MAGNETIC RESONANCE IN MEDICINE 17, 82–94 (1991)

Localized Proton NMR Spectroscopy Using Stimulated Echoes: Applications to Human Skeletal Muscle *in Vivo*

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Received October 10, 1989; revised December 19, 1989

Localized proton NMR spectroscopy using stimulated echoes (STEAM) has been used to study metabolites in different proximal skeletal muscles of normal volunteers at rest. Single scan water-suppressed proton NMR spectra obtained at 1.5 and 2.0 T (Siemens Magnetom) from a 64-ml volume-of-interest (VOI) yield resonances due to triglycerides, phosphocreatine plus a minor contribution from creatine, and betaines comprising carnitine and choline-containing compounds. The observation of the pH-dependent resonances of carnosine required multiple acquisitions and echo times as short as 20 ms. T_1 and T_2 relaxation times of muscle metabolites were obtained by varying the repetition time and echo time of the STEAM sequence, respectively. Although rather long T_2 values such as 180 ms for (phospho-) creatine correspond to natural resonance linewidths of only 2 Hz, the observed linewidths of typically 10–12 Hz are entirely determined by the short T_2 relaxation times (25–30 ms) of the water protons used for shimming. The spectroscopic results from 24 muscle studies on 17 young male volunteers show remarkable intra- and interindividual differences in the absolute signal intensities of mobile lipids. Further metabolic variations were observed for the relative concentrations of betaines (by a factor of 2) and carnosine (by a factor of 3) when total creatine is assumed to be constant.

INTRODUCTION

In recent years *in vivo* NMR spectroscopy (MRS) of muscle has been largely synonymous with applications of phosphorous MRS (*1*, *2*) and surface coil (*3*) "localization." Although previous reports on proton MRS of skeletal muscle in humans (*4–9*) unraveled valuable information complementary to data about the energy metabolism derived from phosphorous spectra, the results were often compromised by technical limitations such as poor resolution (linewidths of 16–30 Hz), spectral distortions (artifacts from binomial water suppression and mathematical resolution enhancement), long echo times, as well as limited (one-dimensional DRESS) or low-quality (SPARS) localization techniques.

The present study was intended to elucidate the quality of localized proton NMR spectra of skeletal muscle in humans that can be achieved on a 1.5-T or 2.0-T whole-body MRI system. In particular we have considered the accessible signal-to-noise ratio (SNR) and corresponding time resolution, the spectral resolution, the technical and biological reproducibility, and the measurement of T_1 and T_2 relaxation times of

0740-3194/91 $3.00

muscle metabolites *in vivo* as prerequisites for quantitative determinations of metabolite concentrations. The approach chosen here is based on the development of image-controlled localized MRS using stimulated echoes (*10*), and its successful application to studies of normal (*11*, *12*) and pathological (*13*, *14*) human brain. The STEAM technique addresses the problems of localization, signal overlap, and contamination due to superficial structures like skin and subcutaneous fat (*15*).

Potential clinical applications of proton MRS of muscle may include early diagnosis of myopathies, physiological studies in sports medicine, and future extensions to a biochemical characterization of cardiac myopathies and infarctions.

EXPERIMENTAL

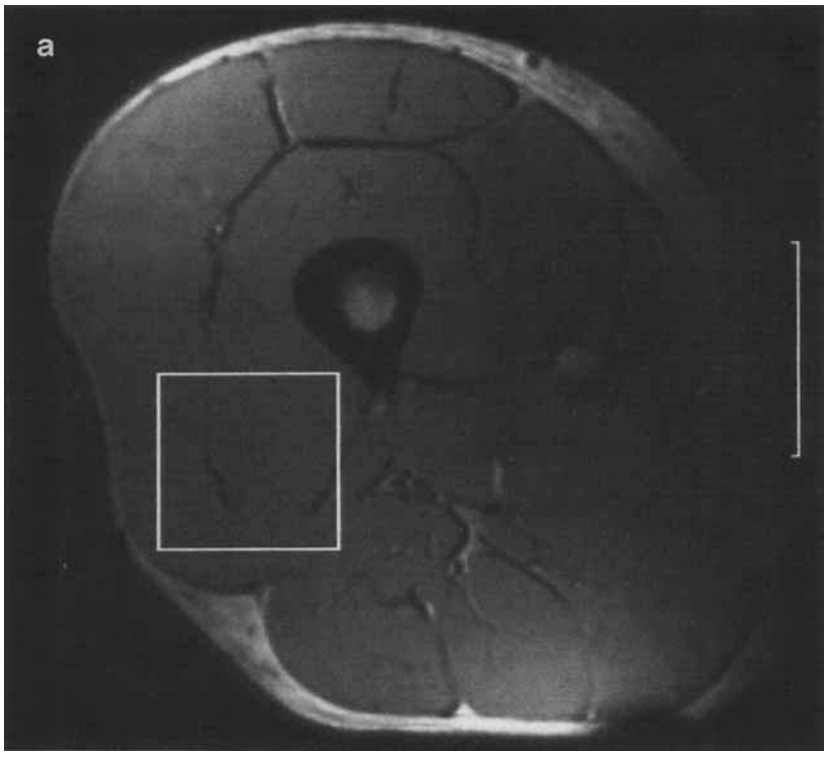
Localized proton NMR spectra were obtained using the STEAM technique (*10*) as described earlier in detail (*11*). Single voxel localization is accomplished in a single scan by means of three orthogonal slices that define a volume-of-interest (VOI) by their intersection. Typically, gradient strengths of 2 mT m^{-1} were employed for a 4 $\times$ 4 $\times$ 4 cm^3 (64 ml) VOI. The timing scheme for the three slice-selective radiofrequency (rf) pulses and the preceding chemical-shift-selective (CHESS) water suppression rf pulse with a bandwidth of 60 Hz reads

$$90° \text{ (CHESS)} - 90° \text{ (\#1)} - \text{TE}/2 - 90° \text{ (\#2)} - \text{TM} - 90° \text{ (\#3)} - \text{TE}/2 - \text{STE}.$$

The investigations were performed at 1.5 T (Erlangen) and 2.0 T (Göttingen) on a Siemens Magnetom MRI/MRS system in both cases. Proton images and spectra were acquired using Helmholtz transmit/receive coils of 17-cm diameter. For the determination of relaxation times the repetition time TR was varied between 750 and 3000 ms, and the echo time TE between 20 and 270 ms (TM = 30 ms). Localization of the VOI in the leg muscles was guided by proton NMR images obtained with an interleaved multislice FLASH sequence (*16*) using a repetition time of TR = 100 ms and a flip angle of 70°. Volunteers consisted of 17 trained and untrained young men (20–35 years) of whom informed consent had been obtained.

RESULTS

Representative results for a 1.5-T-proton MRS study of human skeletal muscle *in vivo* are shown in Fig. 1. The spectra originate from a 4 $\times$ 4 $\times$ 4 cm^3 VOI in the lateral quadriceps muscle of a young male volunteer at rest as depicted in the image in Fig. 1a. Figure 1b refers to a single scan (fully relaxed) acquisition at an echo time of TE = 50 ms, and Fig. 1c demonstrates the SNR improvement that results from 512 acquisitions at a repetition time of TR = 1500 ms. In addition to the dominating methylene signals from mobile lipids at 1.0–1.5 ppm and the residual water peak at 4.7–4.8 ppm, a single-scan spectrum allows an unambiguous identification of the intense resonances of the *N*-methyl group (three protons) of (phospho-) creatine at 3.0 ppm and of the *N*-trimethyl group (nine protons) found in the compound class of betaines such as carnitine and cholines at 3.2 ppm. Further signals in the 13 min spectrum in Fig. 1c are due to resonances of the acyl chains of lipids including terminal methyl moieties at 0.8–1.0 ppm, main methylene protons (1.1–1.5 ppm), α- and β-methylene protons at 2.0–2.5 ppm, and olefinic protons of oleic acid at 5.4 ppm. The



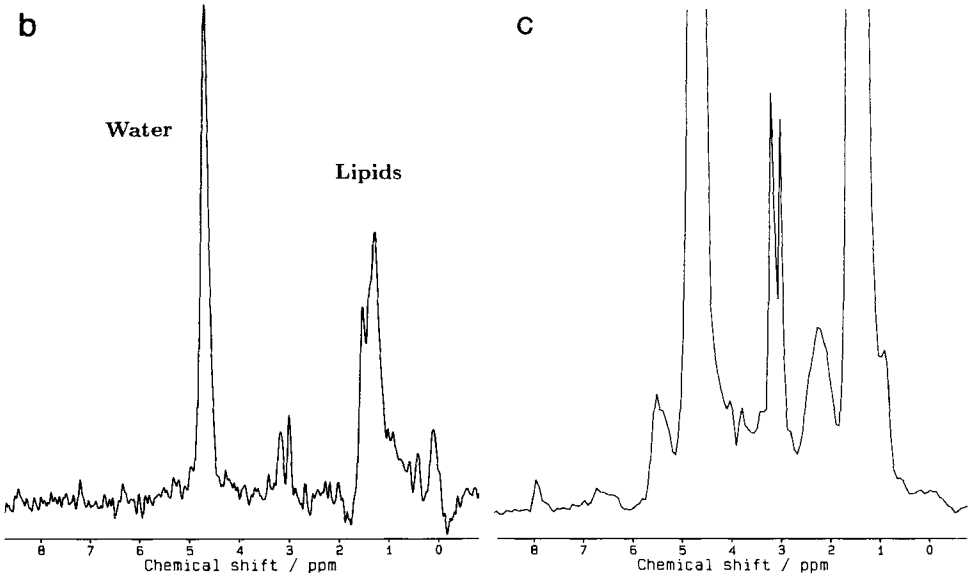


FIG. 1. Sixty-four-megahertz (1.5 T) transverse FLASH NMR image (70°, TR = 100 ms, TE = 11 ms, 256 × 256 data matrix) and localized proton NMR spectra from the upper thigh of a normal volunteer. (a) Axial image indicating a 4 × 4 × 4 cm^3 (64 ml) VOI selected for proton spectroscopy of the lateral quadriceps muscle, (b) corresponding single scan (fully relaxed) spectrum, and (c) 512 acquisitions using TR = 1500 ms. Localization was achieved using the STEAM technique (TM = 30 ms, TE = 50 ms). Resonance signals are due to residual water (4.7–4.8 ppm), terminal methyl (0.8–1.0 ppm), acyl chain methylene (1.1–1.5

C2-bound aromatic ring proton of histidine (one proton) in the muscle dipeptide carnosine (β-Ala-His) resonates at 8.0 ppm. At the relatively long echo time of TE = 50 ms the corresponding C4-bound imidazole proton of carnosine can hardly be detected. The present assignments are in agreement with previous studies of skeletal muscle in humans (*5–7*) and anesthetized rats (*17*), as well as with high-field NMR studies of perchloric acid extracts of various muscle tissues (*18*, *19*).

About 24 studies on the upper thigh of 17 different healthy volunteers were performed to establish intra- and interindividual reproducibilities and dependencies of proton NMR spectra of normal human muscle *in vivo*. Figure 2 shows images and corresponding spectra from three different locations within the upper thigh of the same volunteer as in Fig. 1. They demonstrate intraindividual spectral variations. It turns out that only slight shifts in the position of the VOI either within the lateral quadriceps muscle (Fig. 1b, 2a) or within the great adductor muscle (Figs. 2b, 2c) result in dramatic alterations in the resonance signals of mobile triglycerides. This is best seen in the lipid/creatine ratio using total creatine as a relative internal standard. These findings may be explained by different average concentrations of fat in the respective volumes.

As with the *intraindividual* differences shown above, a series of studies on three different volunteers, depicted in Fig. 3, revealed large *interindividual* spectral variations even when attempting to localize the VOI in approximately the same position in the great adductor muscle. The resulting single-scan proton NMR spectra at TE = 50 ms show considerable variations in the lipid/creatine ratio as might have been anticipated from the intraindividual results.

The determination of T_1 and T_2 relaxation times is documented in Figs. 4 and 5 showing two series of 1.5-T-proton NMR spectra from a 64 ml VOI in the lateral quadriceps muscle of the same volunteer. The spectra have been recorded at repetition times of TR = 750 ms, 1500 ms, and 3000 ms (TE = 50 ms), and at echo times of TE = 50 ms, 135 ms, and 270 ms (TR = 1500 ms), respectively. The inserts covering the aromatic range in Fig. 4 are magnified by a factor of 18. For display purposes the decrease in signal intensities with increasing echo time in Fig. 5 has been accounted for by multiplying the spectra at TE = 135 ms and 270 ms with scaling factors of 2 and 4, respectively, as compared to the spectrum at TE = 50 ms. The evaluated T_1 and T_2 relaxation times of the detected muscle metabolites are summarized in Table 1. No significant differences in relaxation times were observed for the quadriceps and the great adductor muscle. The values were determined from both signal areas and peak heights using a nonlinear Gauss–Newton fitting procedure for T_1 values and a linear least-squares fit for T_2 values. Deviations from the values given in Table 1 are in the range of ±10%.

Additional measurements were performed on a 2.0-T-whole-body system using very short echo times as have recently been obtained for both STEAM spectroscopy (*20*) and subsecond FLASH MRI (*21*). Figure 6 shows single-scan spectra at TE = 20 ms

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ppm), α- and β-methylene (2.0–2.5 ppm), and olefinic protons (5.4 ppm) of lipids, *N*-methyl protons of phosphocreatine and creatine (3.0 ppm), *N*-trimethyl protons of betaines (3.2 ppm) comprising carnitine and choline-containing compounds, and an aromatic resonance from the C2-ring proton of the histidyl moiety (8.0 ppm) in carnosine.

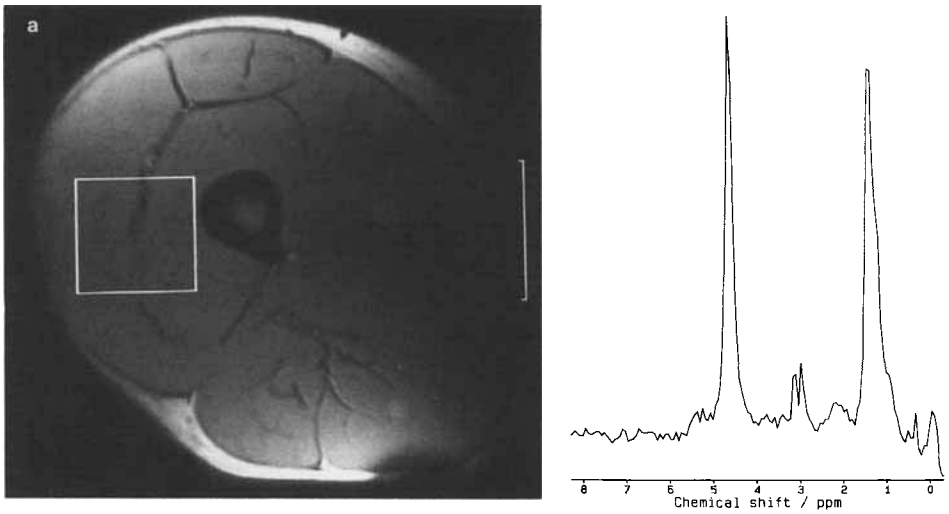

FIG. 2. Sixty-four-megahertz (1.5 T) axial FLASH NMR images (as Fig. 1) and localized proton NMR spectra (64 ml, single scan, TM = 30 ms, TE = 50 ms) from different positions in the upper thigh of a normal volunteer. The spectra demonstrate intraindividual variations in (a) the lateral quadriceps muscle (compare Fig. 1b), and (b, c) the great adductor muscle.

(a) and TE = 50 ms (b) from a 64-ml VOI in the medial quadriceps muscle. The inserts depict the resonances of the aromatic C2 (8.0 ppm)- and C4-bound (7.0 ppm) ring protons of carnosine taken from spectra recorded at a repetition time of TR = 1500 ms using 512 acquisitions. The resulting SNR allows accurate determinations of the short T_2 values of carnosine as listed in Table 1. The T_2 relaxation times for the betaines were identical to those at 1.5 T, while those for (phospho-) creatine were slightly smaller. In most cases the improved resolution and water suppression at 2.0 T allowed additional measurements on the N–CH_2 protons at 3.9 ppm. The pH values of the resting muscles under investigation were found to be 7.0 based on the chemical shift titration curve reported in (*7*).

DISCUSSION

Localized proton NMR spectra from human skeletal muscle are less complex than spectra from human brain (*11*, *12*) since only triglycerides, (phospho-) creatine, and betaines are sufficiently concentrated to be detectable within reasonable measuring times, i.e., within single-scan acquisitions for a 64-ml VOI and within a few minutes for a 8-ml VOI. In fact, the water-suppressed spectra are dominated by a variety of resonances from mobile lipids that preclude the direct observation of other compounds with smaller signals in the same chemical shift ranges. In particular, the detection of lactate would require spectral editing techniques (*22*) in order to evaluate the training status of muscle in sports medicine or its energetic response to a workload in mitochondrial myopathies. Variations in intracellular pH may be monitored via the chemical shifts of the carnosine ring protons (*7*, *23*) with a time resolution of the order of minutes depending on the size of the selected VOI.

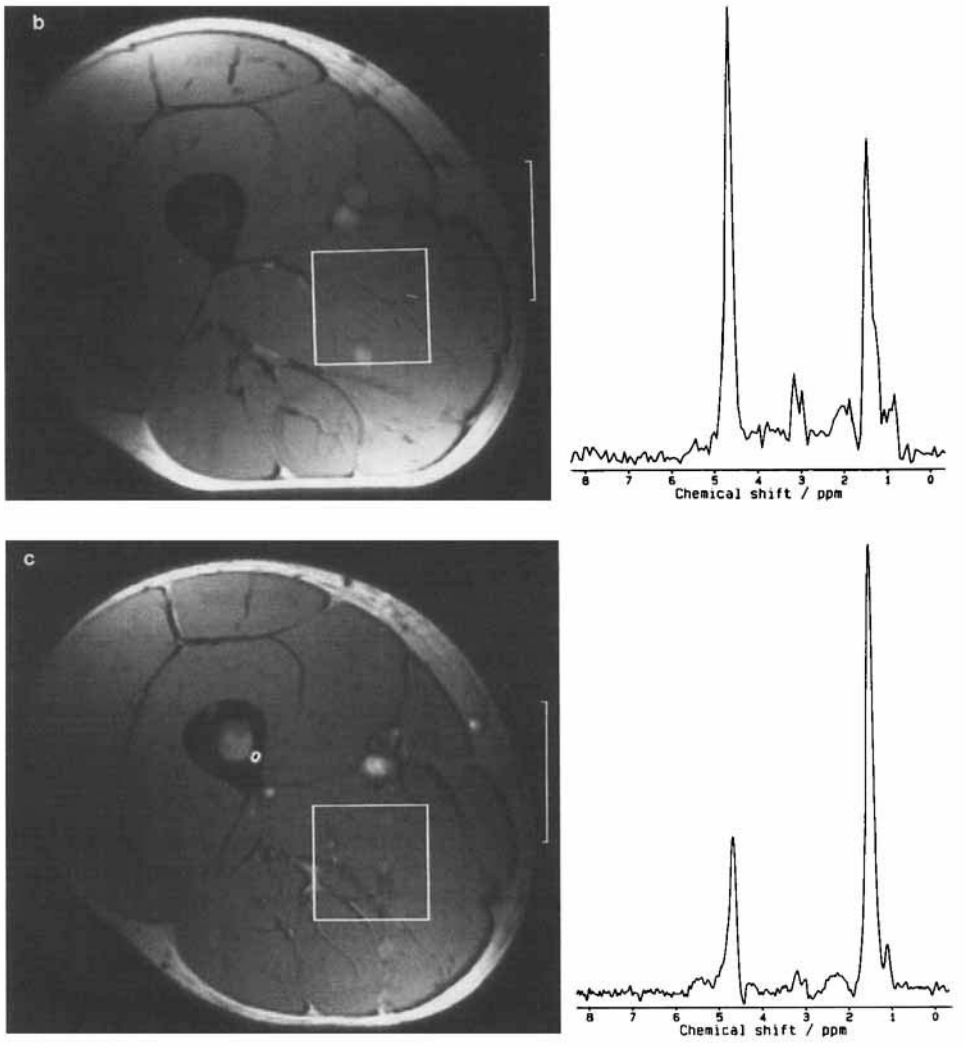

FIG. 2—*Continued*

Spectral Resolution and Relaxation Times

In comparison to brain studies the spectral resolution in proton MRS of muscle *in vivo* is severely degraded by experimental linewidths (10–12 Hz) that are by a factor of 3 greater than in brain. It is noteworthy that the broad lines are not caused by tissue susceptibility problems but, in accordance with the full linewidth at half-height FWHH $= 1/(\pi T_2)$, may be fully ascribed to the short T_2 relaxation times (25–30 ms) of the water protons that are used for shimming. Unfortunately, this "natural" linewidth generally limits the achievable spectral resolution in spite of a rather high mobility of all major metabolites as expressed by their relaxation times in Table 1. For example, the T_2 relaxation times for (phospho-) creatine of 180 ms and for betaines of 110 ms translate into natural linewidths of the order of 2–3 Hz. Even the T_2 values of 50 ms

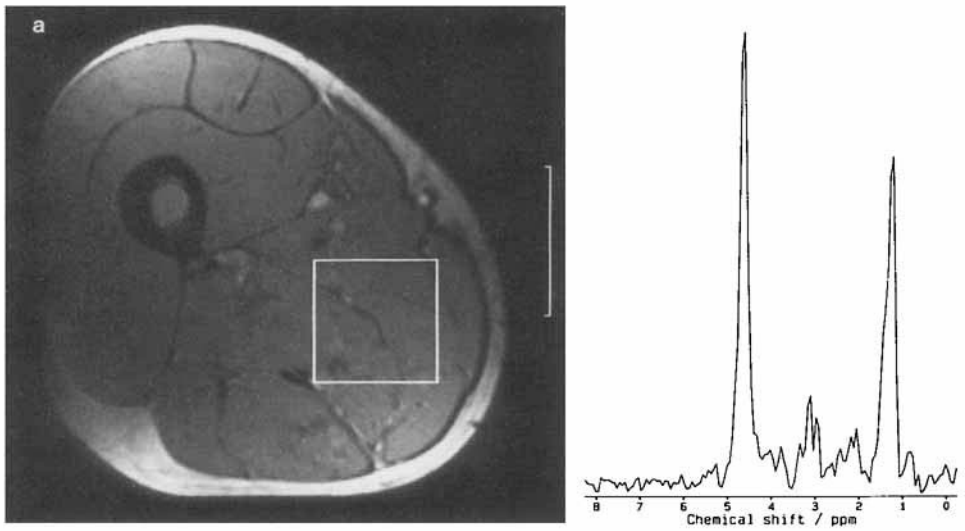

FIG. 3. Sixty-four-megahertz (1.5 T) axial FLASH NMR images (as Fig. 1) and localized proton NMR spectra (64 ml, single scan, TM = 30 ms, TE = 50 ms) from the upper thigh of three different normal volunteers (a–c). The spectra of the great adductor muscle demonstrate interindividual variations in the lipid content.

(C4-proton) and 100 ms (C2-proton) of carnosine are significantly longer than those of water. They are also longer than the 11 ms (C4-proton) and 39 ms (C2-proton) reported for carnosine in human skeletal muscle at 4.7 T (*7*) using a complex combination of techniques for localization and suppression of lipids and water. On the other hand, T_2 relaxation times of 300–500 ms were found for the respective anserine resonances in rat legs *in vivo* at 8.5 T (*17*). However, these data were obtained in CPMG experiments using a 180° pulse repetition time as short as 1.2 ms in order to minimize signal attenuation due to molecular self-diffusion. An explanation of the apparent T_2 shortening from 400 to 100 ms would require a constant (internal) gradient strength of the order of 1–2 mT m^{-1}. Unfortunately, the application of corresponding CPMG sequences to human skeletal muscle would surpass by far the rf safety regulations.

When considering the T_1/T_2 ratio as an indicator for freedom in isotropic molecular tumbling, it is striking that muscle water must be bound much tighter than, e.g., water in neuronal tissues. In fact, a T_1/T_2 ratio of about 30 is higher than for all other "NMR-visible" metabolites in human muscle or brain. The appearance of a short spin–spin relaxation time and a long spin–lattice relaxation time may be related to the association of muscle water to macromolecules or molecular aggregates that mainly affects the overall (translational) motion of the molecule rather than its reorientational mobility. Clearly, restricted mobility is not a general finding in muscle tissue as would be the case for an increase of the intracellular viscosity imposing the same constraints on all metabolites. The T_2 relaxation time of (phospho-) creatine is at the lower boundary of the range of values (190–250 ms) reported for different regions in the human brain (*12*), and its T_1/T_2 ratio of 7.5 is identical to that in neuronal tissue.

The mobility of the *N*-trimethyl resonance in muscle as compared to brain is reduced

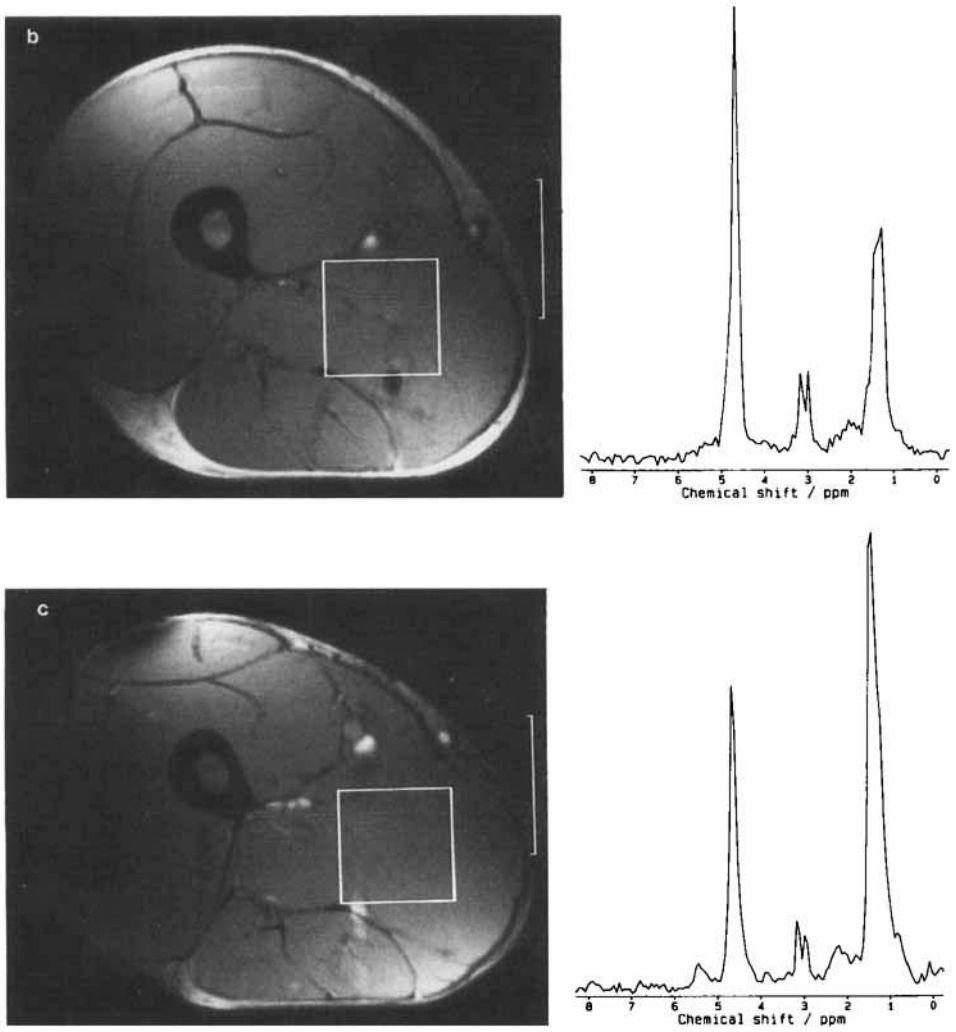

FIG. 3—*Continued*

by a factor of 3 as seen in T_2 and T_1/T_2. This finding probably indicates a shift in the contributing metabolites from choline-containing compounds in brain to carnitine in muscle. A limited mobility of carnitine *in vivo* may reflect its subcellular site of function at the mitochondrial membrane and the carnitine acyl transferases. The *in vitro* observation that in standard subcellular fractionation most of the carnitine would be recovered in the postmicrosomal supernatant (*24*) may be misleading. Finally, the high degree of motional restriction observed for carnosine may be correlated to its possible involvement in complex formation with metal ions such as copper (*25*).

Intra- and Interindividual Variations and Metabolite Concentrations

It is already evident from the inspection of NMR images that muscular tissues are quite heterogeneous comprising fasciae, vascular structures, and depot fat within and

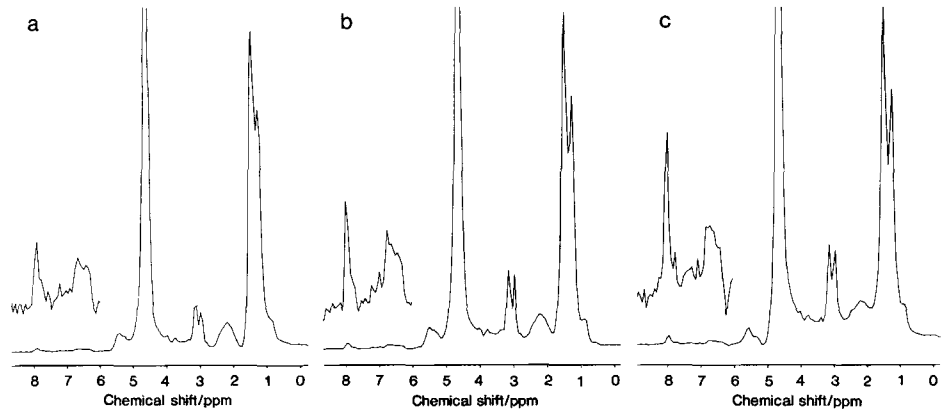

FIG. 4. Sixty-four-megahertz (1.5 T) localized proton NMR spectra (64 ml, TM = 30 ms, TE = 50 ms) from the upper thigh of a normal volunteer. The spectra of the lateral quadriceps muscle recorded at repetition times (a) TR = 750 ms (512 scans), (b) TR = 1500 ms (512 scans), and (c) TR = 3000 ms (256 scans) demonstrate the T_1 dependence of the metabolite resonances. The intensities have been scaled for direct comparability. For display purposes the aromatic regions are magnified by a factor of 18 in the inserts.

around the muscle bundles. Accordingly, the spectra of different locations within the same muscle and volunteer show considerable intraindividual variation in the relative intensity of the lipid signal. Since a different nutritional status and lipid metabolism are expected to complicate the problem, we did not include obese people in the present study. However, even within the rather homogeneous group of young male volunteers the spectra from the same muscles exhibit interindividual differences to a degree similar to that of the intraindividual variations. The observed variations, e.g., in the lipid/creatine ratio, may be due to intracellular triglyceride lipid droplets that are invisible

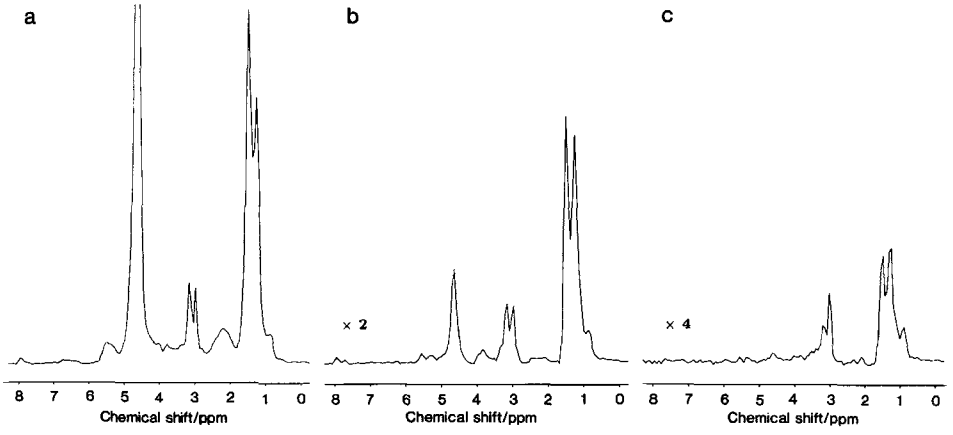

FIG. 5. Sixty-four-megahertz (1.5 T) localized proton NMR spectra (64 ml, TM = 30 ms, TR = 1500 ms, 512 scans) from the upper thigh of a normal volunteer. The spectra of the lateral quadriceps muscle recorded at echo times (a) TE = 50 ms, (b) TE = 135 ms, and (c) TE = 270 ms demonstrate the T_2 dependence of the metabolite resonances. The intensities have been scaled for direct comparability when accounting for the magnifications in (b) and (c), respectively.

TABLE 1

Spin–Lattice (T_1) and Spin–Spin (T_2) Relaxation Times (1.5 T) of Major Metabolites Detected in Localized Proton NMR Spectra of Human Skeletal Muscle *in vivo* at Rest

Compound	Molecular group	δ (ppm)	T_1 (ms)	T_2 (ms)	T_1/T_2
Lipids	CH_2	1.0–1.5	300	90	3.3
Creatines	$N-CH_3$	3.0	1350	180	7.5
	$N-CH_3$	3.0	—	160[a]	—
	$N-CH_2$	3.9	—	100[a]	—
Betaines	$N-(CH_3)_3$	3.2	1150	110	10.5
	$N-(CH_3)_3$	3.2	—	110[a]	—
Carnosine	His, C2–H	8.0	1750	100[a]	17.5
	His, C4–H	7.0	—	50[a]	—
Water	OH	4.7	900	30	30

Note. The proton NMR data for creatines in skeletal muscle predominantly refer to phosphocreatine. Betaines include carnitine and choline-containing compounds. The values represent median values with deviations of about ±10%.

[a] The relaxation times were obtained at 2.0 T.

in NMR images. The triglyceride concentration is approximately 3 times higher in type I than in type II muscle fibers (*26*). The mixture of these fiber types in the upper thigh muscles is known to vary in wide range between individuals, e.g. 29–78% slow twitch fibers in the lateral great muscle (*27*). Obviously, this raises rather difficult problems for standardization if proton MRS were to become a tool in *early* diagnosis of fibrous-fatty degeneration in myopathies. *Advanced* stages of the disease normally present no diagnostic challenge, and would be evident by clinical inspection and MRI alone.

Relative metabolite concentrations were obtained from the NMR spectra by correcting signal areas (or peak heights) with the *in vivo* relaxation times, the number of

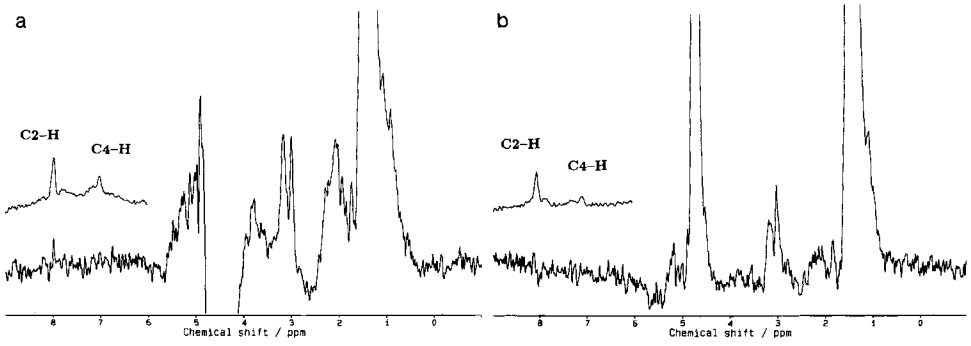

FIG. 6. Eighty-four-megahertz (2.0 T) localized proton NMR spectra (64 ml, single-scan fully relaxed, TM = 30 ms) from the upper thigh of a normal volunteer. The spectra of the medial quadriceps muscle recorded at (a) TE = 20 ms and (b) TE = 50 ms demonstrate the T_2 dependence of metabolite resonances at shorter echo times. The inserts represent the aromatic part of spectra that have been recorded at a repetition time of TR = 1500 ms using 512 scans. They depict both the C2- and C4-ring protons in carnosine at 8.0 and 7.0 ppm, respectively.

contributing protons, and by assuming a constant concentration of total creatine. Summarizing the data for all volunteers the relative concentrations for betaines and carnosine ranged from 5 to 11 and 3 to 11, respectively, as compared to 25 for creatine which has been chosen as a (relative) reference. These large variations by factors of 2–3 are outside experimental errors and clearly reflect remarkable differences in the steady-state metabolite concentrations between different locations, muscles, and volunteers. Despite the large number of studies and data available it has so far been impossible to establish any significant and reproducible correlations between the muscle metabolite concentrations and the different muscles or the overall constitution of a volunteer. There was only a slight tendency to higher concentrations of carnosine and betaines and lower concentrations of lipids in the quadriceps muscle than in the great adductor muscle.

The absolute concentration of total creatine in skeletal muscle has been enzymatically determined in biopsy preparations to range from 25–29 m*M* (*28*, *29*). Phosphorous MRS studies from human distal muscle *in vivo* confirm these values, and also indicate that in intact muscle at rest the creatine kinase equilibrium is shifted to at least 90% in favor of phosphocreatine (*30*). Carnitine is known to act as a cofactor for the transport of fatty acids into the mitochondria, and is present in human muscle in concentrations around 2 m*M* as determined by enzymatic assays (*24*). Carnosine concentrations were determined to 1–8 m*M* (*31*).

Absolute quantification in MRS relies on identical coil loading and coil sensitivity from one subject to the next which in this more qualitative study was not taken care of. For example, the actual distance of the two circular coils varied according to the thickness of the thighs. In addition, due to the inhomogeneities of the rf field the absolute signal strengths in the NMR spectra depended on the position of the VOI relative to the coils precluding absolute interstudy comparisons. Nevertheless, the relative *in vivo* concentrations determined above may be combined with the assumption of a 25 m*M* concentration for (phospho-) creatine yielding 5–11 m*M* for betaines and 3–11 m*M* for carnosine. While the latter agrees with the range of biochemical determinations (*31*), a 5–11 m*M* concentration of betaines clearly indicates that only a smaller part of the *N*-trimethyl resonance is due to carnitine. Major contributions must result from cholines or other betaines. The opposite assumption that the *N*-trimethyl peak may be entirely attributed to the expected 2 m*M* carnitine concentration would lead to total creatine concentrations ranging from 5–10 m*M*. These values would have to be explained by a reduced visibility of creatine. They also show a surprisingly high variability. A further argument against this hypothesis is the fact that a 2 m*M* concentration of a CH_3 group is barely detectable in a single scan even from a 64-ml VOI.

CONCLUDING REMARKS

Localized proton MRS of human skeletal muscle allows the assessment of lipids, of betaines such as carnitine and cholines, and of (phospho-) creatine as well as the recording of the pH-sensitive resonances of carnosine. Although the measurement of T_1 and T_2 relaxation times provides a basis for the *in vivo* determination of absolute metabolite concentrations, standardization may be compromised by marked intra- and interindividual differences. Early diagnosis of myopathies with use of proton MRS

would have to take account for these findings. Open questions remain the absolute concentrations of carnitine and carnosine as well as their remarkable variability.

Although the spectral resolution in proton MRS of muscle is limited by the short T_2 relaxation times of muscle water protons that are used for shimming, the high SNR observed for single-scan acquisitions may be exploited for time-resolved functional studies or transformed into multiscan acquisitions with measuring times of minutes but much smaller VOI sizes. If motion-induced signal losses can be avoided, then ECG-synchronized proton MRS of the heart may be used for a rapid and direct determination of the lipid content in cardiac muscles. The recording of localized phosphorous NMR spectra of the human brain using STEAM sequences with echo times of only 3 ms (*20*) provides a technical solution to this problem that may open new ways for noninvasive monitoring of the deleterious alterations in lipid metabolism in heart ischemia and infarction (*32*).

ACKNOWLEDGMENT

Financial support by the Bundesminister für Forschung und Technologie (BMFT) of the Federal Republic of Germany (Grant 01 VF 8606/6) is gratefully acknowledged.

REFERENCES

1. B. CHANCE, S. ELEFF, AND J. S. LEIGH, *Proc. Natl. Acad. Sci. USA* **77,** 7430 (1980).
2. G. K. RADDA, *Science* **233,** 640 (1986).
3. J. J. H. ACKERMAN, T. H. GROVE, G. G. WONG, D. G. GADIAN, AND G. K. RADDA, *Nature (London)* **283,** 167 (1980).
4. P. SCHMALBROCK, W. W. HUNTER, H. C. CHARLES, F. W. WEHRLI, AND P. J. KELLER, *in* "5th Annual Meeting, Society of Magnetic Resonance in Medicine, Montreal, August 19–22, 1986," p. 983, 1986.
5. P. A. NARAYANA, J. D. HAZLE, E. F. JACKSON, L. K. FOTEDAR, AND M. V. KULKARNI, *Magn. Reson. Imaging* **6,** 481 (1988).
6. P. A. NARAYANA, E. F. JACKSON, J. D. HAZLE, L. K. FOTEDAR, M. V. KULKARNI, AND D. P. FLAMIG, *Magn. Reson. Med.* **8,** 151 (1988); erratum **11,** 134 (1989).
7. J. W. PAN, J. R. HAMM, D. L. ROTHMAN, AND R. G. SHULMAN, *Proc. Natl. Acad. Sci. USA* **85,** 7836 (1988).
8. M. BARANY, B. G. LANGER, R. P. GLICK, P. N. VENKATASUBRAMANIAN, A. C. WILBUR, AND D. G. SPIGOS, *Radiology* **167,** 839 (1988).
9. M. BARANY, P. N. VENKATASUBRAMANIAN, E. MOK, I. M. SIEGEL, E. ABRAHAM, N. D. WYCLIFFE, AND M. F. MAFEE, *Magn. Reson. Med.* **10,** 210 (1989).
10. J. FRAHM, K. D. MERBOLDT, AND W. HÄNICKE, *J. Magn. Reson.* **72,** 502 (1987).
11. J. FRAHM, H. BRUHN, M. L. GYNGELL, K. D. MERBOLDT, W. HÄNICKE, AND R. SAUTER, *Magn. Reson. Med.* **9,** 79 (1989).
12. J. FRAHM, H. BRUHN, M. L. GYNGELL, K. D. MERBOLDT, W. HÄNICKE, AND R. SAUTER, *Magn. Reson. Med.* **11,** 47 (1989).
13. H. BRUHN, J. FRAHM, M. L. GYNGELL, K. D. MERBOLDT, W. HÄNICKE, AND R. SAUTER, *Magn. Reson. Med.* **9,** 126 (1989).
14. H. BRUHN, J. FRAHM, M. L. GYNGELL, K. D. MERBOLDT, W. HÄNICKE, R. SAUTER, AND C. HAMBURGER, *Radiology* **172,** 541 (1989).
15. H. BRUHN, J. FRAHM, M. L. GYNGELL, K. D. MERBOLDT, W. HÄNICKE, AND R. SAUTER, *in* "8th Annual Meeting, Society of Magnetic Resonance in Medicine, Amsterdam, August 12–18, 1989," p. 185, 1989.
16. J. FRAHM, *Naturwissenschaften* **74,** 415 (1987).
17. S. R. WILLIAMS, D. G. GADIAN, E. PROCTOR, B. SPRAGUE, D. F. TALBOT, I. R. YOUNG, AND F. F. BROWN, *J. Magn. Reson.* **63,** 406 (1985).
18. C. ARUS, M. BARANY, W. M. WESTLER, AND J. L. MARKLEY, *Clin. Physiol. Biochem.* **2,** 49 (1984).

19. C. ARUS AND M. BARANY, *J. Magn. Reson.* **57,** 519 (1984).
20. J. FRAHM, T. MICHAELIS, K.-D. MERBOLDT, W. HÄNICKE, M. L. GYNGELL, D. CHIEN, AND H. BRUHN, *NMR Biomed.* **2,** 188 (1989).
21. J. FRAHM, K.-D. MERBOLDT, H. BRUHN, M. L. GYNGELL, W. HÄNICKE, AND D. CHIEN, *Magn. Reson. Med.* **13,** 150 (1990).
22. H. P. HETHERINGTON, M. J. AVISON, AND R. G. SHULMAN, *Proc. Natl. Acad. Sci. USA* **82,** 3115 (1985).
23. K. YOSHIZAKI, Y. SEO, AND H. NISHIKAWA, *Biochim. Biophys. Acta* **678,** 283 (1981).
24. S. DIMAURO, C. SCOTT, A. S. PENN, AND L. P. ROWLAND, *Arch. Neurol.* **28,** 186 (1973).
25. C. E. BROWN AND W. E. ANTHOLINE, *J. Phys. Chem.* **83,** 3314 (1979).
26. B. ESSEN, *Ann. NY Acad. Sci.* **301,** 30 (1977).
27. G. SJØGAARD AND B. SALTIN, *Amer. J. Physiol.* **243,** R271 (1982).
28. R. C. HARRIS, E. HULTMAN, AND L.-O. NORDESJÖ, *Scand. J. Clin. Lab. Invest.* **33,** 109 (1974).
29. J. KARLSSON, *Acta Physiol. Scand.* **81,** Suppl. 358 (1971).
30. D. R. WILKIE, M. J. DAWSON, R. H. T. EDWARDS, R. E. GORDON, AND D. SHAW, *in* "Second Symposium on Cross-Bridge Mechanism in Muscle Contraction" (G. H. Pollack and H. Sugi, Eds.), Vol. 2, p. 333, Plenum Press, New York, 1982.
31. A. A. CHRISTMAN, *Anal. Biochem.* **39,** 181 (1971).
32. A. M. KATZ AND F. C. MESSINEO, *Circ. Res.* **48,** 1 (1981).