resistance. Several Magnetic Resonance (MR) techniques have thus been developed to assess spongy bone status, such as MR interferometry [4,5,6] and the μ-MRI [7,8]. The former is based on T_2^*-weighted Gradient Echo imaging and T_2* quantification of the spongy bone marrow. The latter relies instead on high resolution MR Imaging, which allows a quantitative morphometric analysis of the three-dimensional structure of trabecular bone (TB). Some years ago we presented the first high-field diffusion images of bone marrow in spongy bone samples [9,10] while, very recently, we proposed a new Magnetic Resonance (MR) strategy based on the evaluation of internal gradient (G_i) as extracted from the Spin-Echo decay function to assess the TB density in spongy bone [11]. These papers underlined that the mean *ADC* in spongy bone samples from bovine was of the order of $10^{-10}m^2/s$ [9,10] and emphasized that differences between smaller and larger pores in spongy bone are better characterized by means of diffusion weighted images as compared to T_2-weighted images. Moreover, in these diffusion experiments the selected diffusion length was approximately less than 10 μm, namely less wide than the average dimension of pores in bovine spongy bone (equal to 50-300 μm). Finally, we demonstrated [11] in calf bone samples, that water is more prevalent in the boundary zone while fats are rearranged primarily in the central zone of each pore. Furthermore we showed that water internal gradient (G_i) magnitude from the samples was directly proportional to their TB density [11].
On the base of these observations T_2, T_2*, *ADC* and *G*i measurements for both water and fat components in calf bone samples are illustrated and discussed here to characterize spongy bone.

2. Methods and Materials

We investigated, at 9.4T magnetic field, spongy bone specimens from femoral head of calves, using a micro-imaging probe equipped with maximum gradient strength of 1200 mT/m. T_2, T_2*, *ADC* and *G*i were measured in spatially resolved modality (in plain image resolution equal to 40 micrometers, slice thickness 250 μm) for both water and fat components. Mean values (and their standard deviation, *SD*) of the aforementioned parameters were obtained performing an average over values extracted from 4 slices. Six ex-vivo spongy bone specimens (20mm high, 6mm deep) excised from femoral head of calves (4 specimens) and from distal femur (2 specimens), were cut into three pieces of approximately 7-6mm high and 5mm deep, in order to obtain, for each specimen, three samples characterized by different TB densities (see figure 1). Sample temperature was fixed to 291 K. Gradient Echo images were obtained to evaluate T_2*, using GEFI imaging sequence (*TR*=1000ms, NS=16) at various *TE*s (from 1.8 to 60ms). A MSME (Multi Slice Multi Echo) imaging sequence (*TR*=2000ms, *NS*=8) at various *TE*s (from 2.8 to 120ms), was used to obtain SE decay. Both T_2 and G_i were evaluated from the attenuation of SE signal as a function of different *TE*s. A Pulse Gradient STimulated Echo (PGSTE) imaging sequence was also employed (*TE/TR*=21.9/3000ms, Δ=80ms, δ=4ms, using eight b-values ranging from 200 to 80000s/mm^2, *NS*=16) in order to measure ADC along x axis. Spectra were also obtained to evaluate water and fat percentage from each sample. To obtain T_2, T_2*, *ADC* and *G*i for both fat and water, a Levenberg-Marquard fit was performed using the signal as a superimposition of the two bone marrow components.
The behaviour of all aforementioned MR parameters was investigated as a function of: 1) samples TB density, 2) relative water concentration in bone marrow. To quantify TB density, we calculated the ratio between the perimeter and the area of each pore in the slices. As a result, the ratio (N_p/N_a) between number of voxels defining each pore perimeter (N_p) and number of voxels constituting the corresponding area (N_a) was obtained from all pores included in each of the slices. As a consequence, bone samples characterized by N_p/N_a values ranging from: 0.35 to 0.55, 0.31 to 0.34, 0.28 to 0.30 and 0.24 to 0.27 were indicated as HTD (higher TB), ITDa (intermediate TB), ITDb (intermediate TB) and LTD (lower TB), respectively (see Fig. 1).

3. Results

Figure 2 displays the different behaviour of water and fat measured MR parameters as a function of both TB density and water percentage in bone marrow (*W*%). Water T_2, T_2* and *ADC* are characterized by a decreasing trend when moving from lower TD to higher TB and water G_i increases proportionally with the increase in TB density. Conversely, fat G_i, fat *ADC* and fat T_2* as a function of TB density, do not show a specific trend. Fat *ADC* values result to be independent on both TB density and *W*%, while fat T_2 decreases proportionally with the increase in both TB density and *W*%. Water T_2, T_2*, *ADC* and G_i values extracted from femoral head of calves showed in Fig.2, must be compared with water T_2, T_2*, *ADC* and G_i values extracted from a distal femur samples listed in Table 1.

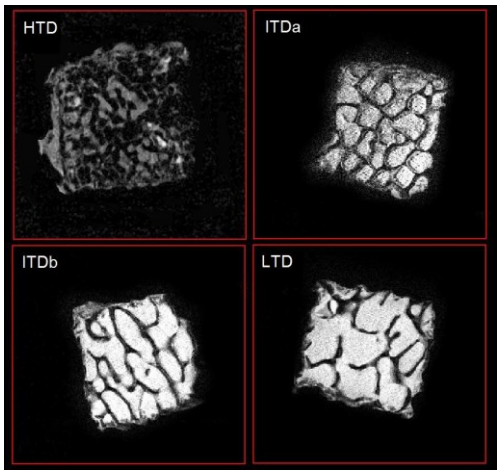

Fig. 1: An example of calf spongy bone specimens used. SE image slices (*TE*=4ms) obtained from: closely spaced spongy bone samples (HTD), intermediate trabecular bone density samples (ITDa, ITDb) and larger inter-trabecular samples (LTD).

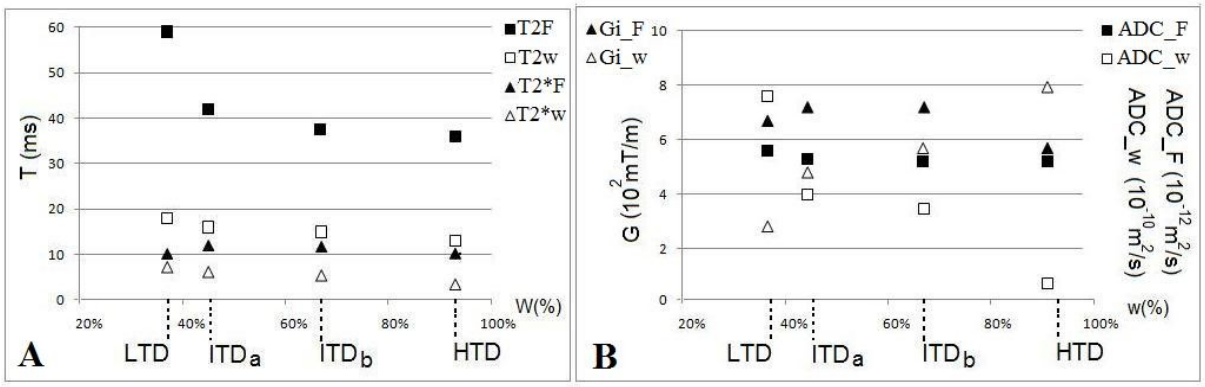

Fig. 2 Results from femoral head samples. A) Fat T_2 and T_2* (filled squares and triangles, respectively) and water T_2 and T_2* (empty squares and triangles, respectively) as a function of both TB density (LTD, ITDa, ITDb, HTD) and water percentage in bone marrow (*W*(%)). B) Fat G_i and *ADC* (filled squares and triangles, respectively) and water G_i and *ADC* (empty squares and triangles, respectively) as a function of both TB density (LTD, ITDa, ITDb, HTD) and *W*(%).

Again, water T_2* is characterized by a decreasing trend when moving from LTD to HTB, however T_2* values in table 1 are lower than those displayed in Fig.2. Furthermore water G_i

	%Water	(T_2*±*SD*)ms	(T_2±*SD*)ms	(*ADC*±*SD*)*10^{-10} m^2/s	(*Gi*±*SD*)mT/m
LTD	15	6.9±1.0	15.0±1.0	0.9±0.2	522±331
ITDb	28	5.2±0.5	16.2±1.0	1.7±0.3	782±134
HTD	57	4.0±0.5	15.9±1.0	3.2±0.1	1093±281

Table 1. Results from one distal femur specimen: water component.

displayed in Table 1 increase proportionally with the increase in TB density, showing higher values as compared to G_i values displayed in Fig.2. Conversely water *ADC* values displayed in Table 1 compared to those in Fig.2 showed an opposite behaviour as a function of TB density. *ADC* behaviour depends on either *W*% (or fat content) or TB density.

4. Discussion

Data illustrated here demonstrate the key role of diffusion to obtain both structural and quality information from spongy bone. Fat *ADC* is approximately two orders of magnitude less than water *ADC*, and the former results to be independent on both TB density and *W*%. Conversely, water *ADC* is characterized by a fast (of the order of $10^{-9}m^2/s$) or a restricted (of the order of 10^{-10} -10^{-11} m^2/s) diffusion regime as a function of fat quantity. Considering that water is more prevalent in the boundary zone, while fats are rearranged primarily in the central zone of each spongy bone pore [11] it is possible to understand the important role of water diffusion to characterize spongy bone. Water diffusion partially averages the rapid spin dephasing effect due to susceptibility difference between bone and water. The faster the diffusion, the higher the average effect. As a consequence water T_2* and water G_i values related to a selected TB density, strongly depend on water diffusion regime. Besides, in spongy bone, water diffusion regime is strictly linked to the width of the boundary pore zone (between solid bone and fat), where water is more prevalent. The smaller the boundary zone, the lower the average effect. Data reported in Table 1 and Fig.2 can be fully explained using the above argumentations. As an example, because distal femur specimens are characterized by a higher fat content compared to that of femoral head samples, water diffusion regime is more restricted in samples of Table 1 than in those displayed in Fig.2. As a consequence, for a fixed TB density, T_2* in Table 1 is lower than T_2* in Fig.2. Moreover, G_i in Table 1 is higher than G_i in Fig.2.

3. Conclusions

T_2*, *ADC* and G_i extracted from bone marrow water component and, more strongly, water G_i may be reliable markers to evaluate trabecular bone density and to assess the status of spongy bone. Conversely T_2*, *ADC* and G_i from fat component did not provide any useful information related to TB density.

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