Chemical shift imaging at 4.7 tesla of brown adipose tissue

Andrea Sbarbati,[1,*,†] Uliano Guerrini,[*] Pasquina Marzola,[*] Roberto Asperio,[§] and Francesco Osculati[*]

Institute of Anatomy and Histology,[*] University of Verona; Institute of Normal Human Morphology,[†] University of Ancona, and Institute of Veterinary Surgery,[§] University of Milan, Italy

Abstract In vivo distinction between small deposits of brown adipose tissue (BAT) and surrounding tissues may be difficult. In this article, we propose an experimental paradigm, based on techniques of chemical shift magnetic resonance imaging (CSI), which can improve the methods presently available for the study of BAT. Male rats were examined in an imager-spectrometer equipped with a 4.7 T magnet. Proton spectra of isolated BAT deposits showed that both fat and water protons contributed significantly to the genesis of the magnetic resonance signal. An unequivocal definition of BAT deposits was obtained by three (respectively, spin-echo, water-selective, and fat-selective) images. The spin-echo (SE), T1-weighted image provided the best anatomical description of the structures. The images selective for fat-protons displayed the degree of lipid accumulation in each area. The images selective for water-protons provided an internal control of adipose tissue localization. The proposed paradigm allows an unequivocal definition of BAT deposits and appears particularly useful in studies where experimental manipulation (i.e., cold acclimation or drug treatment) produces changes in this tissue.—**Sbarbati, A., U. Guerrini, P. Marzola, R. Asperio, and F. Osculati.** Chemical shift imaging at 4.7 tesla of brown adipose tissue. *J. Lipid Res.* 1997. **38:** 343–347.

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Two types of adipose tissue are found in mammals: white adipose tissue (WAT) and brown adipose tissue (BAT) (1, 2). In vivo identification of BAT deposits as distinct from WAT is not feasible by means of X-ray imaging or thermography (3, 4).

In previous studies, we demonstrated that magnetic resonance imaging (MRI) defines BAT deposits in the living animal (5) and high spatial resolution MRI proved effective in anticipating some cellular characteristics of brown adipocytes at different ages (6). Moreover, we provided evidence that the magnitude of BAT deposits can be determined by a combination of MRI and morphometry, and that MRI also allows investigation of the functional morphology of the tissue in vivo (7).

The above-mentioned results were obtained by means of T1 weighted (T1W), spin-echo (SE) pulse sequences. However, in standard SE images, signals from protons of both water and fat are simultaneously detected and cannot be differentiated. The identification of BAT consequently rests mainly on differences in relaxation times. A number of specific pulse sequences that allow for image fat and water protons separately have been proposed. These chemical shift imaging (CSI) techniques directly couple spectroscopic information (chemical shift) with spatial information and have been proposed for quantification of lipids in biological tissues (8–11). In the present report, CSI techniques are applied for the first time to the study of BAT. The aim of the present work was to develop a better methodology for the study of BAT giving unequivocal information on space location and extention of this tissue.

MATERIALS AND METHODS

Male Wistar rats were maintained at room temperature and fed ad libitum. The animals were pre-anesthetized with ether, anesthetized with ketamine, and examined in an SIS 200/330 imager spectrometer (SIS Co., Freemont, CA), equipped with a 4.7 T Oxford magnet with a 33-cm bore. All images were acquired with TR/TE = 1000/30 ms. Slices were 2-mm thick. In order to

Abbreviations: BAT, brown adipose tissue; CSI, chemical shift imaging; MRI, magnetic resonance imaging; NMR, nuclear magnetic resonance; ppm, parts per million; SE, spin-echo; T, tesla; WAT, white adipose tissue.

[1]To whom correspondence should be addressed.

acquire chemical shift selective images, the slice selection gradient during the first pulse (90°) in the SE sequence was eliminated and a 90° chemical shift selective pulse was used. This pulse was gaussian shaped and 4 ms long resulting in a 500 Hz band excitation, small enough to discriminate between fat and water signals which, at 4.7 T, are separated by about 700 Hz. Fat-selective and water-selective images were obtained by setting the center of the excitation bandwidth on the resonances of fat and water protons, respectively.

Proton spectra of interscapular BAT, periepididymal WAT, and leg muscle were obtained, after removal of these tissues from killed animals, on the same instrument used for imaging procedures. In order to establish a quantitative relationship between the water/fat integrated peak and the percentage of water/fat protons contained in the sample, we have used a phantom constituted by water-in-oil reverse micelles suspensions (12). According to ref. 12, reverse micelles suspensions were prepared by dissolving the surfactant bis-2(ethylhexyl) sulfosuccinate sodium salt (AOT) in isooctane and adding a distilled water solution of 1.5 mM $CuSO_4$. AOT and isooctane were purchased from Fluka. Six samples were prepared containing, respectively, 46.1, 50.0, 60.0, 70.0, 80.0, and 90.0 ml of isooctane per 100 ml of solution. The concentration of AOT was kept constant at 0.57 M in all the samples and the contribution of AOT to proton spectra was neglected (12). Proton spectra of these solutions were obtained by using the same experimental conditions as for excised tissues. The water and oil peaks of each spectrum were integrated and normalized at 1. The correlation between the oil proton integrated peak, Y, and the known oil content of each sample, X (expressed as [oil volume]/[oil volume + water volume]), was analyzed by least squares analysis. The correlation was found to be linear and was expressed by the formula: $Y = 0.0451 + 0.937\ X$ ($r^2 = 0.998$). As the ratio between water and isooctane proton density is about 1 (1.02 at 20°C), the oil volume fraction represents the oil proton fraction.

RESULTS

Proton spectra of isolated BAT deposits (**Fig. 1a**) show that both fat and water protons contribute significantly to the genesis of the nuclear magnetic resonance (NMR) signal. The NMR signal of WAT (Fig. 1b) originates mainly from fat protons while the NMR signal of muscle (Fig. 1c) is largely due to water protons, the fat proton signal being virtually absent. As described in Materials and Methods, proton spectra integrated peaks are linearly correlated (slope 0.937) to the oil proton

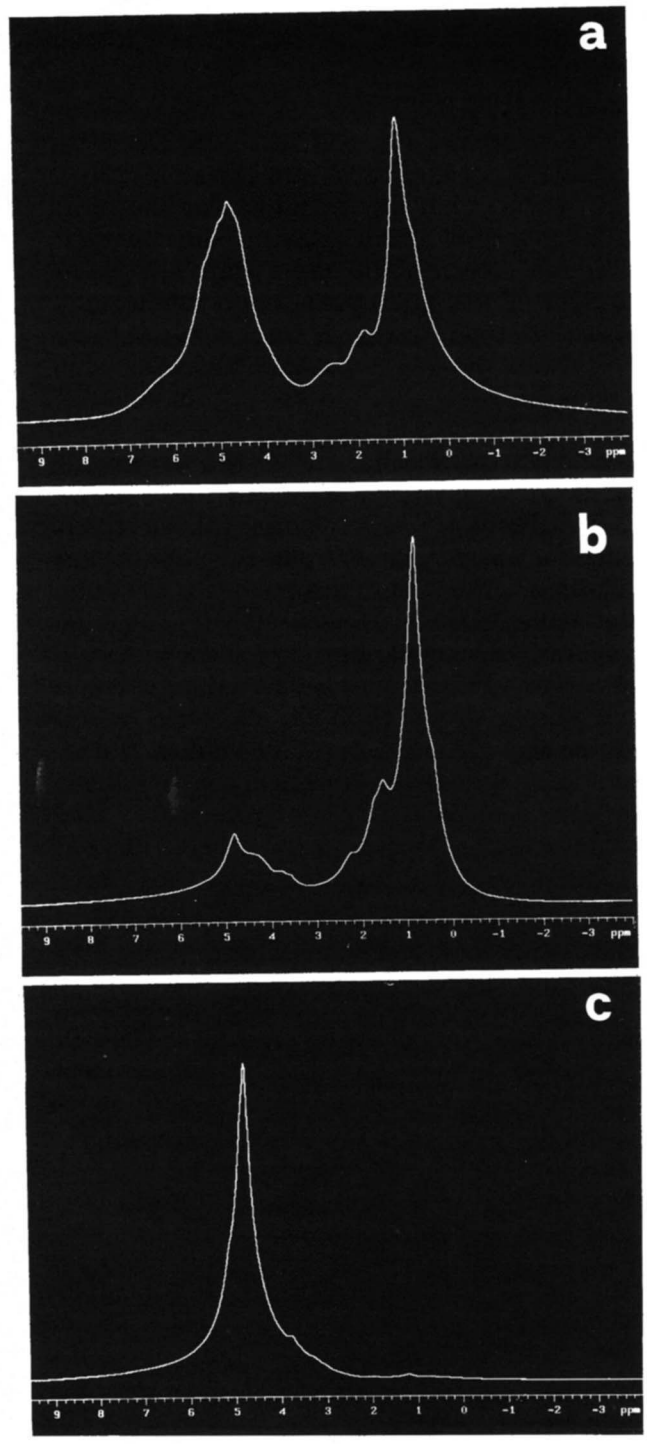

Fig. 1. Proton spectra of interscapular BAT (a), periepididymal WAT (b), and leg muscle (c). The water-proton and fat-proton resonances are at 4.7 and 1.2 ppm, respectively. In these representative samples, obtained from the same rat, the water-proton percentage was roughly 46% in BAT, 20% in WAT, and 99% in muscle as estimated from the integrals of spectral peaks.

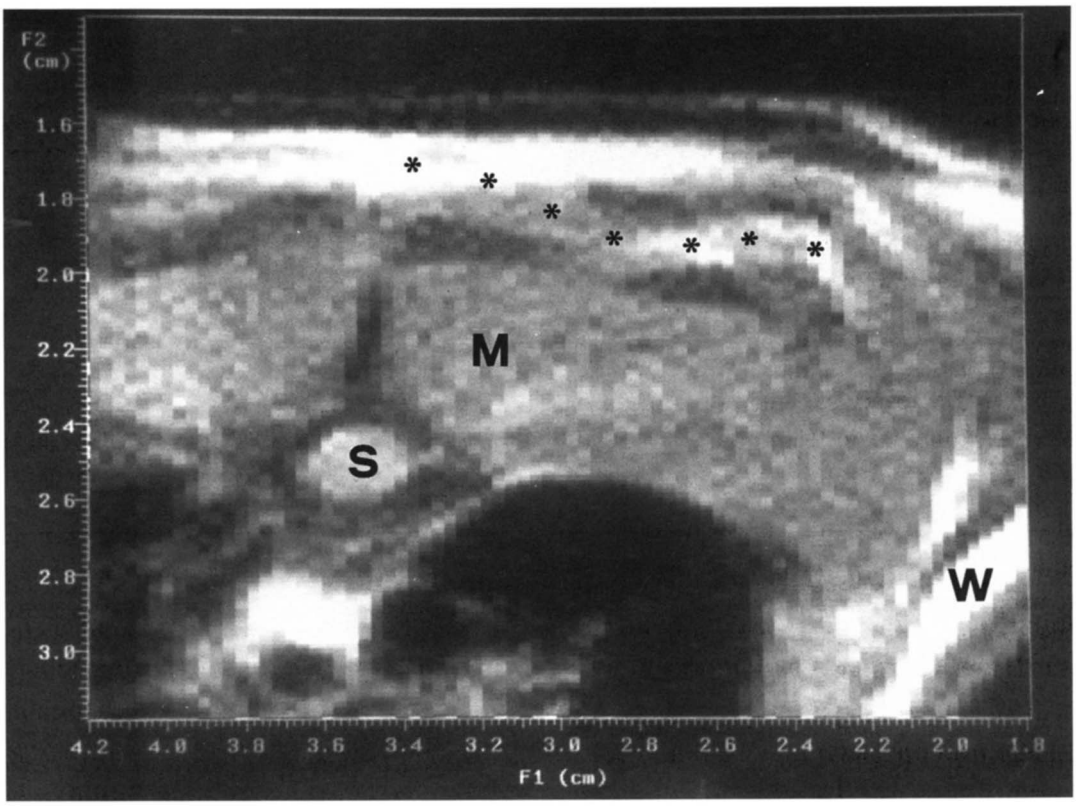

Fig. 2. Axial section, at the level of the thoracic region, of a living rat. In this SE image, the interscapular BAT is recognizable (asterisks). The intensity of the signal emitted from the BAT ranges from those of muscle (M) to those of WAT (W); S, spinal cord.

fraction in the model system; consequently, for the tissues examined ex vivo, we estimated the fat and water proton content from spectra (see Fig. 1 caption). In the standard SE sequence (**Fig. 2**), large BAT deposits can usually be identified. The signal intensity of BAT is quite variable showing differences also in the same deposit depending on the functional status of the tissue. In SE images, BAT may appear isointense with muscle, WAT, or other structures, such as thymus gland or spinal cord. In particular, it is difficult to differentiate BAT from muscle. This variability makes the recognition of small BAT deposits difficult.

Our experimental paradigm for an unequivocal definition of BAT requires that SE, water-selective, and fat-selective images are acquired (**Fig. 3**). The SE, T1-weighted image (Fig. 3a) provides the best anatomical description of the structures, but BAT appears isointense with other tissues.

The image selective for fat protons (Fig. 3b) displays the degree of lipid accumulation in each area. A homogeneous, high intensity signal is emitted from WAT. BAT deposits are easily identified because they emit a signal less intense than WAT. The other tissues physiologically present in the cervical and thoracic regions give signal intensity at the noise level, with our experimental conditions.

The images selective for water protons (Fig. 3c) provide an internal control of adipose tissue localization. In this type of image, adipose tissue deposits appear as darker areas in sharp contrast to the strong hydrated muscular parenchyma.

DISCUSSION

Quantification of BAT in the living body would be of great importance to assess the role of the tissue in the dynamics of energy balance and body weight. However, investigations have been hindered thus far by the lack of a reliable, in vivo procedure for BAT definition.

In the past we have obtained good distinction between BAT and WAT using SE sequences, however, in standard SE sequences, tissue information is based on the proton density and on the relaxation times. The chemical shift information exploited in NMR spectroscopy (13) is not utilized in SE pulse sequences that do not retain the chemical shift differences between the

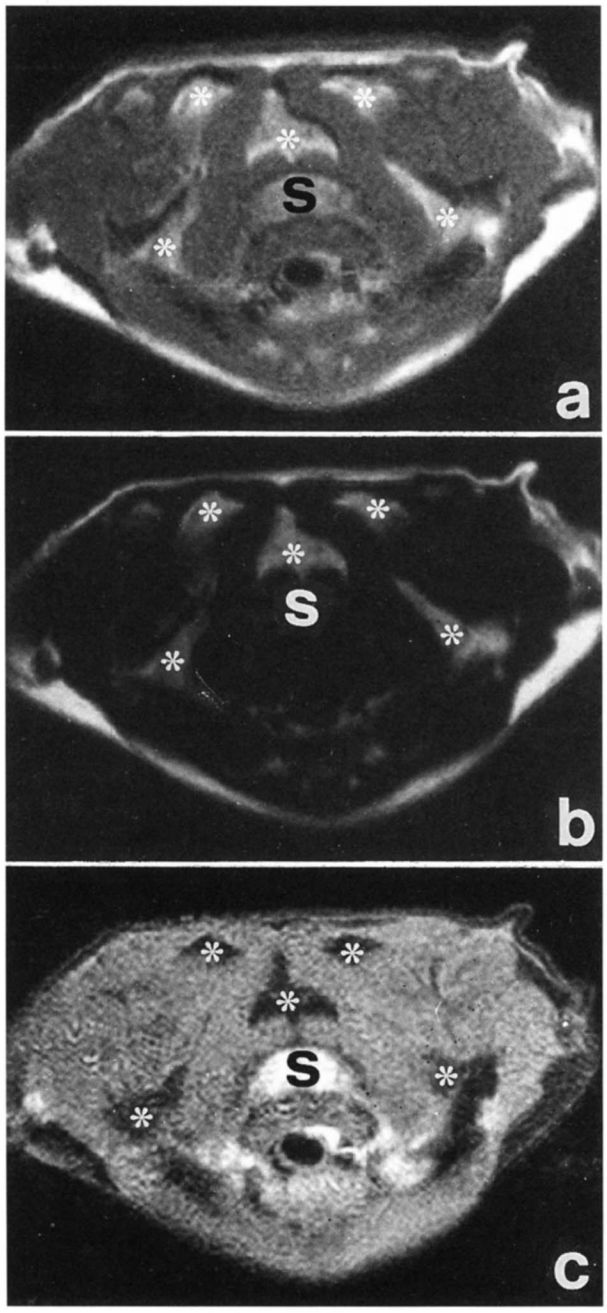

Fig. 3. Axial section, at the level of the cervical region, of a living rat. a) SE image. b) Selective fat-protons image. c) Selective water-protons image. In all the pictures the cervical pad (asterisk) and other deposits of BAT are visualised. Note the high intensity signal emitted from spinal cord (S) in panel c).

tissues. Therefore, if the SE sequence is usually sufficient for studying WAT, it has limits in visualization of BAT in which a great percentage of the signal may be due to water protons. Also, the simple differentiation of BAT from other tissues may be difficult. NMR spectra clearly demonstrate that the NMR characteristics of BAT are largely different from those of WAT or from muscle. Using conventional MRI techniques, the WAT signal originates almost totally from fat protons and the signal of muscle originates from water protons. The BAT signal originates from both fat and water protons. By removing the part of the signal due to water, CSI allows a more nearly correct evaluation of BAT deposit because the image originates directly from the lipid content of the tissue. The proposed paradigm allows an unequivocal definition of BAT deposits and it appears particularly useful in studies where experimental manipulation (e.g., cold acclimation or drug treatment; ref. 14) produces changes in the dimension and fat content of BAT deposits. For example, in age-related processes, BAT undergoes progressive modification of the water/lipid ratio (15). Therefore, the utility of a method that can differentiate water from fat signal in the study of BAT is evident. In addition, CSI is useful when high magnetic fields are used. At high magnetic fields, an easier distinction among the peaks is possible and CSI can also allow for elimination of chemical shift artifacts that are more severe at high magnetic fields (16).

In conclusion, the proposed paradigm, based on CSI, appears as an improved methodology for the study of BAT giving more reliable information on location and lipid contents of this tissue.

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REFERENCES

1. Cannon, B., and J. Nedergaard. 1984. The biochemistry of an inefficient tissue: brown adipose tissue. *Essays Biochem.* **20:** 110–165.
2. Heaton, J. 1972. The distribution of brown adipose tissue in the human. *J. Anat.* **112:** 35–39.
3. Rothwell, N. J., and M. J. Stock. 1979. A role for brown adipose tissue in diet-induced thermogenesis. *Nature.* **281:** 31–35.
4. Dauncey, M. J., C. Haseler, D. P. Page Thomas, and G. Parr. 1983. Influence of meal on skin temperatures estimated from quantitative IR-thermography. *Experientia.* **39:** 860–862.
5. Osculati, F., F. Leclercq, A. Sbarbati, C. Zancanaro, S. Cinti, and K. Antonakis. 1989. Morphological identification of brown adipose tissue by magnetic resonance imaging in the rat. *Eur. J. Radiol.* **9:** 112–114.
6. Osculati, F., A. Sbarbati, F. Leclercq, C. Zancanaro, C. Accordini, K. Antonakis, A. Boicelli, and S. Cinti. 1991. The correlation between magnetic resonance imaging and ul-

trastructural patterns of brown adipose tissue. *J. Submicroscop. Cytol. Pathol.* **23:** 167–174.
7. Sbarbati, A., A. M. Baldassarri, C. Zancanaro, A. Boicelli, and F. Osculati. 1991. In vivo morphometry and functional morphology of brown adipose tissue by magnetic resonance imaging. *Anat. Rec.* **231:** 293–297.
8. Dixon, W. T. 1984. Simple proton spectroscopic imaging. *Radiology.* **153:** 189–194.
9. Buxton, R. B., G. L. Wismer, T. J. Brady, and B. R. Rosen. 1986. Quantitative proton chemical-shift imaging. *Magn. Reson. Med.* **3:** 881–900.
10. Brix, G., S. Heiland, M. E. Bellemann, T. Koch, and W. J. Lorenz. 1993. MR imaging of fat-containing tissues: valuation of two quantitative imaging techniques in comparison with localized proton spectroscopy. *Magn. Reson. Imaging.* **11:** 977–991.
11. Wong, W. F., S. R. Northrup, R. C. Herrick, A. P. Glombicki, R. P. Wood, and J. D. Morrisett. 1994. Quantitation of lipid in biologic tissue by chemical shift magnetic resonance imaging. *Magn. Reson. Med.* **32:** 440–446.
12. Roe, J. E., W. E. Prentice, and J. P. Hornak. 1996. A multipurpose MRI phantom based on a reverse micelle solution. *Magn. Reson. Med.* **35:** 136–141.
13. Zancanaro, C., R. Nano, C. Marchioro, A. Sbarbati, A. Boicelli, and F. Osculati. 1994. Magnetic resonance spectroscopy investigations of brown adipose tissue and isolated brown adipocytes. *J. Lipid Res.* **35:** 2191–2199.
14. Sbarbati, A., F. Leclercq, F. Osculati, and I. Gresser. 1995. Interferon a/b-induced abnormalities in adipocytes of suckling mice. *Biol. Cell* **83:** 163–167.
15. Sbarbati, A., M. Morroni, C. Zancanaro, and S. Cinti. 1991. Rat interscapular brown adipose tissue at different ages: a morphometric study. *Int. J. Obes.* **15:** 581–588.
16. Volk, A., B. Tiffon, J. Mispelter, and J. M. Lhoste. 1987. Chemical shift-specific slice selection. A new method for chemical shift imaging at high magnetic field. *J. Magn. Reson.* **71:** 168–174.