing for early aggressive treatment and improving
317 patient outcome [65].

318 The main obstacles to the widespread adoption of WB-MRI are related to access to scanners, and
319 lack of radiological expertise. Scans can be long but with careful planning and the use of fast
320 imaging sequences (such as Dixon scans), whole body scans with both morphological and functional
321 imaging can be achieved in as little as 30 minutes [66].

322 WB-MRI data sets represent a daunting amount of information for radiologists to read. However
323 with standardisation in MRI acquisition and validated biomarkers, automatic segmentation will help
324 radiologists analyse image sets rapidly and understand disease phenotypes at a population level.

325 There are a number of techniques which are being refined but the most promising are based on
326 machine learning [67].

327

328 IV. Conclusion

329 Bone imaging is changing. MRI can provide anatomical and functional information and
330 multiparametric and quantitative techniques offer a new insight into bone disease. These techniques
331 have the potential for improvement of disease diagnosis, assessment of disease activity and
332 treatment response, and for prognostication. Using computational methods it may be possible to
333 create a comprehensive anatomical map of disease with quantitative metrics on disease activity and
334 bone quality. These data have the potential for early treatment stratification and therapeutic
335 escalation where necessary.

336 The challenge for radiologists is identifying which parameters add clinical value. Currently there are a
337 number of techniques, which provide interesting data about disease processes but there is a lack of
338 evidence comparing these techniques in a quantitative way to determine the quality of diagnostic
339 information. Furthermore, there is a lack of economic analyses comparing different techniques and
340 on the evaluation of the impact on patient outcome. Further research is necessary to assess the true
341 impact of quantitative bone measurements on disease management and outcome.

342

343

344

345

346 Figures

347 Figure 1

348 MRI images of the lower limb of a 26-year-old male with osteosarcoma before and after two cycles
349 of chemotherapy: Coronal T2W showing a metaphyseal lesion (a1, arrow), which has not changed in

350 size post treatment (a2). Axial m-Dixon fat only image (b1) shows no appreciable difference post
351 treatment (b2). Axial DWI b1000 image (c1) showing a penumbra of high signal in the lateral aspect
352 of the lesion before treatment which is of lower signal post treatment (c2). Axial ADC map (d1)
353 shows corresponding low ADC in the periphery suggestive of cellular tumour, which increases post
354 treatment suggestive of response to treatment (d2) (Images courtesy of Dr. Harbir Sidhu, University
355 College London Hospital).

356 Figure 2

357 Representative MR images showing a bone lesion in the right pelvis of a patient with Multiple
358 Myeloma before and 8 weeks after treatment. Focal lesion (arrow) demonstrated on (A) coronal
359 pre-contrast fat-only mDixon, (B) pre-contrast water-only mDixon, (C) post-contrast water only
360 mDixon and (D) b1000 diffusion weighted imaging at baseline (A1–D1) and 8 weeks (A2–D2) in a
361 patient who achieved very good partial response after induction chemotherapy (images courtesy of
362 Dr. Dr Arash Latifoltojar, University College London Hospital).

363 Figure 3

364 Whole body chemical shift-encoded MR (CSE-MR) images from a patient with multiple myeloma. Fat
365 only (A), water only (B) images, and fat fraction maps (C), are shown from left to right. A diffuse
366 pattern of cellular infiltration of the vertebral bodies and iliac wings is demonstrated bilaterally in
367 contrast to the normal fatty composition of the femoral and tibial bone marrow (images courtesy of
368 Dr. Arash Latifoltojar, University College London Hospital).

369 Figure 4

370 Quantifying disease in Spondyloarthritis by Fat fraction mapping (PDFF – proton density fat fraction).
371 Coronal images of the sacroiliac joints show areas of periarticular bone marrow oedema (a,b).
372 Arrowed regions show high signal on the STIR image (a) and a reduction in fat fraction (b).

373 Conversely, areas of fat metaplasia (c,d), which arise in areas of previous inflammation, show high
374 signal on the T1-weighted image (c), and increased fat fraction (d).

375 Figure 5

376 Cortical bone maps generated from phase sensitive dual inversion recovery subtraction using
377 Ultrashort Echo time (UTE) MRI. Axial image of the tibia and fibula showing high signal in the cortical
378 bone and no signal from surrounding fat or muscle (images courtesy of Professor Graeme Bydder,
379 UCSD).

380

381 References

- 382 [1] Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, et al. Years lived with disability
383 (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the
384 Global Burden of Disease Study 2010. *Lancet* (London, England) 2012;380:2163–96.
385 doi:10.1016/S0140-6736(12)61729-2.
- 386 [2] Tofts P. *Quantitative MRI of the Brain: Measuring Changes Caused by Disease*. 2005.
387 doi:10.1002/0470869526.
- 388 [3] Taichman RS. Blood and bone: two tissues whose fates are intertwined to create the
389 hematopoietic stem-cell niche. *Blood* 2005;105:2631–9. doi:10.1182/blood-2004-06-2480.
- 390 [4] Clarke B. Normal bone anatomy and physiology. *Clin J Am Soc Nephrol* 2008;3 Suppl 3:S131–
391 9. doi:10.2215/CJN.04151206.
- 392 [5] Lin S, Ouyang T, Kanekar S. Imaging of Bone Marrow. *Hematol Oncol Clin North Am*
393 2016;30:945–71. doi:10.1016/j.hoc.2016.03.012.
- 394 [6] Tofts PS, Brix G, Buckley DL, Evelhoch JL, Henderson E, Knopp M V, et al. Estimating kinetic

- 395 parameters from dynamic contrast-enhanced T(1)-weighted MRI of a diffusable tracer:
396 standardized quantities and symbols. *J Magn Reson Imaging* 1999;10:223–32.
- 397 [7] Komarov FI, Bkarev IN, Smolianitskiĭ AI. NIH Consensus Development Panel on Osteoporosis
398 Prevention, Diagnosis, and Therapy. *Jama* 2001;285:785–95. doi:10.1097/00007611-
399 200106000-00005.
- 400 [8] Oei L, Koromani F, Rivadeneira F, Zillikens MC, Oei EHG. Quantitative imaging methods in
401 osteoporosis. *Quant Imaging Med Surg* 2016;6:680–98. doi:10.21037/qims.2016.12.13.
- 402 [9] McCalden RW, McGeough JA, Barker MB, Court-Brown CM. Age-related changes in the
403 tensile properties of cortical bone. The relative importance of changes in porosity,
404 mineralization, and microstructure. *J Bone Joint Surg Am* 1993;75:1193–205.
- 405 [10] Cooper DML, Kawalilak CE, Harrison K, Johnston BD, Johnston JD. Cortical Bone Porosity:
406 What Is It, Why Is It Important, and How Can We Detect It? *Curr Osteoporos Rep*
407 2016;14:187–98. doi:10.1007/s11914-016-0319-y.
- 408 [11] Patsch JM, Burghardt AJ, Yap SP, Baum T, Schwartz A V, Joseph GB, et al. Increased cortical
409 porosity in type 2 diabetic postmenopausal women with fragility fractures. *J Bone Miner Res*
410 2013;28:313–24. doi:10.1002/jbmr.1763.
- 411 [12] Yao K, Troupis JM. Diffusion-weighted imaging and the skeletal system: a literature review.
412 *Clin Radiol* 2016;71:1071–82. doi:10.1016/j.crad.2016.07.007.
- 413 [13] Le Bihan D. Apparent diffusion coefficient and beyond: what diffusion MR imaging can tell us
414 about tissue structure. *Radiology* 2013;268:318–22. doi:10.1148/radiol.13130420.
- 415 [14] Tanner JE. Self diffusion of water in frog muscle. *Biophys J* 1979;28:107–16.
416 doi:10.1016/S0006-3495(79)85162-0.
- 417 [15] Panagiotaki E, Walker-Samuel S, Siow B, Johnson SP, Rajkumar V, Pedley RB, et al.

- 418 Noninvasive quantification of solid tumor microstructure using VERDICT MRI. *Cancer Res*
419 2014;74:1902–12. doi:10.1158/0008-5472.CAN-13-2511.
- 420 [16] Wang C-S, Du L-J, Si M-J, Yin Q-H, Chen L, Shu M, et al. Noninvasive assessment of response
421 to neoadjuvant chemotherapy in osteosarcoma of long bones with diffusion-weighted
422 imaging: an initial in vivo study. *PLoS One* 2013;8:e72679. doi:10.1371/journal.pone.0072679.
- 423 [17] Pawlyn C, Fowkes L, Otero S, Jones JR, Boyd KD, Davies FE, et al. Whole-body diffusion-
424 weighted MRI: a new gold standard for assessing disease burden in patients with multiple
425 myeloma? *Leukemia* 2016;30:1446–8. doi:10.1038/leu.2015.338.
- 426 [18] Giles SL, Messiou C, Collins DJ, Morgan VA, Simpkin CJ, West S, et al. Whole-Body Diffusion-
427 weighted MR Imaging for Assessment of Treatment Response in Myeloma. *Radiology*
428 2014;271:131529. doi:10.1148/radiol.13131529.
- 429 [19] Horger M, Weisel K, Horger W, Mroue A, Fenchel M, Lichy M. Whole-body diffusion-weighted
430 MRI with apparent diffusion coefficient mapping for early response monitoring in multiple
431 myeloma: preliminary results. *AJR Am J Roentgenol* 2011;196:W790-5.
432 doi:10.2214/AJR.10.5979.
- 433 [20] Wehrli FW. Structural and functional assessment of trabecular and cortical bone by micro
434 magnetic resonance imaging. *J Magn Reson Imaging* 2007;25:390–409.
435 doi:10.1002/jmri.20807.
- 436 [21] Vendhan K, Bray TJP, Atkinson D, Punwani S, Fisher C, Sen D, et al. A diffusion-based
437 quantification technique for assessment of sacroiliitis in adolescents with enthesitis-related
438 arthritis. *Br J Radiol* 2016;89:20150775. doi:10.1259/bjr.20150775.
- 439 [22] J. P. Bray T, Vendhan K, Ambrose N, Atkinson D, Punwani S, Fisher C, et al. Diffusion-weighted
440 imaging is a sensitive biomarker of response to biologic therapy in enthesitis-related arthritis.

- 441 Rheumatology 2016;kew429. doi:10.1093/rheumatology/kew429.
- 442 [23] Ai F, Ai T, Li X, Hu D, Zhang W, Morelli JN. Value of diffusion-weighted magnetic resonance
443 imaging in early diagnosis of ankylosing spondylitis. *Rheumatol Int* 2012;32:4005–13.
444 doi:10.1007/s00296-011-2333-9.
- 445 [24] Boutault J-R, Baunin C, Bérard E, Vial J, Labarre D, Domenech C, et al. Diffusion MRI of the
446 neck of the femur in Legg-Calve-Perthes disease: a preliminary study. *Diagn Interv Imaging*
447 2013;94:78–83. doi:10.1016/j.diii.2012.10.003.
- 448 [25] Hodgson R, Grainger A, O’Connor P, Barnes T, Connolly S, Moots R. Dynamic contrast
449 enhanced MRI of bone marrow oedema in rheumatoid arthritis. *Ann Rheum Dis*
450 2008;67:270–2. doi:10.1136/ard.2007.077271.
- 451 [26] Moehler TM, Hawighorst H, Neben K, Egerer G, Hillengass J, Max R, et al. Bone marrow
452 microcirculation analysis in multiple myeloma by contrast-enhanced dynamic magnetic
453 resonance imaging. *Int J Cancer* 2001;93:862–8.
- 454 [27] Guo J, Reddick WE, Glass JO, Ji Q, Billups CA, Wu J, et al. Dynamic contrast-enhanced
455 magnetic resonance imaging as a prognostic factor in predicting event-free and overall
456 survival in pediatric patients with osteosarcoma. *Cancer* 2012;118:3776–85.
457 doi:10.1002/cncr.26701.
- 458 [28] Fayad LM, Jacobs MA, Wang X, Carrino JA, Bluemke DA. Musculoskeletal Tumors: How to Use
459 Anatomic, Functional, and Metabolic MR Techniques. *Radiology* 2012;265:340–56.
460 doi:10.1148/radiol.12111740.
- 461 [29] Teixeira P, Beaumont M, Gabriela H, Bailiang C, Verhaeghe J, Sirveaux F, et al. Advanced
462 Techniques in Musculoskeletal Oncology: Perfusion, Diffusion, and Spectroscopy. *Semin*
463 *Musculoskelet Radiol* 2015;19:463–74. doi:10.1055/s-0035-1569250.

- 464 [30] Dixon WT. Simple proton spectroscopic imaging. *Radiology* 1984;153:189–94.
465 doi:10.1148/radiology.153.1.6089263.
- 466 [31] Reeder SB, Pineda AR, Wen Z, Shimakawa A, Yu H, Brittain JH, et al. Iterative decomposition
467 of water and fat with echo asymmetry and least-squares estimation (IDEAL): Application with
468 fast spin-echo imaging. *Magn Reson Med* 2005;54:636–44. doi:10.1002/mrm.20624.
- 469 [32] Wren TAL, Chung SA, Dorey FJ, Bluml S, Adams GB, Gilsanz V. Bone marrow fat is inversely
470 related to cortical bone in young and old subjects. *J Clin Endocrinol Metab* 2011;96:782–6.
471 doi:10.1210/jc.2010-1922.
- 472 [33] Shen W, Chen J, Gantz M, Punyanitya M, Heymsfield SB, Gallagher D, et al. MRI-measured
473 pelvic bone marrow adipose tissue is inversely related to DXA-measured bone mineral in
474 younger and older adults. *Eur J Clin Nutr* 2012;66:983–8. doi:10.1038/ejcn.2012.35.
- 475 [34] Cohen A, Dempster DW, Stein EM, Nickolas TL, Zhou H, McMahon DJ, et al. Increased marrow
476 adiposity in premenopausal women with idiopathic osteoporosis. *J Clin Endocrinol Metab*
477 2012;97:2782–91. doi:10.1210/jc.2012-1477.
- 478 [35] Griffith JF, Yeung DKW, Antonio GE, Lee FKH, Hong AWL, Wong SYS, et al. Vertebral bone
479 mineral density, marrow perfusion, and fat content in healthy men and men with
480 osteoporosis: dynamic contrast-enhanced MR imaging and MR spectroscopy. *Radiology*
481 2005;236:945–51. doi:10.1148/radiol.2363041425.
- 482 [36] Bredella MA, Lin E, Gerweck A V., Landa MG, Thomas BJ, Torriani M, et al. Determinants of
483 bone microarchitecture and mechanical properties in obese men. *J Clin Endocrinol Metab*
484 2012;97:4115–22. doi:10.1210/jc.2012-2246.
- 485 [37] Devlin, MJ, Cloutier, AM, Thomas N. Caloric restriction leads to high marrow adiposity and
486 low bone mass in growing mice. *J Bone Min Res* 2010;25.

- 487 [38] Bredella M a., Fazeli PK, Miller KK, Misra M, Torriani M, Thomas BJ, et al. Increased bone
488 marrow fat in anorexia nervosa. *J Clin Endocrinol Metab* 2009;94:2129–36.
489 doi:10.1210/jc.2008-2532.
- 490 [39] Takasu M, Kaichi Y, Tani C, Date S, Akiyama Y, Kuroda Y, et al. Iterative decomposition of
491 water and fat with echo asymmetry and least-squares estimation (IDEAL) magnetic resonance
492 imaging as a biomarker for symptomatic multiple myeloma. *PLoS One* 2015;10:e0116842.
493 doi:10.1371/journal.pone.0116842.
- 494 [40] Latifoltojar, A, Hall-Craggs, M, Rabin, N, Popat, R, Bainbridge, A, Dikaios, N, Sokolska, M,
495 Rismani, A, D'Sa, S, Punwani, S, Yong K. Whole body magnetic resonance imaging in newly
496 diagnosed multiple myeloma: early changes in lesional signal fat fraction predict disease
497 response. *Br J Haematol* 2016. doi:10.1111/bjh.14401.
- 498 [41] Bray TJP, Bainbridge A, Punwani S, Ioannou Y, Hall-Craggs MA. Simultaneous Quantification of
499 Bone Edema/Adiposity and Structure in Inflamed Bone Using Chemical Shift-Encoded MRI in
500 Spondyloarthritis. *Magn Reson Med* 2017. doi:10.1002/mrm.26729.
- 501 [42] Carl M, Bydder GM, Du J. UTE imaging with simultaneous water and fat signal suppression
502 using a time-efficient multispoke inversion recovery pulse sequence. *Magn Reson Med*
503 2016;76:577–82. doi:10.1002/mrm.25823.
- 504 [43] Wang C-K, Li C-W, Hsieh T-J, Chien S-H, Liu G-C, Tsai K-B. Characterization of bone and soft-
505 tissue tumors with in vivo ¹H MR spectroscopy: initial results. *Radiology* 2004;232:599–605.
506 doi:10.1148/radiol.2322031441.
- 507 [44] Bae WC, Chen PC, Chung CB, Masuda K, D'Lima D, Du J. Quantitative ultrashort echo time
508 (UTE) MRI of human cortical bone: correlation with porosity and biomechanical properties. *J*
509 *Bone Miner Res* 2012;27:848–57. doi:10.1002/jbmr.1535.

- 510 [45] Weiger M, Stampanoni M, Pruessmann KP. Direct depiction of bone microstructure using MRI
511 with zero echo time. *Bone* 2013;54:44–7. doi:10.1016/j.bone.2013.01.027.
- 512 [46] Subhawong TK, Wang X, Durand DJ, Jacobs MA, Carrino JA, Machado AJ, et al. Proton MR
513 spectroscopy in metabolic assessment of musculoskeletal lesions. *AJR Am J Roentgenol*
514 2012;198:162–72. doi:10.2214/AJR.11.6505.
- 515 [47] Bangerter NK, Tarbox GJ, Taylor MD, Kaggie JD. Quantitative sodium magnetic resonance
516 imaging of cartilage, muscle, and tendon. *Quant Imaging Med Surg* 2016;6:699–714.
517 doi:10.21037/qims.2016.12.10.
- 518 [48] Lee CW, Lee J-H, Kim DH, Min HS, Park B-K, Cho HS, et al. Proton magnetic resonance
519 spectroscopy of musculoskeletal lesions at 3 T with metabolite quantification. *Clin Imaging*
520 2010;34:47–52. doi:10.1016/j.clinimag.2009.03.013.
- 521 [49] Mulkern R V, Meng J, Bowers JL, Oshio K, Zuo C, Li H, et al. In vivo bone marrow lipid
522 characterization with line scan Carr-Purcell-Meiboom-Gill proton spectroscopic imaging.
523 *Magn Reson Imaging* 1997;15:823–37.
- 524 [50] Li X, Kuo D, Schafer AL, Porzig A, Link TM, Black D, et al. Quantification of vertebral bone
525 marrow fat content using 3 Tesla MR spectroscopy: reproducibility, vertebral variation, and
526 applications in osteoporosis. *J Magn Reson Imaging* 2011;33:974–9. doi:10.1002/jmri.22489.
- 527 [51] Rico-Sanz J, Thomas EL, Jenkinson G, Mierisová S, Iles R, Bell JD. Diversity in levels of
528 intracellular total creatine and triglycerides in human skeletal muscles observed by (1)H-MRS.
529 *J Appl Physiol* 1999;87:2068–72.
- 530 [52] Manhard MK, Nyman JS, Does MD. Advances in imaging approaches to fracture risk
531 evaluation. *Transl Res* 2017;181:1–14. doi:10.1016/j.trsl.2016.09.006.
- 532 [53] Chesnut CH, Majumdar S, Newitt DC, Shields A, Van Pelt J, Laschansky E, et al. Effects of

533 salmon calcitonin on trabecular microarchitecture as determined by magnetic resonance
534 imaging: results from the QUEST study. *J Bone Miner Res* 2005;20:1548–61.
535 doi:10.1359/JBMR.050411.

536 [54] Ma J, Wehrli FW, Song HK. Fast 3D large-angle spin-echo imaging (3D FLASE). *Magn Reson*
537 *Med* 1996;35:903–10.

538 [55] Krug R, Carballido-Gamio J, Burghardt AJ, Kazakia G, Hyun BH, Jobke B, et al. Assessment of
539 trabecular bone structure comparing magnetic resonance imaging at 3 Tesla with high-
540 resolution peripheral quantitative computed tomography ex vivo and in vivo. *Osteoporos Int*
541 2008;19:653–61. doi:10.1007/s00198-007-0495-9.

542 [56] Chang G, Honig S, Brown R, Deniz CM, Egol KA, Babb JS, et al. Finite element analysis applied
543 to 3-T MR imaging of proximal femur microarchitecture: lower bone strength in patients with
544 fragility fractures compared with control subjects. *Radiology* 2014;272:464–74.
545 doi:10.1148/radiol.14131926.

546 [57] Schmidt GP, Schoenberg SO, Schmid R, Stahl R, Tiling R, Becker CR, et al. Screening for bone
547 metastases: whole-body MRI using a 32-channel system versus dual-modality PET-CT. *Eur*
548 *Radiol* 2007;17:939–49. doi:10.1007/s00330-006-0361-8.

549 [58] Dutoit JC, Verstraete KL. Whole-body MRI, dynamic contrast-enhanced MRI, and diffusion-
550 weighted imaging for the staging of multiple myeloma. *Skeletal Radiol* 2017;46:733–50.
551 doi:10.1007/s00256-017-2609-6.

552 [59] Perez-Lopez R, Lorente D, Blackledge MD, Collins DJ, Mateo J, Bianchini D, et al. Volume of
553 Bone Metastasis Assessed with Whole-Body Diffusion-weighted Imaging Is Associated with
554 Overall Survival in Metastatic Castration-resistant Prostate Cancer. *Radiology* 2016;280:151–
555 60. doi:10.1148/radiol.2015150799.

- 556 [60] Padhani AR, Makris A, Gall P, Collins DJ, Tunariu N, de Bono JS. Therapy monitoring of skeletal
557 metastases with whole-body diffusion MRI. *J Magn Reson Imaging* 2014;39:1049–78.
558 doi:10.1002/jmri.24548.
- 559 [61] Giles SL, Messiou C, Collins DJ, Morgan VA, Simpkin CJ, West S, et al. Whole-Body Diffusion-
560 weighted MR Imaging for Assessment of Treatment Response in Myeloma. *Radiology*
561 2014;271:785–94. doi:10.1148/radiol.13131529.
- 562 [62] Zamagni E, Cavo M. The role of imaging techniques in the management of multiple myeloma.
563 *Br J Haematol* 2012;159:499–513. doi:10.1111/bjh.12007.
- 564 [63] Walker R, Barlogie B, Haessler J, Tricot G, Anaissie E, Shaughnessy JD, et al. Magnetic
565 Resonance Imaging in Multiple Myeloma: Diagnostic and Clinical Implications. *J Clin Oncol*
566 2007;25:1121–8. doi:10.1200/JCO.2006.08.5803.
- 567 [64] Yang H-L, Liu T, Wang X-M, Xu Y, Deng S-M. Diagnosis of bone metastases: a meta-analysis
568 comparing 18FDG PET, CT, MRI and bone scintigraphy. *Eur Radiol* 2011;21:2604–17.
569 doi:10.1007/s00330-011-2221-4.
- 570 [65] Karpitschka M, Godau-Kellner P, Kellner H, Horng A, Theisen D, Glaser C, et al. Assessment of
571 therapeutic response in ankylosing spondylitis patients undergoing anti-tumour necrosis
572 factor therapy by whole-body magnetic resonance imaging. *Eur Radiol* 2013;23:1773–84.
573 doi:10.1007/s00330-013-2794-1.
- 574 [66] Pace L, Nicolai E, Luongo A, Aiello M, Catalano OA, Soricelli A, et al. Comparison of whole-
575 body PET/CT and PET/MRI in breast cancer patients: Lesion detection and quantitation of
576 18F-deoxyglucose uptake in lesions and in normal organ tissues. *Eur J Radiol* 2014;83:289–96.
577 doi:10.1016/j.ejrad.2013.11.002.
- 578 [67] Pedoia V, Majumdar S, Link TM. Segmentation of joint and musculoskeletal tissue in the study

579 of arthritis. Magn Reson Mater Physics, Biol Med 2016;29:207–21. doi:10.1007/s10334-016-
580 0532-9.

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