In Vivo Proton Relaxation Times Analysis of the Skin Layers by Magnetic Resonance Imaging

Stéphanie Richard, Bernard Querleux, Jacques Bittoun, Ilana Idy-Peretti, Odile Jolivet, Eva Cermakova, and Jean-Luc Lévêque

Laboratoires de Recherche de L'OREAL (SR, BQ, JLL), Aulnay-Sous-Bois; and CIERM (JB, IIP, OJ, EC), Hôpital de Bicêtre, Le Kremlin-Bicêtre, France

If in vivo magnetic resonance imaging is nowadays a powerful non-invasive method in medical diagnosis, its application in order to study the skin in vivo is not yet in common use because skin imaging requires a high resolution, at least in the direction perpendicular to the skin surface. We have therefore designed a specific imaging module, which, connected to a standard whole-body imager at 1.5 Tesla, allows us to obtain in vivo magnetic resonance images of skin on most parts of the body. With a depth resolution of about 70 μm, we are able to differentiate the skin layers: epidermis, dermis, subcutaneous fat, and even a thickened stratum corneum on palm as well as on heel.

This paper reports the T1 and T2 water proton relaxation times of the different skin layers, in vivo, which are magnetic resonance parameters extracted from the images. Results show that skin layers are characterized by shorter T2 relaxation times than other biologic soft tissues. On the contrary, the measured T1 values are in the same range as in other tissues. These short T2 values may be assigned to the fibrous protein content of the skin and particularly of the dermis. This study on normal skin is the precursor of further works such as the influence of aging. As regards skin pathologies, it will be a powerful tool to follow the evolution of skin diseases under treatment. *J Invest Dermatol 97:120–125, 1991*

Over the last decade the nuclear magnetic resonance (NMR) technique has become a powerful method in medical diagnosis. Magnetic resonance (MR) imaging is of great interest because one can non-invasively obtain not only a spatial localization of the different tissues but also quantitative information on tissues by measuring their proton relaxation times T1 and T2. These are intrinsic parameters of each tissue, providing, for instance, useful information about correlation with water content or interactions of water protons with macromolecules [1–4].

The use of the NMR technique in order to study the skin in vivo is recent because, due to its small thickness, skin imaging requires high spatial resolution and highly sensitive systems. Previous studies presented images of normal [5–7] and pathologic skin [8,9] where either the resolution was too poor to differentiate epidermis from dermis or resolution was sufficient but only extremities could be imaged. Finally, a preliminary in vitro study demonstrated the ability to differentiate normal from diseased skin by MR spectroscopy [10]. As far as skin is concerned, published results of T1 and T2 values are very variable and were obtained in vitro, with no differentiation between the skin layers [1,11–14]. For subcutaneous fat, T1 and T2 values are reported in normal [1,15] and tumorous fat [16].

The aim of this work is to quantify and analyze water proton relaxation times T1 and T2 of the different skin layers in vivo. Such measurements are of interest, first, because they enable an optimization of the imaging sequence parameters so that the quality of in vivo skin images can be improved and, secondly, because NMR is a non-perturbing approach to the physico-chemical properties of the water proton in the different skin layers.

In this paper, we briefly describe the specific instrumentation that allows us to obtain high-resolution images where the different layers and some structural elements of the skin are clearly visualized. Distinction between dermis and epidermis, and even stratum corneum (SC) in areas where it is very thick, permits the first measurements to our knowledge of the T1 and T2 relaxation times of these layers in vivo. Thus, we present the results obtained on nine healthy subjects, on two areas of the body. We discuss the results for the different skin layers and finally compare their variations according to the area.

MATERIALS AND METHODS

Equipment The MR whole-body imager is a Signa system (General Electric medical systems, Milwaukee, WI) operating at 1.5 Tesla. In vivo skin imaging requires a high resolution, at least in the depth of the skin. We obtain a spatial resolution better than 0.1 mm in this direction by adding a specific imaging module. It is made with a surface gradient coil in order to increase the resolution in the direction perpendicular to the skin surface, and with a surface radiofrequency coil that improves the signal-to-noise ratio. Both are connected in place of the standard corresponding coils of the system.

This specific imaging module, described in previous papers [5,17], enables one to acquire high-resolution MR images of the skin in vivo on most parts of the body, and thus to differentiate its layers [18].

Manuscript received July 16, 1990; accepted for publication February 5, 1991.

Reprint requests to: Dr. J. L. Lévêque, Laboratoires de Recherche de L'OREAL, 1, Avenue Eugène Schueller, 93600 Aulnay-Sous-Bois, France.

Abbreviations:
NMR: nuclear magnetic resonance
MR: magnetic resonance
SC: stratum corneum
TR: repetition time
TE: echo time
D: dimension
ROI: region of interest

0022-202X/91/$03.50

Measurement Protocol We used an MR acquisition sequence that is characterized by a repetition time TR, and an echo time TE. The signal intensity, S, on an image of a homogeneous tissue is given by [19]

$$S = N(H)e^{-TE/T2}(1 - (1 + f)\, e^{-TR/T1}) \qquad (1),$$

where T1 and T2 are tissue relaxation times, N(H) is a function of the proton density, and f is a factor taking into account the non-homogeneity of the radiofrequency pulse.

T2 (Spin-Spin Relaxation Time) Measurement: T2 value is determined according to relation (1) by acquiring a set of images with different values of TE. Five images were thus acquired with TE ranging from 9–20 ms and TR fixed at 100 ms, for calf epidermis and dermis. In order to avoid too long an examination time, only four images were acquired for T2 measurements on the heel, with TE ranging from 9–35 ms and TR fixed at 100 ms. A three-dimensional (3D) gradient-echo sequence was used for measurements in stratum corneum, epidermis, and dermis in order to obtain short TE values and thin slices of 1.2 mm in thickness.

Preliminary measurements were carried out on phantoms in order to assess the validity of our method to measure short T2 values. In order to obtain a phantom with a short T2 value, we used a high concentration of agar gel (4%) [20] and have introduced a paramagnetic ion. We chose manganese ($MnCl_2$ solution, 0.1 10^{-3} M) because it affects T2 relaxation more than T1 [21]. With our specific instrumentation and a 3D gradient-echo sequence, we obtained a mean T2 value of 19 ± 1 ms. It was not significantly different from the value (18 ± 0.2 ms) given by the standard coils of the system and a spin-echo sequence.

Due to effects of the chemical frequency shift between fat and water protons [22] in hypodermis, it was necessary to use a 2D spin-echo sequence in this tissue in calf; eight images were acquired with TE ranging from 15–70 ms and TR fixed at 100 ms. With this sequence the thinnest available slice thickness is 3 mm.

T1 (Spin-Lattice Relaxation Time) Measurement: T1 can also be calculated from relation (1) by acquiring seven images while only varying TR from 100–4000 ms on calf and five images with TR ranging from 100–3000 ms on heel. TE is fixed at 15 ms. Due to the long values of TR required by T1 measurement, a standard 2D spin-echo sequence was used instead of a 3D gradient-echo sequence.

Thus, as indicated in literature [19,23], a minimum of four points was used for relaxation times calculations.

Protocol: During the examination time (about 1 h per location) the skin to be analyzed lay on the specific imaging module fixed on the examination cradle. All MR images were obtained with an 18 × 50 mm field of view (in-depth resolution = 70 μm), at room tem-

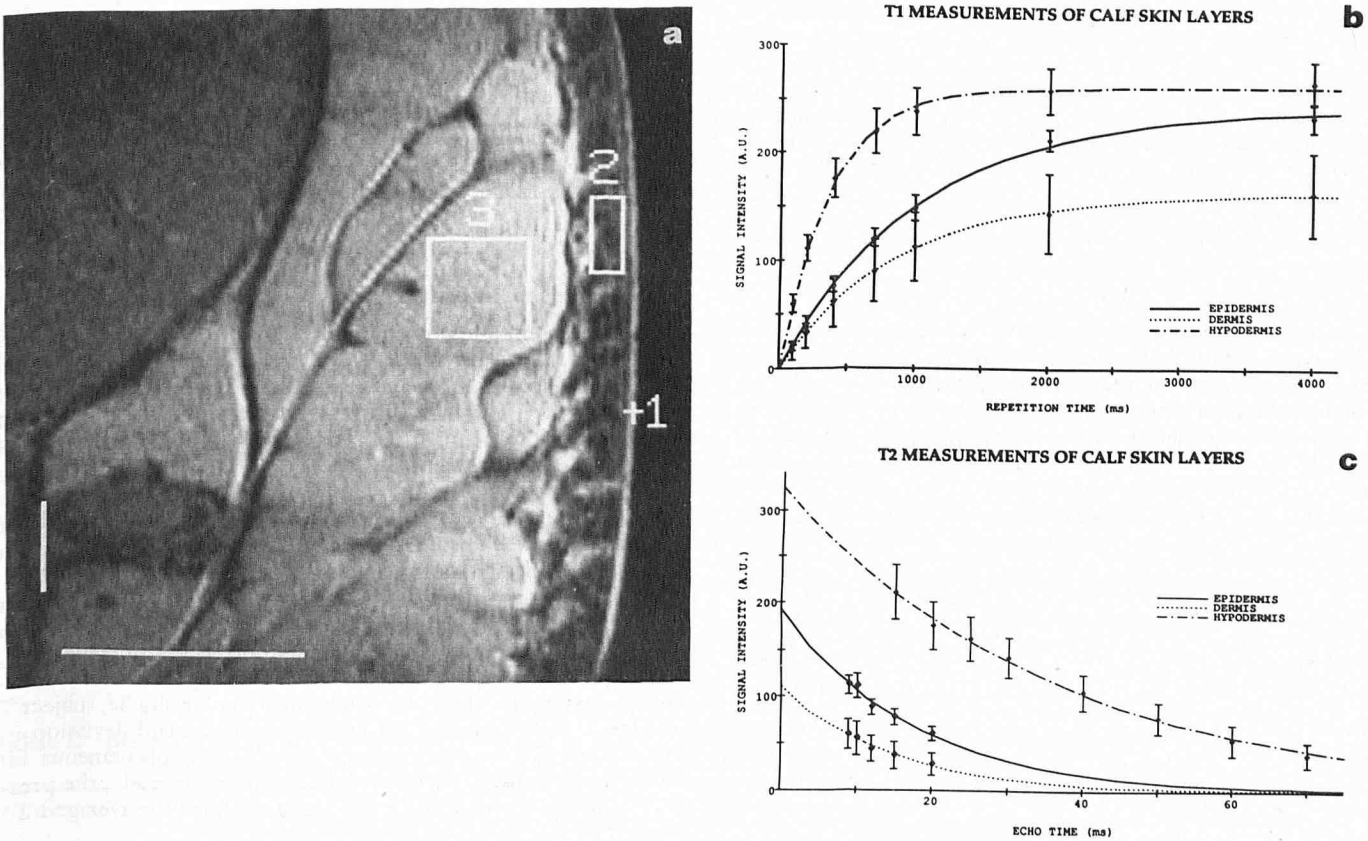

Figure 1. Skin on calf. *a*, magnetic resonance image of skin in vivo on calf. Layers of skin are clearly delineated: the outer bright thin layer corresponds to epidermis, measuring about 100 μ in thickness. Cursor 1 represents one of the 40 pixels selected in epidermis. Dermis (region 2) appears as a dark layer about 1 mm thick. It is quite inhomogeneous because of grey inclusions that are pilosebaceous units. Hypodermis (region 3) appears bright and its darker fibrous septae are clearly visible. Regions 2 and 3 represent some of the five ROI selected in each layer for relaxation time calculations. *Bars,* 5 mm. *b*, plots of typical experimental points and the resulting fitted curves from one of the five ROI for dermis and hypodermis and from averaged 40 pixels in epidermis. Data values for T1 calculation are obtained with a spin-echo sequence for seven values of TR. *c*, plots of typical experimental points and the fitted curves from a ROI (or averaged 40 pixels in epidermis) obtained for T2 calculation with a gradient-echo sequence and five values of TE. In hypodermis, data values are obtained with a spin-echo sequence and eight values of TE (see text). *Error bars* around the *data points,* standard deviation between pixels of the selected ROI.

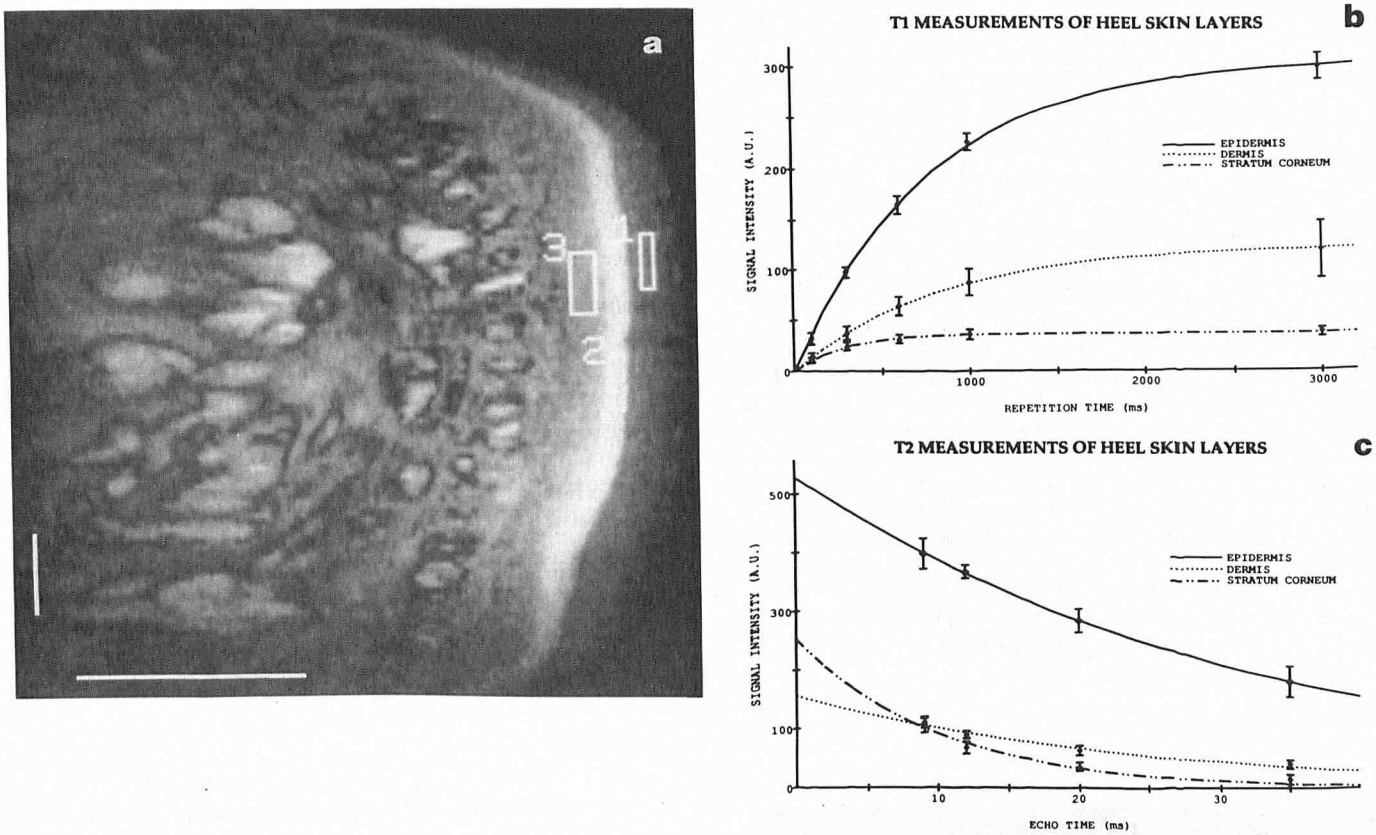

Figure 2. Skin on heel. *a*, magnetic resonance image of skin in vivo on heel. The outer grey layer is stratum corneum, which appears particularly thick in this area; it corresponds to region 1. The brighter layer between stratum corneum and dermis is epidermis, which is also particularly thick (about 500 μ in thickness); it corresponds to region 2. Dermis (region 3) appears more homogeneous than in calf. The border between dermis and hypodermis is less delineated than in calf due to the particular texture of hypodermis. Regions 1, 2, and 3 represent some of the five ROI selected in each layer. *Bars,* 5 mm. *b, c,* plots of experimental points and the fitted curves obtained for T1 calculation *(b)* with a spin-echo sequence and five different TR; and for T2 calculation *(c)* with a gradient-echo sequence and four TE values. Presented data are issued from one of the five ROI in each layer. *Error bars,* standard deviation between pixels of the selected ROI.

perature (21°C). A region of interest (ROI) was then selected on each layer (epidermis, dermis, subcutaneous fat, and stratum corneum) in order to perform measurements. Five ROI were taken on each location for each subject in order to minimize variations due to tissue heterogeneity. Because the epidermis of calf is too thin to contain a ROI, signal intensity of about 40 pixels was measured and averaged.

Data calculations were performed with the use of the standard Signa software, by fitting the signal intensities from a ROI to relation (1).

Subjects MR imaging of in vivo skin were acquired on two areas (heel and calf) for each of nine healthy volunteers (four women, five men). The mean age was 35 $\pm$ 6 years. The range was chosen intentionally short in order to cancel the influence of this variable.

Statistics The significance of the results was tested by ANOVA. A probability of $p < 0.01$ was considered significant for every test. The values of the relaxation times T1 and T2 were compared according to the different layers, for each area. A comparison was then established between the areas.

RESULTS

T1 and T2 values of the different skin layers in vivo Figures 1*a* and 2*a* show two MR images of the skin. Figure 1*a* corresponds to skin on calf, where, from right to left, we delineate an outer bright thin layer that corresponds to the epidermis. Then, dermis appears as a dark layer under epidermis. Many inclusions are clearly delineated inside the dermis; some of them are in continuity with epidermis and represent pilosebaceous units. Finally, hypodermis appears bright with its black fibrous septae. Figure 2*a* corresponds to skin on heel, where stratum corneum, epidermis, and dermis are clearly delineated too. The skin is particularly thick in this area, mainly due to the stratum corneum. Example of typical ROI in the different layers are drawn on these images.

Figures 1*b,c,* and 2*b,c* represent the typical fitting of signal intensities v. TR or TE according to relation (1), used for T1 and T2 calculations for each tissue and area. Each curve is obtained from the selected ROI, drawn on image.

In Fig 3*a–d,* the mean values of T1 and T2 for the nine subjects are presented (each point being the T1 or T2 average value calculated from five different ROI or from 40 pixels in calf epidermis), according to layer and location, in order to compare the intra-individual variability and the inter-individual one. In Fig 3*d*, subject 7 presents a higher value of T1 and a greater standard deviation in hypodermis. This subject has a relatively thin subcutaneous fat layer, which appears not homogeneous; more precisely, the presence of many fibrous septae might have modified the averaged T1 relaxation time.

The mean values of the nine subjects and their SD are summarized in Table I.

Tissue Dependence of T1 and T2 Relaxation Times There is no significant difference for the T1 relaxation time values related to epidermis and to dermis ($p > 0.01$). On the contrary, the T2 relaxation times of these tissues are significantly different ($p < 0.01$), the T2 in epidermis being greater than in dermis for each area.

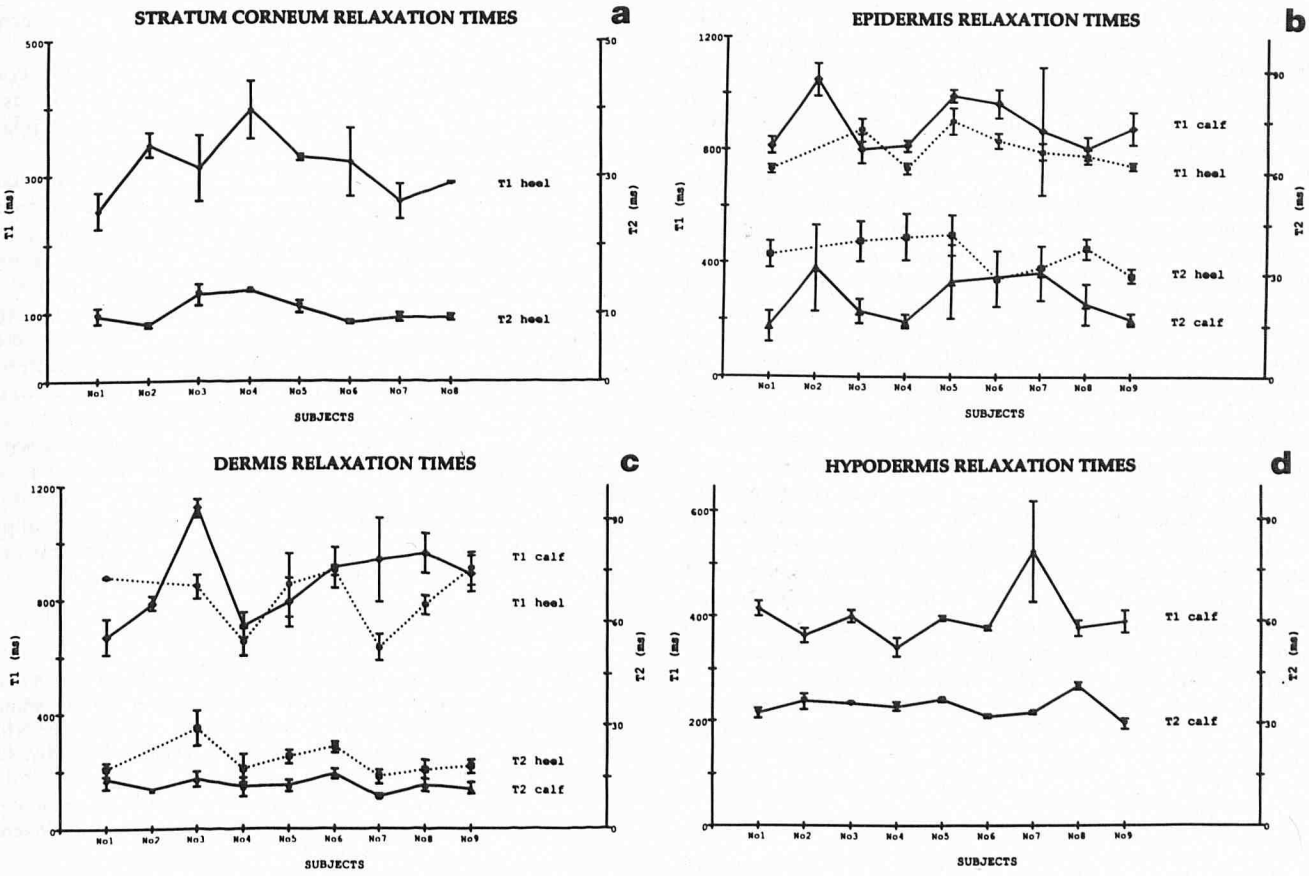

Figure 3. Mean values of relaxation times T1 and T2 according to subject, tissue, and location in stratum corneum *(a)*, in epidermis *(b)*, in dermis *(c)*, and in hypodermis *(d)*. Each *point* represents the averaged relaxation time obtained from five ROI and *error bars*, the standard deviation (except for *b* where each point represents the relaxation time obtained from 40 pixels in calf epidermis). These four graphs show that inter-individual variations are larger than intra-individual ones, indicating that biologic variations between subjects are the main cause of variance. Note also the great overlap between T1 values, whereas T2 values are clearly separated between heel and calf, for both epidermis and dermis.

Among the skin layers, subcutaneous fat has the longest T2 relaxation time and stratum corneum has the shortest one. T1 measurements of these tissues show similar short values.

Location Dependence There is a great overlap between the mean ± SD values of T1 in calf and in heel, for both epidermis and dermis. Thus, the difference between the T1 values of the two areas is not significant ($p > 0.01$). For T2, the mean values are significantly different according to the location ($p < 0.01$). The T2 relaxation time is greater in calf than in heel; T2 is prolonged by about 55% in dermis and 60% in epidermis.

Because subcutaneous fat is not well delineated in heel, and stratum corneum of calf is too thin to be visualized, no comparison related to the location could be established for these tissues.

Table I. Mean Values of the Relaxation Times T1 and T2 of the Different Skin Layers in Heel and Calf

	Heel		Calf	
	T1(ms)	T2(ms)	T1(ms)	T2(ms)
Stratum corneum	313 ± 47[a]	10.2 ± 2		
Epidermis	720 ± 53	35.6 ± 5	887 ± 92	22.3 ± 7
Dermis	728 ± 104	17.9 ± 5	870 ± 143	13 ± 2.4
Hypodermis			393 ± 34	35.4 ± 3.6

[a] Standard deviation calculated from nine subjects, five ROI per subject. Compared to other biological tissues, both epidermis and dermis have relatively long T1 values and short T2 values.

DISCUSSION

Many authors have shown that T1 and T2 measurements in biologic tissues with an NMR imaging system have large variations according to the imaging system and its characteristics, and have a large variance between the different subjects [1,4,23,24]. However, most of the semiology of the different organs in MR imaging is based on the variations of the relaxation times.

Thus, as a new method of investigation of normal and pathologic skin, high-resolution MR imaging of the skin requires a preliminary determination of the MR characteristics of normal skin, which constitutes most of the work presented here. Moreover, some preliminary conclusions on skin physico-chemical properties can be drawn from these results.

The accuracy of our T2 measurements was first tested with phantoms, because gradient-echo is not the usual measurement protocol. Phantom T2 relaxation times show values not significantly different with normal imaging conditions (spin-echo sequence) and with a 3D gradient-echo sequence. Furthermore, the T2 values of hypodermis, as well as the T1 values obtained in our conditions, are very similar to those reported by other authors in standard imaging systems [1,15].

Finally, though the values presented in Table I show large standard deviations in our population, statistical analysis from data presented on Fig 3*a*–*d* indicates that the inter-individual variance is greater than the intra-individual one. That means that measurement errors are less important than biologic variance in tissues. Thus we consider that our results can be favorably compared to those obtained in standard imaging conditions and allow one to discuss some particularities observed in dermis and epidermis.

The main feature observed is the discrepancy between T1 and T2 values. The T1 to T2 ratio of both epidermis and dermis is higher than the usual ratios obtained in biologic soft tissues; this is mainly due to short T2 values. Indeed, results have shown that both layers have similar relatively long T1 values in the range of soft biologic tissues. The same water content in both epidermis and dermis [25,26] can explain such values because T1 relaxation time is primarily related to the total water content of the tissues [2,3].

On the contrary, the T2 value in dermis is short and significantly lower than in epidermis. As the most common dry constituent of dermis is collagen (about 70% dry weight) [25], its primary role in T2 value may be evoked. Collagen is a fibrous protein, organized into a complex network of interacting elements. The presence of large amounts of fibrillar macromolecules in tissues increases the surface area per unit of tissue weight, and consequently the water-protein interaction surface [14]. As the T2 relaxation time decreases with water proton mobility [2], the high content of collagen in dermis would contribute significantly to its short T2 value.

Concerning the T2 value of dermis, the role of collagen is enhanced by the location dependence of this relaxation time. In dermis, differences in the distribution of collagen fibril diameters were correlated to their mechanical properties: dermis in the heel is subjected to pressure and friction, and collagen fibrils are thin and associated with an enriched proteoglycan matrix [27,28]. Dermis in the calf is subjected to high tensile strength and has thicker collagen fibrils, closely packed [27,28]. The thin collagen fiber network of dermis in the heel seems to be less densely packed, providing a greater degree of motional freedom for water protons. So, a smaller motional constraint of protons would explain the longer T2 value for dermis in the heel. Meanwhile, other factors, such as the types and water-retention properties of proteoglycans, may also affect relaxation times.

Another discrepancy is the great difference in signal intensity observed on MR images between epidermis and dermis. This difference cannot be explained by T1 values, for they are quite similar in both tissues. At the same time, the contrast between the two layers cannot be fully explained by the T2 relaxation times because we are using short TE. So only proton density could explain it. Dermis and epidermis have a similar water content [25,26], but NMR only measures mobile protons, whereas the signal of bound protons that have a very short T2 value cannot be detected due to the dead time of the receiver. MR spectroscopic analyses of collagen [29,30] have shown that water is tightly bound to collagen. Indeed, water constitutes an integral part of the collagen 3D structure, and the amount of adsorbed water onto the macromolecule surface is high, compared to that of globular proteins, for instance.

The presence of bound protons was confirmed in the tendon, which is also a high-collagen-content tissue; its T2 has a very short component, between 150 and 500 nsec [31]. So the lack of signal in the dermis can be related to its bound proton fraction, which has a T2 value too short to be detected. Consequently, although epidermis and dermis have a similar water content, the fraction of bound protons seems to be more important in dermis than in epidermis according to the contrast observed on MR images.

Calf epidermis also has a relatively short T2 value, whereas it contains no collagen molecules. This may be due to the presence of few fibrous proteins, which are essentially tonofilaments and precursors of keratin. On the contrary the heel epidermis T2 is lengthened and is closer to usual T2 values obtained in biologic soft tissues. This longer value, compared to that of calf epidermis, could be related to physiologic differences between heel and calf epidermis. It has been shown that regions submitted to friction, such as heel, have an increased rate of epidermis cell proliferation associated with changes in proteins composition and conformation, leading to the formation of epidermal callus [32–34]. This adaptative response would affect the relaxation process. Nevertheless, due to its superficial position and small thickness, calf epidermis relaxation time measurements represent limit conditions. Even though great care has been taken with signal intensity measurements, it cannot be excluded that artifacts such as the partial volume effect of the papillary dermis as well as the susceptibility effect on the epidermis/air interface may shorten the T2 value.

Finally, as regards dermis and epidermis, stratum corneum presents short values not only of T2 but also of T1, for it has a very low amount of water [35] and, consequently, has short relaxation times [2,3].

In conclusion, our interest in measuring the proton relaxation times of in vivo skin by a noninvasive method stemmed from two considerations. First, differences among relaxation times are key to MR imaging contrast and knowledge of them is useful in order to adjust parameters of the imaging sequence. Secondly, they play a pivotal role in the understanding of the molecular-level organization of biologic tissues. We have found short T2 values that distinguish the whole skin from other soft tissues, and also differentiate each skin layer. More particularly, the very short T2 value of dermis has been related to the highly ordered structure of water around collagen fibrils. These measurements on healthy subjects will serve for other studies on normal skin, such as the influence of age. Concerning skin diseases, we expect important modifications of proton relaxation times, which are related to the physico-chemical properties of pathologic skin such as the inflammatory process or alterations of the collagen network.

REFERENCES

1. Bottomley PA, Foster TH, Argersinger RE, Pfeifer LM: A review of normal tissue hydrogen NMR relaxation times and relaxation mechanisms from 1-100 MHz: Dependence on tissue type, NMR frequency, temperature, species, excision, and age. Med Phys 11:425–448, 1984
2. Fullerton GD, Potter JL, Dornbluth NC: NMR relaxation of protons in tissues and other macromolecular water solutions. Magn Reson Imaging 1:209–228, 1982
3. Ling GN, Tucker M: Nuclear magnetic resonance relaxation and water contents in normal mouse and rat tissues and in cancer cells. J Natl Cancer Inst 64:1199–1207, 1980
4. Bottomley PA, Hardy CJ, Argersinger RE, Allen-Moore G: A review of ^{1}H nuclear magnetic resonance relaxation in pathology: are T1 and T2 diagnostic? Med Phys 14:1–37, 1987
5. Querleux B, Yassine MM, Darrasse L, Saint-Jalmes H, Sauzade M, Leveque JL: Magnetic resonance imaging of the skin. A comparison with the ultrasonic technique. Bioeng Skin 4:1–14, 1988
6. Hyde JS, Jesmanowicz A, Kneeland JB: Surface coil for MR imaging of skin. Magn Reson Med 5:456–461, 1987
7. Wood B, Zhao S, Mackenzie IS, Waller ML: High resolution imaging of the skin at 4.7 T. 7th Annual Meeting of the Society of Magnetic Resonance in Medicine, San Francisco, Vol. 2. 1988, p 1063
8. Schwaighoffer BW, Fruehwald FXJ, Pohl-Markl H, Neuhold A, Wicke L, Landrum WL: MRI evaluation of pigmented skin tumors, preliminary study. Invest Radiology 24:289–293, 1989
9. Zemtsov A, Lorig R, Bergfield WF, Bailin PL, Ng TC: Magnetic resonance imaging of cutaneous melanocytic lesions. J Dermatol Surg Oncol 15:854–858, 1989
10. Kim YH, Orenberg EK, Faull KF, Wade-Jardetzky NG, Jardetzky O: ^{1}H NMR spectroscopy: an approach to evaluation of diseased skin in vivo. J Invest Dermatol 92:210–216, 1989
11. Hollis DP, Economou JS, Parks LC, Eggleston JC, Saryan LA, Czeisler JL: Nuclear magnetic resonance studies of several experimental and human malignant tumors. Cancer Res 33:2156–2160, 1973
12. Frey HE, Knispel RR, Kruuv J, Sharp AR, Thompson RT, Pintar M: Brief communication: Proton spin-lattice relaxation studies of nonmalignant tissues of tumourous mice. J Natl Cancer Inst 49:903–906, 1972
13. Eggleston JC, Saryan LA, Hollis DP: Nuclear magnetic resonance investigations of human neoplastic and abnormal nonneoplastic tissues. Cancer Res 35:1326–1332, 1975
14. Cameron IL, Ord VA, Fullerton GD: Characterization of proton NMR relaxation times in normal and pathological tissues by correlation with other tissues parameters. Magn Reson Imaging 2:97–106, 1984
15. Dooms GC, Hricak H, Margulis AR, de Geer G: MR imaging of fat. Radiology 158:51–54, 1986

16. Dooms GC, Hricak H, Sollitto RA, Higgins CB: Lipomatous tumors and tumors with fatty component: MR imaging potential and comparison of MR and CT results. Radiology 157:479–483, 1985
17. Yassine MM, Darrasse L, Saint-Jalmes H, Sauzade M, Querleux B, Leveque JL: In vivo skin imaging at 0.1 tesla. 6th Annual Meeting of the Society of Magnetic Resonance in Medicine, New York, Vol. 1. 1987, p 466
18. Bittoun J, Saint-Jalmes H, Querleux B, Darrasse L, Jolivet O, Idy-Peretti I, Wartski M, Richard S, Leveque JL: In vivo high-resolution imaging of the skin in a whole-body MR system at 1.5T. Radiology 176:457–460, 1990
19. MacFall JR, Wehrli FW, Breger RK, Johnson GA: Methodology for the measurement and analysis of relaxation times in proton imaging. Magn Reson Imaging 5:209–220, 1987
20. Mitchell MD, Kundel HL, Axel L, Joseph PM: Agarose as a tissue equivalent phantom material for NMR imaging. Magn Reson Imaging 4:263–266, 1986
21. Schneiders NJ: Solutions of two paramagnetic ions for use in nuclear magnetic resonance phantoms. Med Phys 15:12–16, 1988
22. Dixon WT: Simple proton spectroscopic imaging. Radiology 153:189–194, 1984
23. Breger RK, Rimm AA, Fischer ME, Papke RA, Haughton VM: T1 and T2 measurements on a 1.5 T commercial MR imager. Radiology 171:273–276, 1989
24. Breger RK, Wehrli FW, Charles HC, MacFall JR, Haughton VM: Reproducibility of relaxation and spin-density parameters in phantoms and the human brain measured by MR imaging at 1.5 T. Magn Reson Med 3:649–662, 1986
25. Pearce RH, Grimmer BJ: Age and the chemical constitution of normal human dermis. J Invest Dermatol 58:347–361, 1972
26. Warner RR, Myers MC, Taylor DA: Electron probe analysis of human skin: determination of the water concentration profile. J Invest Dermatol 90:218–224, 1988
27. Flint MH, Craig AS, Reilly HC, Gillard GC, Parry DAD: Collagen fibril diameters and glycosaminoglycan content of skins—indices of tissue maturity and function. Connect Tiss Res 13:69–81, 1984
28. Parry DAD, Barnes GRG, Craig AS: A comparison of the size distribution of collagen fibrils in connective tissues as a function of age and a possible relation between fibril size distribution and mechanical properties. Proc R Soc Lond 203:305–321, 1978
29. Wise WB, Pfeffer PE: Measurement of cross-relaxation effects in the proton NMR of water in fibrous collagen and insoluble elastin. Macromolecules 20:1550–1554, 1987
30. Westover CJ, Dresden MH: Collagen hydration: pulsed nuclear magnetic resonance studies of structural transitions. Biochim Biophys Acta 365:389–399, 1974
31. Fullerton GD, Cameron IL, Ord VA: Orientation of tendons in the magnetic field and its effect on T2 relaxation times. Radiology 155:433–435, 1985
32. Mackenzie IC: The effects of frictional stimulation on mouse ear epidermis. I. Cell proliferation. J Invest Dermatol 62:80–85, 1974
33. Bullough WS: The control of epidermal thickness. Br J Dermatol 87:187–199, 1972
34. Mercer EH: Protein synthesis and epidermal differentiation. In: Montagna W, Lobitz WC (eds.). The epidermis. Academic Press, New York, 1964, pp 161–178
35. Walkley K: Bound water in stratum corneum measured by differential scanning calorimetry. J Invest Dermatol 59:225–227, 1972