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Heteronuclear Cross Polarization for Enhanced Sensitivity of *in Vivo* ^{13}C MR Spectroscopy on a Clinical 1.5 T MR System

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The potential of heteronuclear {^{1}H-^{13}C} cross polarization was studied for optimization of the signal-to-noise ratio in *in vivo* ^{13}C MR spectroscopy at the clinical field strength of 1.5 T. Experiments on the human calf showed a significant chemical-shift selective signal enhancement on triglyceride signals of 3.9 by heteronuclear cross polarization, compared to a standard pulse-acquire sequence. Studies on a neonatal piglet brain showed an enhancement by cross polarization of 2.2 for the detection of ^{13}C-1-glucose. This enhancement allowed a fourfold improvement in time resolution in dynamic ^{13}C MR of ^{13}C-1-glucose inflow in piglet brain. Phantom experiments demonstrated the efficiency of this technique for interleaved detection of two spectral regions. Tests with a volume coil showed the feasibility of signal enhancement by cross polarization over a large volume of interest. © 1998 Academic Press

Key Words: **heteronuclear cross polarization; ^{13}C; human; decoupling; nuclear Overhauser enhancement.**

INTRODUCTION

In vivo ^{13}C MR spectroscopy enables one to obtain metabolic information of humans not easily obtained by other non-invasive methods. The large chemical-shift range of ^{13}C MR spectra allows the resolution of resonances of a large number of substances, and thereby the study of some metabolites of which the signals are difficult to resolve in ^{1}H MR spectroscopy.

One of the major drawbacks of ^{13}C MR spectroscopy applied to humans is its low sensitivity, that is a consequence of the low natural abundance (1.1%) and the low gyromagnetic constant γ of the carbon nucleus (γ^1H/γ^{13}C = 4). Furthermore, most carbons have attached protons, resulting in multiplet structures which further decrease sensitivity and complicate spectrum analyses. Most ^{13}C MR studies performed on humans use proton decoupling to remove the multiplet-splittings, thereby enhancing sensitivity and resolution (*1–5*). With nuclear Overhauser enhancement (NOE) the sensitivity of ^{13}C MR spectroscopy can be further increased (*6–8*).

Heteronuclear polarization transfer provides another way to enhance sensitivity. This polarization transfer can be performed from the sensitive to the insensitive nucleus or vice versa (*9, 10*). The theoretically highest signal gain can be obtained when excitation and detection are performed at the sensitive nucleus (*11–15*).

This study focuses on the polarization transfer from protons to carbons to enhance sensitivity. Only a few studies have been reported in which this was applied to *in vivo* ^{13}C MR spectroscopy of humans, e.g., by the SINEPT method (*16, 17*) and by the DEPT method (*18, 19*). An alternative approach, not based on pulse-interrupted free precession, but by which transfer of magnetization is achieved by heteronuclear isotropic mixing, has recently been reported to be feasible *in vivo* at high magnetic field on a small bore NMR system (*20*). An adiabatic variant of this heteronuclear cross polarization has been proposed for *in vivo* applications (*21*). The technique of heteronuclear cross polarization has some advantages compared to the pulse-interrupted free precession methods: (i) the resulting peaks are not in mixed phase as may occur in SINEPT which makes analyses of the signals easier; (ii) the magnetization transfer function is less critically dependent on the scalar coupling; (iii) less sensitive to motion and pulse imperfections; (iv) the transfer does not depend on the multiplicity like in DEPT; (v) relaxation losses during the magnetization transfer period are limited (*20*); (vi) one can use decoupling without the need for a delay (which results in a further decrease in enhancement) between the polarization transfer pulses and the decoupling/acquisition period as in SINEPT (*16*).

The objective of this study was to explore the potential of heteronuclear {^{1}H-^{13}C} cross polarization, using a WALTZ isotropic mixing period, to enhance the sensitivity of ^{13}C MR spectroscopy on a clinical MR system at 1.5 T. The WALTZ-4 based cross polarization sequence is shown in Fig. 1.

RESULTS

Phantom Studies

Figure 2A shows an ^{1}H decoupled ^{13}C MR pulse-acquire spectrum of a phantom filled with an aqueous solution of glucose, lactate, and glutamate, obtained with a surface coil

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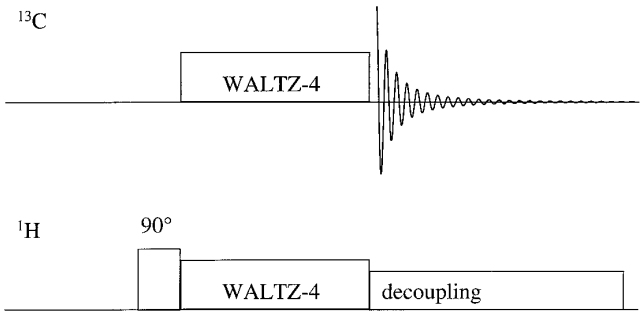

FIG. 1. The WALTZ-4 based heteronuclear cross polarization sequence. Duration of the WALTZ-4 contact pulse was 6.5 ms, which is optimal for proton–carbon coupling constants of 150 Hz.

set-up. Resonances of carbon spins from all substances are clearly visible. Spectra in Figs. 2B and 2C show the results of the enhancement by the use of chemical-shift selective cross polarization applied in an interleaved mode for two specific spectral regions, i.e., at the C-1 resonance of glucose (94 ppm) and at the C-3 resonance of lactate (20 ppm). Enhancement factors of both resonances were 3.1 and 3.6, respectively. The present use of WALTZ-4 based cross polarization makes the experiment chemical-shift selective; polarization transfer occurs in a small spectral region ($\gamma B_1/2\pi \approx 900$ Hz).

Cross polarization experiments targeting a single frequency region were performed with the surface coil set-up on phantoms containing sunflower oil and a glycogen solution. On the oil phantom enhancement factors of 3.8 for the unsaturated as well as the saturated lipid carbon resonances could be achieved, which is close to the theoretical maximum enhancement of ≈4. The signal gain of 2.1 obtained for the C-1 signal of glycogen in solution was considerably lower.

Experiments with the volume coil and ^{13}C MR spectroscopy acquisition extended with 2D-CSI localization resulted in the data presented in Fig. 3. Spectral maps of the C═C spectral region at 130 ppm of the 2D-CSI data sets are shown; the spectra drawn in white were obtained with ^{1}H broadband decoupled ^{13}C 2D-CSI. Spectra drawn in red were obtained with the polarization transfer pulse sequence optimized for the C═C spectral region; broadband ^{1}H decoupling was also applied. All voxels showed a significant signal increase; signal gains of the individual voxels ranged from 2.68 to 3.85, mean signal gain was 3.18 ($SE = 0.04$). The lowest signals in both experiments are in the top row voxels. These voxels are outside the sensitive volume of the ^{13}C half volume coil which was wrapped around the bottom side of the phantom; coil wires are marked in the figure.

In Vivo Studies

Postmortem cross polarization studies on the brain of a neonatal piglet resulted in a signal enhancement of 2.2 for the C-1 signals of ^{13}C-labelled glucose, present in the brain (Figs. 4A and 4B). Figure 4C shows a stack plot of spectra recorded during infusion of ^{13}C-labelled glucose using heteronuclear cross polarization applied *in vivo* to the brain of a piglet. The time resolution was 3 min. The first spectrum was obtained before the start of the ^{13}C-1-glucose infusion. The subsequent spectra clearly show increasing glucose signals during the infusion period and a decrease thereafter.

In vivo ^{13}C MR spectra of the unsaturated triglyceride carbons originating from superficial adipose tissue of the human leg are shown in Fig. 5. For the spectrum in Fig. 5A no decoupling was used whereas for the spectrum in Fig. 5B, WALTZ-4 proton decoupling was applied during the signal acquisition period. Spectrum C was obtained using both cross polarization and WALTZ-4 decoupling. Enhancement factors for spectra B and C are respectively 2.7 and 10.6 with respect to the non-decoupled spectrum. A gain larger in magnitude than 2 for decoupling can be explained by partial NOE. Cross polarization accounted for a signal gain of 3.9 in the latter experiment. Until now, no signal enhancement could be achieved *in vivo* for the C-1 signal of glycogen in human muscle compared to direct acquisition with enhancement by decoupling and NOE.

DISCUSSION

This study shows that the sensitivity of ^{13}C MR spectroscopy at the clinical field strength of 1.5 T can be improved significantly with the use of heteronuclear cross polarization from protons to carbons. The gains obtained for triglyceride signals are close to the theoretical value, $\gamma^{1}H/\gamma^{13}C = 4$. The

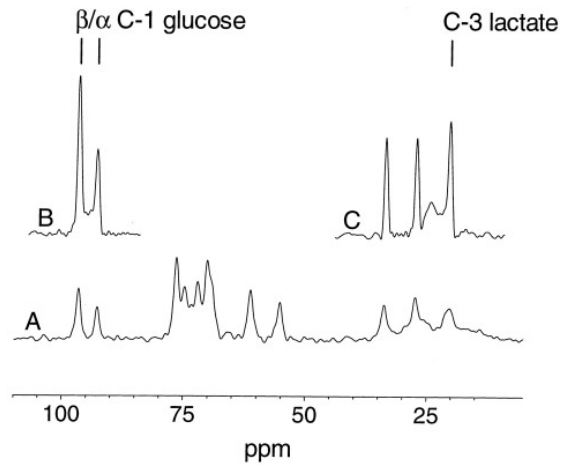

FIG. 2. ^{13}C MR spectra recorded from a spherical phantom containing an aqueous solution of glucose 300 m*M*, lactate 250 m*M*, and glutamate 200 m*M*. Shown are spectra obtained with a pulse-acquire sequence (A) compared to spectra (B), (C) obtained with chemical-shift selective cross polarization. Measurement parameters were as follows: 256 FIDs were accumulated for spectrum (A), for spectra (B) and (C) in an interleaved fashion: 2 times 2 blocks of 128 accumulations at each frequency, the ^{13}C frequency was placed at the C-1-glucose region (94 ppm) and at the C-3-lactate region (20 ppm) with a difference of 1200 Hz. Spectral width was 8 kHz, 512 data points were collected, and the repetition time was 1 s.

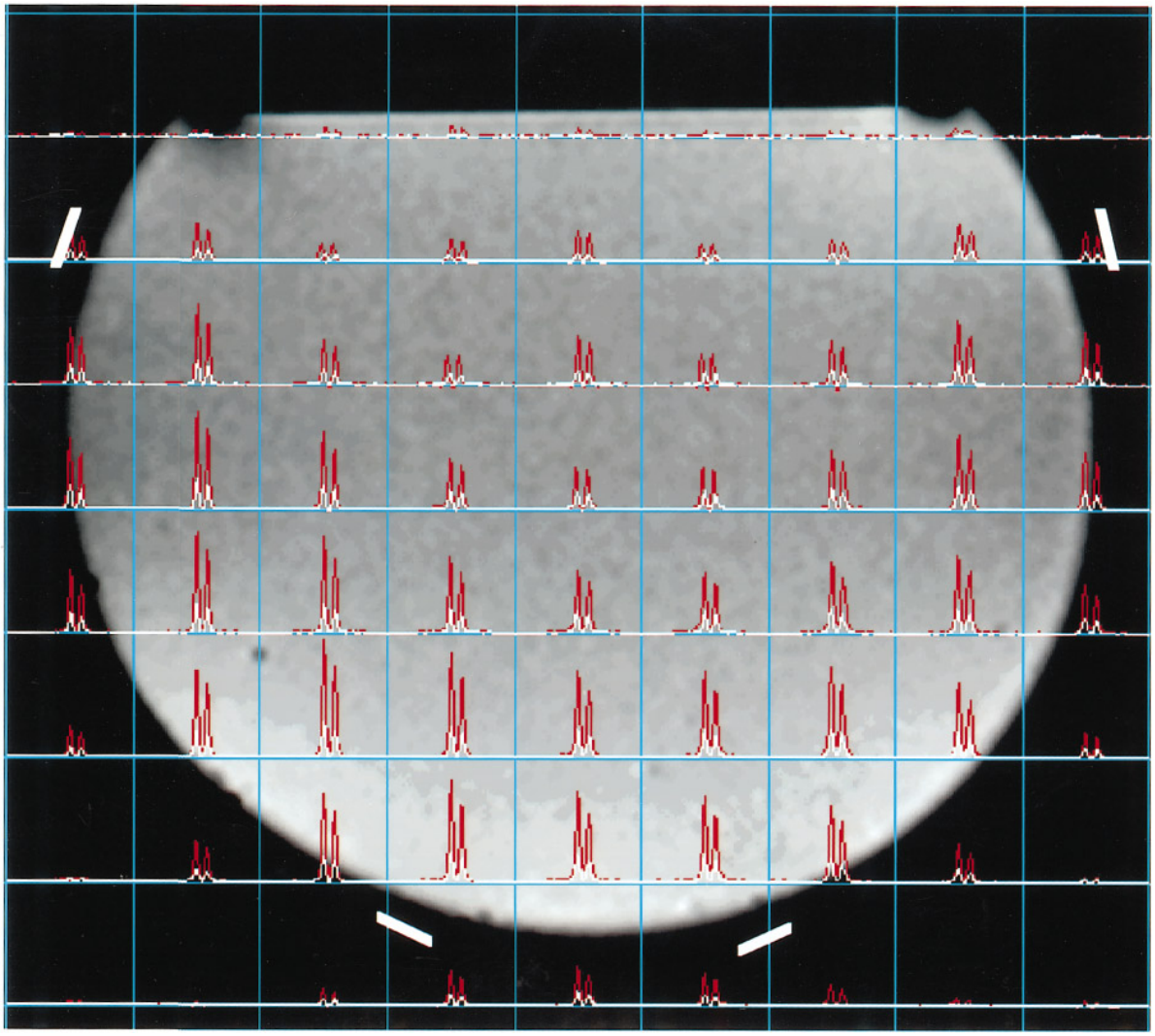

FIG. 3. Spectral maps obtained from a large spherical phantom containing sunflower oil, measured using an ^{1}H/^{13}C volume coil configuration. Spectra acquired using heteronuclear cross polarization are drawn in red, compared to the spectra obtained with a pulse-acquire technique shown in white, plotted with the same scaling. Positions of the ^{13}C half volume coil wires are indicated. The matrix size of the CSI data was 16 × 16 × 1024, spectral width was 6 kHz, field of view of 240 mm resulting in a voxel size of 15 × 15 mm, repetition time in both pulse-acquire and heteronuclear cross polarization was 1.5 s, and total measurement time of a CSI data set was 6 min, 36 s. Cross polarization was optimized for the C═C spectral region around 130 ppm.

total gain achieved with cross polarization, decoupling, and NOE was 10.6 for the unsaturated triglyceride carbons in *in vivo* ^{13}C MR spectra of the human calf. Polarization transfer experiments performed on the human calf with which we compare these results are from Bomsdorf *et al.* (*16*). They reported a total signal enhancement of 6 for the unsaturated fatty acid signals with the use of SINEPT and ^{1}H decoupling at 4 T.

Knüttel *et al.* (*22*) demonstrated the detection of unsaturated carbons of fatty acids by an indirect method of selecting ^{13}C attached protons. Theoretically a higher signal enhancement is possible with this method but the resulting spectrum showed some disadvantages. Firstly, only one of the lines of the doublet is visible in the proton detected spectrum; the other line coincides with the water resonance. Secondly, at the low resolution in proton detection, one cannot discriminate between mono- and poly-unsaturated fatty acids, as is possible in the cross polarization enhanced ^{13}C spectrum, see Fig. 3.

In general, indirect detection of carbons in ^{1}H spectra requires good water suppression; it often results in distorted baselines and suffers a low spectral resolution (*22–24*). With labelled compounds often broadband ^{13}C decoupling is required during proton detection to obtain maximum signal gain and resolution. This requires much more power than broadband ^{1}H decoupling during ^{13}C signal acquisition and is of concern regarding RF deposition safety guidelines.

In phantom studies, detection of the C-1 signal of glycogen using heteronuclear cross polarization, decoupling, and partial

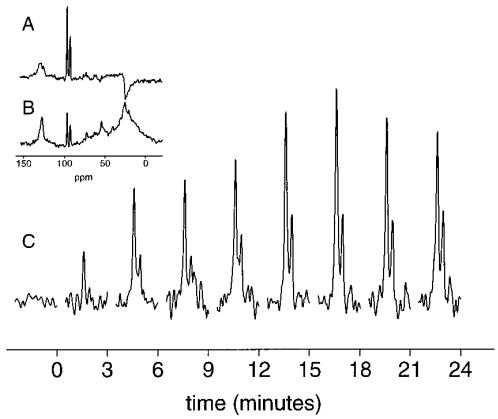

FIG. 4. ^{13}C MR spectra of a postmortem neonatal piglet brain containing ^{13}C-1-glucose are shown in Figs. 4A and 4B. Spectrum (A) was obtained using heteronuclear cross polarization and spectrum (B) using direct detection and proton decoupling. *In vivo* ^{13}C MR spectra recorded from a neonatal piglet brain before, during, and after administration of ^{13}C-1-glucose are shown in Fig. 4C. Only the C-1-glucose spectral region is shown, 80–110 ppm. Experimental parameters: 240 acquisitions, repetition time of 750 ms.

NOE resulted in a signal gain of 2.1 compared to direct detection with decoupling and partial NOE. No signal gain was achieved *in vivo* with respect to ^{1}H decoupling and partial NOE. A significantly lower transfer efficiency for glycogen C-1 as compared to lipid signals was reported for the SINEPT polarization transfer scheme (*16, 17*). This is likely due to a shorter (effective) T_2 of the glycogen C-1 compared to the triglyceride carbons. Knüttel *et al.* showed that detection of liver glycogen is possible using proton-detected ^{13}C spectroscopy (*23*).

The most promising application of cross polarization *in vivo* is probably the possibility to enhance the time resolution in dynamic ^{13}C-labelling studies. The increase in magnitude of the C-1 signals of glucose in the neonatal piglet brain by a factor of 2.2 improves the temporal resolution for detection of these signals by a factor over 4. An improved time resolution was also reported in a ^{13}C-labelling study of RIF-1 tumors with the use of heteronuclear cross polarization (*20*).

Although the effective chemical-shift range of the cross polarization method as applied in this study is limited, it is demonstrated that more chemical-shift regions can be covered by interleaved cross polarization at different frequencies. The total measurement time, for cross polarization of two chemical-shift regions, can still be shorter than the time to obtain a ^{13}C MR spectrum with the same signal-to-noise ratio, using the pulse-acquire method. Chemical-shift selective enhancement by cross polarization at two frequencies has also been reported by Artemov *et al.* (*20*).

In the cross polarization experiments broadband ^{1}H WALTZ-4 decoupling was applied during the acquisition period to enable a comparison with the conventional ^{1}H decoupled ^{13}C MR spectroscopic pulse-acquire technique. Because of the chemical-shift selective character of the present cross polarization method broadband decoupling is not really necessary. The use of CW decoupling relaxes RF power deposition and therefore higher duty cycles can be used which further decreases measurement times.

Part of the results in this study was obtained with a double surface coil set-up, to achieve a high signal-to-noise ratio. However, one problem arises with surface coils and cross polarization techniques: the RF fields of both coils have to match to fulfill the Hartmann–Hahn condition. A partly mismatch may have contributed to the lack of enhancement for the C-1 signal of glycogen from the muscle as compared to an enhancement of 2.1 for this signal of glycogen in solution.

To achieve easy matching of the RF fields over a large volume of interest, we explored the use of a homogeneous ^{1}H coil in which a ^{13}C half volume coil was placed. Calibration of the necessary power to be applied to the ^{1}H coil was straightforward. For comparison of the signal gain in the individual CSI voxels the RF pulse amplitudes used on the ^{13}C half volume coil, in the pulse-acquire experiment, and the cross polarization experiment were set to the same value. Signal gain was similar for all voxels in the sensitive area of the ^{13}C coil, showing good Hartmann–Hahn matching of the RF fields over the large volume of the phantom.

In this study both non-localized spectra and CSI localized spectra were measured. Improvements in localization can be

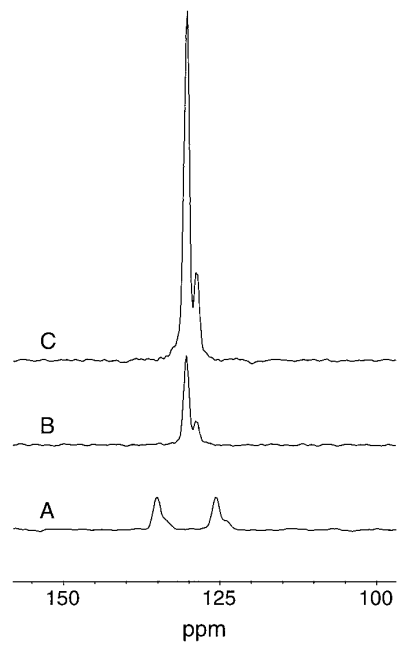

FIG. 5. Natural abundance ^{13}C MR spectra of the human calf. Spectra were obtained (A) without ^{1}H decoupling, (B) with broadband ^{1}H decoupling using WALTZ-4, and (C) with heteronuclear cross polarization optimized for the C═C spectral region (130 ppm) and broadband ^{1}H decoupling, using the surface coil setup. Spectra are plotted on the same scaling. Experimental parameters: 64 acquisitions, repetition time of 3 s, 512 data points, and spectral width 8 kHz. Subject was a 23-year-old female weighing 52 kg. The study was performed after an informed consent was obtained.

made by using a slice selective proton excitation pulse or other means of volume selection like ISIS (*8, 9, 18, 19, 25*) which can be placed before the polarization transfer element in the sequence.

EXPERIMENTAL

Measurements were performed on an 1.5-T Magnetom SP 4000 (Siemens, Erlangen, Germany) equipped with a second RF transmit channel. Two types of coil configurations were used for the different ^{13}C/^{1}H double resonance experiments, depending on the volume of interest. The surface coil set-up consisted of an 11-cm diameter ^{13}C transmit/receive surface coil and a 17 $\times$ 23 cm ^{1}H transmit/receive butterfly coil. As a volume coil an ^{1}H linear vertically polarized birdcage constructed within the original housing of a Siemens SP head coil was used, combined with a transmit/receive ^{13}C horizontally polarized curved half volume coil (*26*).

Two pulse sequences were used in this study. First, a ^{13}C pulse-acquire sequence with optional proton decoupling during the acquisition period and secondly a cross polarization sequence as described in (*27*). The mixing period in the latter sequence consisted of one WALTZ-4 cycle with a duration of 6.5 ms; decoupling was also optional in this sequence.

For the direct detection experiments the power of the hard ^{13}C excitation pulse (duration 260 μs) was optimized for maximal signal intensity. This optimized power level was also used for the contact pulse in polarization transfer experiments. The RF field strength of the ^{1}H coil for decoupling as well as polarization transfer was optimized for a certain volume using a localized calibration technique (*28*) which enables optimal settings for polarization transfer despite the use of two differently shaped coils.

Phantom Studies

To demonstrate the feasibility of this sensitivity enhancement technique several phantom studies were performed. To test the surface coil set-up we used a cylindrical phantom with diameter 10 cm, containing sunflower oil, or a solution of 109 m*M* glycosyl units rabbit liver glycogen. Another phantom spherical in shape, with diameter 8 cm, contained a solution of glucose, lactate, and glutamate. The latter phantom was used to study interleaved cross polarization at two different frequencies.

To investigate the effect of the larger matching volume of the RF fields of the volume coil set-up we used a large spherical phantom (diameter 15 cm) filled with sunflower oil. Localized spectra were acquired using 2 dimensional chemical-shift imaging (CSI); the phase encoding was applied after the polarization transfer from ^{1}H to the ^{13}C nucleus to compare the results with conventional ^{13}C 2D-CSI measurements.

In Vivo Studies

Postmortem decoupling and cross polarization studies were performed with the surface coil set-up on a neonatal piglet brain, containing ^{13}C-1 labelled glucose, to optimize the Hartmann–Hahn matching. *In vivo* ^{13}C MR spectroscopy was performed on the brain of an anesthetized neonatal piglet to monitor the influx of labelled ^{13}C-1-glucose. The piglet was anesthetized with pentobarbital, and catheterized in the carotid artery. Inside the magnet, anesthesia was maintained by pump-ventilating with ethrane. During spectroscopy, rectal temperature and ECG were monitored. Over a period of 15 minutes, glucose 20% (30% enriched) was infused with an infusion speed of 0.5 ml/min. In this case cross polarization was performed at one frequency region to achieve the highest time resolution possible.

Furthermore, ^{13}C MR spectra of the human calf were obtained to show that signal enhancement using cross polarization is realizable on humans *in vivo.* In this experiment, the SAR at the body surface of RF power deposition from the ^{1}H surface coil was estimated to be 2.9 Watt/kg, as calculated according to the procedure described in (*28*) and this is well below safety guidelines (*29*). This SAR value was mainly due to the decoupling part of the sequence, and not the WALTZ-4 contact period. Contribution of the ^{13}C channel was negligible because of the much lower duty cycle and its lower frequency (*30*).

Spectral analyses was performed using Siemens Luise software (Siemens, Erlangen, Germany). Spectra were zero-filled to 4K data points, multiplied by a gauss function of 64 ms, phase and baseline corrected; peaks of interest were fitted to a gaussian lineshape. Chemical-shift imaging data were filtered with a hamming filter prior to fourier transformation. Spectra of individual voxels were analyzed as mentioned above. Chemical-shift scaling was performed placing the methyl signal of tetramethylsilane at 0 ppm.

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